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Contents

Volume I

Block 2 - Tuesday, July 5, 11:00 – 12:30

Session 5.4. Pathogens in exotic regions

Block 6 - Wednesday, July 6, 14:00 – 15:30

Session 6.1. Feed additives

Session 6.2. Ruminant Health

Session 6.3. (MR)SA

Volume II

Part IV Poster Presentations

Tuesday, July 5

Volume III

Part IV Poster Presentations

Wednesday, July 6

Part IV

Poster Presentations

IMPACT OF HIGH-FIBRE DIET ON EXPLORATORY BEHAVIOUR IN FATTENING PIGS

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SUMMARY

The natural behaviour of pigs includes a large range of foraging and exploratory activities. In contrast, pigs in conventional housing spend little time feeding, and unlike their natural food, conventional fattening feed stuffs contain only small amounts of fibre. Their need to forage is therefore often misdirected towards mates. In this study it was tested if an increased amount of fibre in the pigs´ feed changes the animals´ motivation for feeding and exploration.

A group of 14 fattening pigs received a compound feed containing 9 % high-fibre lignocellulosis, while the control group was fed with a standard fattening compound feed. During two separate weeks, both groups had access to a toy, which was equipped with a standard ALT-pedometer to count its movements.

The results show that the pigs fed with additional fibre played significantly less than conventionally fed pigs. Both groups played approximately the same amount in both weeks and the use of the toy did not decrease over time. The use of the toys was highest between 9:00 and 17:00 o'clock, matching the pigs` natural circadian rhythm, however, movements of the toys where also recorded during the night.

It can be assumed, that pigs fed with a higher amount of fibre play less because their motivation to forage is lower. High-fibre food is known to cause longer feeding times and a higher satiation. Possibly feeding a higher amount of fibre can thus prevent misdirected foraging behaviour and behavioural disorders in fattening pigs.

INTRODUCTION

Pigs in natural environments spend major parts of their active time foraging. The species-specific foraging behaviours include rooting and exploring with the snout [8]. Although pigs in modern housing systems spend only short time actually feeding, these behaviours are still performed regularly [10, 11]. Since the customary barren environments do not give pigs the opportunity to root, exploratory behaviours are often misdirected towards mates or parts of the enclosures and behavioural anomalies can be developed [9]. It is known that hunger and ´boredom` in pigs, caused by restricted feeding and barren environment, leads to stereotypic or aggressive behaviour and a lack in animal welfare [3, 7]. Various studies on sows show that an increased amount of fibrous substances in the feed can decrease such stereotypes as well as active behaviour in general [1, 2, 5, 6]. It is assumed that high fibre feed reduces hunger and feeding motivation and associated motivations for exploratory behaviours [4]. However, those studies were almost exclusively done on sows, because they are fed restrictively for physiology-related reasons and stereotypes are a widespread problem there. In contrast, fattening pigs are mostly fed *ad libitum*. Behavioural disorders, however, are also frequently found in growing pigs. Customary fattening feedstuffs are high in energy and low in fibre, and are consumed in short times. The motivation for foraging-related behaviours is not satisfied through feeding alone and therefore directed towards other things.

To test whether the motivation for exploratory behaviour can be reduced by high-fibre feed also in fattening pigs, the use of a movable toy was assessed for a group of high-fibre fed pigs (test group) and a group of conventionally fed pigs (control group). The intensity of using the toy was regarded as an indicator for exploratory motivation of the animals.

MATERIAL AND METHODS

The experiment was conducted on 28 Duroc x (Landrace x Large White) castrated male pigs from September to December 2010. The animals were randomly assessed to two groups of the same medium body weight in the beginning of the fattening period. Both groups were housed in similar outdoor enclosure for an adaption period of two weeks and a fattening period of 12 weeks from 39.9 ± 4.1 kg to 103.3 ± 12.5 kg life weight. Both enclosures had concrete floor and were equipped with an automatic feeder, a drinker and an air conditioned hut with slatted floor that served as resting area. Enclosures were changed every three weeks. While the control group was fed with a standard fattening compound feed, the test group's feed contained 9 % lignocellulosis and was thus higher in crude fibre (table 1).

Table 1: Ingredient and nutrient composition of the control feed and the test feed.

	Control feed	High-fibre feed
Dry matter (%)	86.76	87.57
Ingredient composition (g/kg)		
Wheat	439	349
Rye bran	150	150
Wheat bran	120	120
Barley	80	80
Rape cake	75	75
Soybean extraction meal	66	66
Sugar beet molasses	30	30
Rye	17	17
Lignocellulosis	-	90
Nutrient composition (% DM[1])		
Ash	5.13	5.04
Nitrogen	2.98	2.86
Protein	18.64	17.86
Crude fibre	5.18	8.39
Crude fat	2.37	2.05
NDF[2]	22.42	28.99
ADF[3]	6.86	11.54
ADL[4]	2.09	3.45
Energy (ME MJ/kg)	12.6	11.58

1=dry matter, 2=neutral detergent fibre, 3=acid detergent fibre, 4=acid detergent lignin

During week 9 and week 12, both groups were provided an additional toy: a square iron rod of 4 x 4 cm and 130 cm length, hanging freely from above approximately 30 cm above the ground. The rods were equipped with standard ALT-Pedometers that counted every movement of the rod in 15 minutes intervals.

RESULTS

Both groups showed interest in the toy and used it frequently by moving it with the nose, trying to bite and chew it and sense it with the snout.

While the group that received high fibre feed moved the rod 12,978 times during the first period and 14,901 times during the second period, the control group used it much more often, 27,513 and 23,288 times (figure 1). This is a difference of the factor 2.1 in the first period and of the factor 1.6 in the second period. This difference is statistically significant (t-test; n=16, p=0.001) for the whole period as well as for period 1 only (t-test, n=8, p=0.002). For period 2 only the difference is not significant (t-test, n=8, p> 0.05).

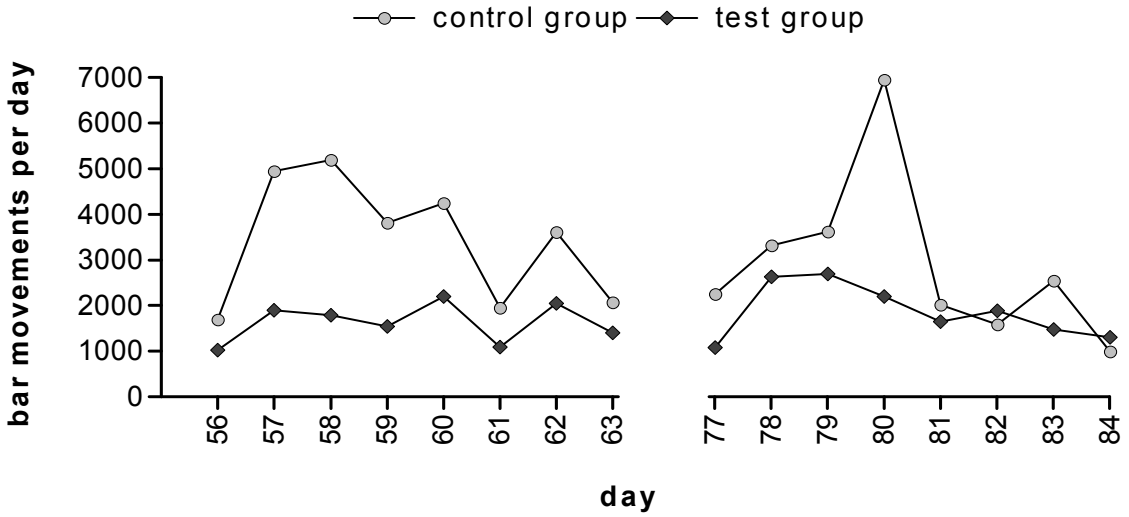

Figure 1: Use of the toy, total movements per day.

The rod was mostly used during the day, with peaks between 10:00 and 11:30 o'clock, and between 12:30 and 16:00 o'clock, but also occasionally during the night, as shown in figure 2.

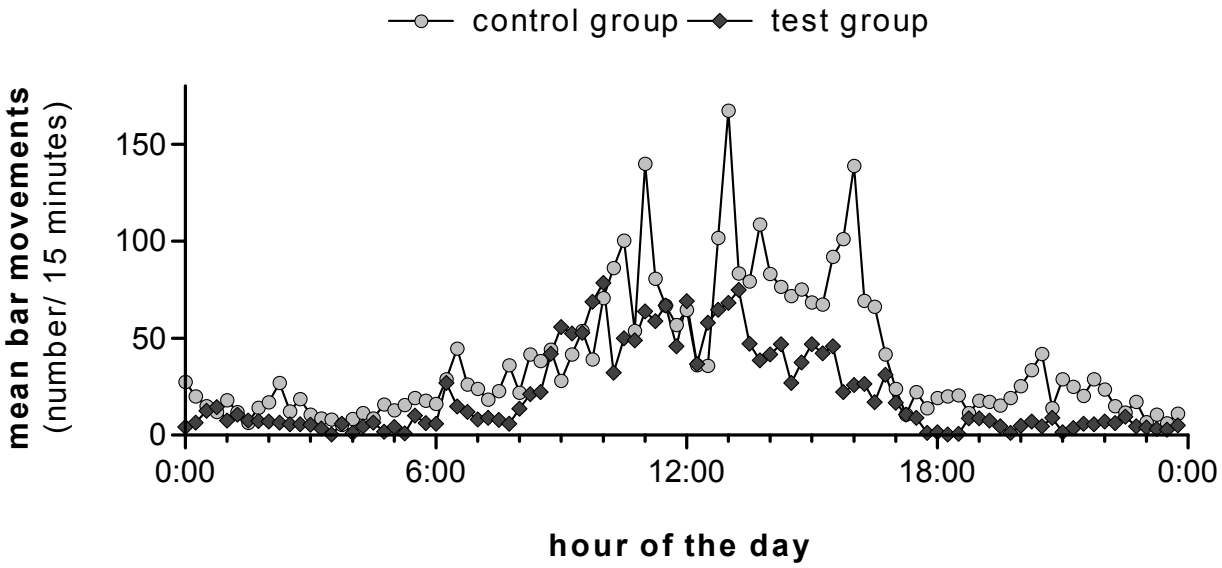

Figure 2: Average use of the toy in the course of the day.

DISCUSSION

Explorative behaviour belongs to the species-specific behaviour repertoire of the pig. It is closely connected to feeding behaviour and foraging [8]. Since pigs in modern housing systems spend only short times feeding, the motivation to forage is not satisfied. This need is therefore directed against other movable objects [9]. The toy used in the described experiment serves as an indicator for the motivation to manipulate objects and to forage. The results show that pigs fed with a higher amount of crude fibre manipulated the object significantly less than the control croup. This might indicate a lower motivation to forage. High fibre feed is known to influence the activity behaviour of pigs [1, 5]. It is assumed that high fibre feed increases satiation and thus decreases the motivation for foraging behaviour [4, 5]. Pigs fed with such a feed rest more and show less activity and less behavioural disorders [2, 6].

CONCLUSIONS

It can be proposed that a feed high in crude fibre decrease the pigs' motivation for exploration and manipulation of objects such as the offered toy. With the decrease of that motivation, behavioural disorders might occur less and the welfare of the animal might increase.

An assessment of toy-use on individual basis rather than for the whole group might deliver more accurate results.

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BACK TEST vs TONIC IMMOBILITY TEST: BEHAVIOURAL RESPONSE IN TWO DIFFERENT RESTRAIN SITUATIONS

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SUMMARY

Backtest (BT) and Tonic Immobility Test (TIT) allow to classify piglets in different type of coping style (HR=High-Resisting; LR=Low-Resisting for the BT; non-TI= don't show tonic immobility and TI=showing tonic immobility for the TIT) on the basis of their reaction to restrain. There are no data concerning the relationship between TIT and BT responses in piglets. The aim of this study was to investigate this potential relationship. Sixty piglets of commercial crossbred (Duroc x (Landrace x Large White)) reared in the same farm were examined. The BT was performed on all piglets at 10 and 17 days of age; the TIT was carried out at 9 days (20 piglets), at 13 days (20 piglets) and at 19 days (20 piglets) of age. Results of the tests were not influenced by their reciprocal temporal sequence. There was not a correspondence between the BT and TIT categories. This result suggests that the two tests measure reactions to different challenging stimuli and they may be used as indicators of different types of response to restrain. In the BT, the duration of vocalization, struggling, and relaxation were significantly different ($P<0.05$) between HR and LR piglets.

INTRODUCTION

Pigs vary largely in behavioural reactions when exposed to the same stressful situation. Individual differences in adaptive or coping reactions of pigs have received growing attention, as the identification of basic characteristics that predict (mal)adaptation to husbandry conditions could be relevant both for pig husbandry and pig welfare. In order to identify these characteristics, the Backtest (BT) (Figure 1a) and the Tonic Immobility test (TIT) (Figure 1b) have been used [4]. In both tests young piglets are restrained in supine position. The behavioural reaction of piglets in these tests is thought to reveal part of their "coping style" or 'personality'. Some piglets usually referred to as 'high-resisters' (HR), struggle a lot during the Backtest, whereas others respond with immobility, the so-called 'low-resisters' (LR) [6]. At a later age, HR and LR pigs have been shown to differ in their behavioural and neuroendocrine reactions to a variety of challenges [5, 6, 7, 9, 10, 11]. The response profiles of HR and LR largely resemble the diverging coping styles, often referred to as "(pro)active" versus "passive/reactive", respectively, that have been identified in pigs and in other species [1, 8]. Likewise, it has been proposed that the tonic immobility is one possible way of assessing whether individual pigs are more likely to adopt a more active (low susceptibility/short duration of Tonic Immobility test, struggle, move fast) or a more passive behavioural strategy (high susceptibility to/long duration of Tonic Immobility test, tense, move more slowly) in a challenging situation [2, 3]. There are no data concerning the possible link between the Tonic Immobility and response to the Backtest in piglets. The aim of this study was to investigate this potential relationship.

MATERIAL AND METHODS

Sixty piglets from 10 litters of commercial crossbred pigs (Duroc x (Landrace x Large White)) were tested. Male piglets were castrated at approximately 5 days of age. Sow and piglets were housed in conventional farrowing pens (1.5 m x 2.5 m) with the sows restricted in farrowing crates. The Backtest was performed at 10 and 17 days of age; the Tonic Immobility test was performed before (at 7 days of age - 20 piglets), between (at 13 days of age - 20 piglets) and after (at 19 days of age - 20 piglets) the Backtest. During the Backtest each piglet was restrained on its back by placing the right hand over the throat and the other loosely on the hind legs (Figure 1a). Classification of pigs was based on the number of escape attempts (i.e. bouts of struggling with at least the hind legs) they displayed during 60 s. A pig was classified as high-resisting (HR) if it performed more than four escape attempts in the two tests, with a minimum of two attempts in one test. If a pig struggled less than four times in two tests, with a maximum of two attempts in one test, it was labelled low-resisting (LR) [1]. In the Tonic Immobility test the experimenter placed the piglet on its back onto a V-shaped wooden cradle (70 cm long, angle approximately 80°) (Figure 1b). Then the experimenter put a sand-filled cloth bag (15 x 20 cm^2, ca. 500 g) on the piglet's chin, gently stretched its back legs and then let go of both the hind legs and the sand bag. If the pig became immobile, the duration of immobility was recorded from this point onwards (we call these pigs 'TI pigs'). As soon as the piglet struggled, the bag was removed and the duration of immobility recorded. If the piglet did not struggle within 5 min, the test was terminated, and duration of 300 s was allocated to this

pig. Some piglets did not show the immobility response described above ('non-TI pigs') [2]. They struggled while they were being placed onto the cradle, or as soon as they touched the cradle. It was not possible to get them through the process described above. In this experiment, they were recorded as having duration of immobility of 0 s. Due to non-normality of the data, non-parametric statistics were used for the analysis. Chi-square analysis was used to evaluate the effect of the reciprocal sequence of tests on the classification results for Backtest and Tonic Immobility test, and to analyse the relationship between Backtest scores and TIT. Mann-Whitney U Test was used to evaluate the differences in behaviour between High and Low resisters pigs and Low resisters pig.

RESULTS

The reciprocal sequence of the tests did not influence their results (Backtest: $\chi2=3.61$, d.f.= 2, n=60, P=0.16; Tonic Immobility test: $\chi2=1.08$, d.f.=2, n=60, P=0.58).

In the Backtest the duration of vocalization, struggling and relaxation were significantly different between HR and LR piglets, validating the methodology (Table 1).

Due to the Tonic Immobility test methodology, the duration of struggling, relaxation and vocalizations were not analyzable. Moreover, among "TI-pigs" we found a high variability in the time of reaction (9.57 ± 9.21 sec). There were no relationship between Backtest scores and susceptibility to immobility ($\chi2=1.15$, d.f.=1, n=60, P=0.29). Only 28.3% of "low resisters" pigs showed immobility response during the Tonic Immobility test and, on the other hand, only 20.0 % of "high resisters" pigs struggled immediately afterwards placed onto a V-shaped wooden cradle.

DISCUSSION

Even if Backtest and Tonic Immobility test are present in literature for a long time [6, 7], there are no data concerning the relationship between their classifications. During Backtest and Tonic Immobility test piglets are subjected to a restrain situations and both tests adopt a bimodal classification creating two groups whose characteristics have been compare each other (HR as non-TI, LR as TI) [2, 3]. Nevertheless, our results indicated that behavioural response of an animal to one test was not predictive of the behavioural response of the same animal to the other test. It is important to underline that there are relevant differences between the two tests: the Backtest has a fixed duration while the duration of Tonic Immobility test is extremely variable. Moreover, in the Backtest the animal is restrains by a human hand while in the Tonic Immobility test a piglet is restrain by means of a sand-filled cloth bag and V-shaped wooden cradle. These differences can affect the behavioural response of the animals which may perceive different emotions during the tests (e.g. fear of humans during BT, anxiety for the restrain situation during TIT) and therefore to show different reactions.

CONCLUSIONS

There was not a correspondence between the BT and TIT categories. In particular, the "TI-pigs" class was heterogeneous and it included piglets with different coping styles. Based on our results it can be assumed that the two tests measure the reactions of pigs to different challenging stimuli and they may be seen as indicators of different types of response.

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1a  **1b** 

Figure 1: The Backtest (1a) and the Tonic Immobility Test (1b)

Table 1: Comparison between HR and LR reactions to the Backtest (Mean ± SD)

Behaviour	HR	LR	U	P
Vocalization (sec)	49.76 ± 12.34	31.28 ± 9.98	150	0.02
Struggling (sec)	27.43 ± 11.56	13.57 ± 5.5	31	0.0001
Relaxation (sec)	8.13 ± 3.78	32.17 ± 13.78	35	0.005

DIFFERENCE OF SURFACE BODY TEMPERATURE IN PIGLETS DUE TO THE BACKTEST AND ENVIRONMENTAL CONDITION

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SUMMARY

One-hundred-height piglets were subjected to Backtest (BT) at 10 and 17 days of age in two periods, at the environmental temperatures of 25°C and 22°C, and 30°C and 24°C, respectively. Before and after the BT, infrared thermo-images (IRT) of eye, back, womb and right side of each subject were taken. Rectal temperature, sex and live weight were also recorded. The data of body surface temperatures were processed using the Mixed procedure of SAS for repeated analysis. The model included the random effect of the subject, the fixed effects of period (1, 2), age at the BT (10 d, 17 d), BT (before and after), BT reactivity (LR, HR), sex (male, female), and the covariate live weight within age at the BT. The major sources of skin temperature variations were the period and the weight at the BT. There was a significant difference ($P < 0.05$) in skin temperature before and after the BT at the positions of right ear and eye. It was found slightly lower after the BT due to a vasoconstriction caused by the stress of immobilization. The LR and HR piglets showed different surface body temperatures ($P<0.05$) only at the level of the right armpit. The results show the possibility to use the body surface temperature recorded by IRT in piglets stress assessment but highlight the need to take in account the environmental thermal conditions in order to control this primary source of variability.

INTRODUCTION

In pigs, some stable individual traits such as coping style may have a predictive value of the individual's adaptive capacity and its susceptibility to show abnormal behaviours in stressful situation. In order to identify the coping style in pigs, the "Backtest" [3] has been frequently used. It is carried out on piglets at the age of 10 and 17 days keeping manually the subject in a supine position for one minute, while observing the behavioural response of the animal which can vary from immobility to extreme struggle. Piglets who struggle a lot during immobilization are usually referred as 'high-resisters' (HR), whereas piglets which tend to respond with immobility are called 'low-resisters' (LR). HR subjects are more aggressive and unwilling to adapt to environmental change while LR subjects tend to explore the environment and show a pronounced behavioural flexibility in responding to environmental stimuli [2]. It is well-known that stress can induce vascular changes in different parts of the body which are followed by changes in deep (rectal) and skin temperatures [1]. A measure of pig skin temperature can be obtained using an infrared camera that detects, without contact, the thermal energy emitted by the body. This is converted into an electronic signal to create a digital image that represents the gradient of the body temperature with an accuracy of less than 0.1 °C. The purpose of this study was to evaluate by thermography the variation in skin temperature of piglets subjected to a stressor such as the immobilization required by the Backtest.

MATERIAL AND METHODS

The study was carried out on 108 piglets which were divided into two groups of 53 and 55 subjects examined at the same farm in two different periods. At the Backtest, carried out at 10 and 17 days of age, subjects that showed more than four escape attempts, at least two attempts per test, were classified as HR. If the subjects have struggled less than 4 times, with less than two escape attempts per test, were classified as LR [3]. Skin temperature was assessed by examination of a thermal imaging recorded by a thermal camera (ThermaCAM P25, FLIR Systems, Milan) settling the skin emissivity at 0.98, placing the subject at m 1.5 of distance and taking in account the environmental temperature which was recorded by a thermo-hygrometer (Horegon Scientific ThGr800). The environmental temperatures, during the tests carried out at 10 and 17 days of age were 25 °C and 22 °C in the first period and 30 °C and 24 °C in the second period, respectively. Before and after the Backtest, infrared images of eye, back, womb and right side of each subject were taken. The images were analyzed with the program ThermaCAM Researcher Basic (Flir System, Milan). In the present work only the values of maximum temperature of the body areas examined are presented. Rectal temperature, sex and live weight were also recorded. The data were processed using the Mixed procedure of SAS for repeated analysis [4]. The model included the random effect of the subject, the fixed effects of period (1, 2), age at the BT (10 d, 17 d), BT (before and after), BT reactivity (LR, HR), sex (male, female), and the covariate live weight within age at the BT. In the

model were also included the interactions of first degree. The interaction between period and age at BT was found statistically significant (P <0.001) for all measures of skin temperature. The same model was used for the rectal temperature with the only exclusion of the fixed effect BT. Moreover, the correlation coefficients between the maximum values of skin temperature and rectal temperature were calculated.

RESULTS

Table 1 shows the values of F obtained by the statistical analysis. For all measurements of skin temperature the most important sources of variation were the period, the age at the BT and the respective interaction. This highlights the strong influence exerts by the environmental condition on the skin temperature at the time of test within and between the periods. Significant differences (P<0.05) in skin temperature before and after the Backtest were found at the position of right ear, back and eye. LR and HR pigs showed different skin temperatures (P<0.05) only in the area located at the right armpit. The weight or the sex of subjects did not show any significant effects. Rectal temperature was affected significantly (P<0.05) only by the age at the BT showing the lowest value at 10 days in comparison to the value recorded at 17 days of age (39.05 °C *vs* 39.40 °C). Table 2 shows the effects of the interaction between the period and the age at the BT on maximum skin temperature. There was a correspondence between these values and the variation of environmental temperatures recorded between the two tests. In both first and second period, the tests at 17 days of age were carried out with lower environmental temperature respect to those carried out at 10 days of age. In the table 3 the effects of Backtest, reactivity and sex on maximum skin temperature are reported. The Backtest lead to a significant but little decrease of skin temperature in the area of the right ear and in the eye. A wider variation of skin temperature was observed in the area of the back due to the contact, and the resulting heat loss, with the surface on which the piglets were placed supine. With the only exception of the back position, the values of skin temperature were higher in HR than LR subjects, but these differences reached the statistical significance (P<0.05) only in the position at the right armpit. In general, the correlation coefficients between skin and rectal temperatures were low. The lowest was observed for the eye (r = +0.02) and the highest for the armpits (r = + 0.33).

DISCUSSION

The results of this study confirm the effect of environmental temperature on the skin surface that lead to the differences between the Backtests carried out at different times and ages. The decrease in skin temperature observed in the areas behind the ear and in the eye after the test seems to be related to a vasoconstrictive reaction caused by the stress of immobilization.

A drop of superficial temperature in the eye due to stressful treatments was found in calf [6] and in sheep [5]. With regard to the reactivity, the slightly higher values of skin temperature observed in subjects HR can be attributed to a physical activity related to the escape attempts from the restraint.

CONCLUSION

The results achieved show the possibility to use the body surface temperature recorded by IRT in piglets stress assessment but it requires a careful assessment of the environmental thermal conditions in order to control this main source of variability.

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Table 1: F values of analysis of variance carried out on skin and rectal temperatures (°C).

Position	Period	Age at the Backtest	Backtest	Reactivity	Sex	Period x Age at the Backtest
Ear (right)	15.49***	146.34***	8.40**	0.99	0.45	28.82***
Ear (left)	29.63***	78.09***	1.34	0.10	0.14	31.50***
Back	64.34***	349.94***	65.97***	0.59	0.65	58.55***
Womb	135.09***	20.22***	0.92	1.70	3.56	71.28***
Armpit (right)	16.51***	22.74***	0.78	4.51*	0.37	59.90***
Armpit (left)	14.74***	110.14***	2.32	2.10	0.92	52.95***
Flanc (right)	31.18***	309.7***	1.04	0.49	0.31	19.41***
Eye (right)	112.53***	187.20***	4.77*	1.37	0.27	84.09***
Rectal temp.	2.86	4.61*	--	0.80	1.15	--

***: P<0.001; **: P<0.01; *: P<0.05.

Table 2: Effect of interaction between Period x Age of Backtest on the skin temperatures (°C).

Position	Age at the Backtest (d)	Period 1	Period 2
Ear (right)	10	35.81^{ax}	36.47^{bx}
	17	35.42^{y}	35.44^{y}
Ear (left)	10	35.74^{ax}	36.54^{bx}
	17	35.53^{x}	35.61^{y}
Back	10	34.99^{ax}	36.07^{bx}
	17	34.42^{ay}	34.72^{by}
Womb	10	35.69^{ax}	37.01^{bx}
	17	35.86^{ay}	36.43^{by}
Armpit (right)	10	36.42^{ax}	36.91^{bx}
	17	36.33^{ay}	36.26^{by}
Armpit sx	10	36.41^{ax}	36.84^{bx}
	17	36.31^{y}	36.26^{y}
Flanc (right)	10	35.57^{ax}	36.22^{bx}
	17	34.94^{ay}	35.18^{by}
Eye (right)	10	32.45^{ax}	33.71^{bx}
	17	32.18^{ay}	32.39^{by}

^{a,b} - ^{j,x} Mean values without a common superscript within rows and columns differ (P<0.05).

Table 3: Effects of Backtest, Reactivity and Sex on the skin temperature (°C)

Position	Backest		Reactivity		Sex	
	Before	After	LR	HR	Male	Female
(No. piglets)	108	108	59	49	60	48
Ear (right)	$35.87^{\mathbf{A}}$	$35.71^{\mathbf{B}}$	35.74	35.83	35.82	35.76
Ear (left)	35.89	35.82	35.85	35.86	35.84	35.87
Back	$35.26^{\mathbf{A}}$	$34.84^{\mathbf{B}}$	35.61	35.56	35.61	35.56
Womb	36.23	36.27	36.20	36.30	36.17	36.33
Armpit (right)	36.48	36.43	$36.42^{\mathbf{a}}$	$37.54^{\mathbf{b}}$	36.41	36.50
Armpit (left)	36.47	36.50	36.39	36.42	36.50	36.47
Flanc (right)	36.50	35.46	35.45	35.51	35.46	35.50
Eye (right)	$32.75^{\mathbf{a}}$	$32.62^{\mathbf{b}}$	32.64	32.72	32.70	32.66
Rectal temp.	--	--	39.01	39.34	38.98	38.97

A,B: P<0,01; a, b: P<0.05.

THE EFFECT OF THERMAL STRESS ON SHEEP WELFARE

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SUMMARY

Stress is a serious risk to health and can cause metabolic disorders including cardio – vascular diseases and give rise to the serious systemic infection, and even mental illness. One of the many causes of stress reactions is the increase of temperature causing the thermal stress. The aim of the study was to examine the animal response to thermal stress in 30 °C and 50 °C including physiological and blood parameters. The experimental animals were Polish Merino sheep. The experiment was carried out in climatic chamber. During the experiment daily microclimatic conditions were monitored. It was found that in the comparison with the control group (17 °C), high temperatures resulted in significant differences in both the physiological measurements as well as in the blood analysis.

INTRODUCTION

Stress is a sign of the reduced welfare. Source of information determining the level of stress and its intensity is manifested through physiological mechanisms of animal body, avoiding or adapting to the existing stressful factors by changing the internal balance of the organism. Stress is a serious risk to health and can cause metabolic disorders including cardio – vascular diseases and give rise to the serious systemic infection, and even mental illness. One of the many causes of stress reactions is the increase of temperature causing the thermal stress. Adult sheep, depending on the breed, tolerate a range from –12 to up to 32 °C [12]. The phenomenon of mammalian sensitivity to heat and related stress as well as the functioning of the thermoregulatory system is quite widely reported in the literature [5, 6, 10]. The measurements of temperatures in experimental animals are necessary, particularly in heat stress conditions. Fluctuations in skin temperature and rectal temperature are compatible with growth, lactation and reproductive capacity of animals, what has repeatedly been confirmed in studies on the effects of temperature (thermal and chronic stress) on the animals [11]. These parameters are an excellent indicators of thermal equilibrium of the body, suggesting adverse environmental conditions which directly impinge on the health and welfare of the animals. It was also found that the thermal stress causes symptoms of hyperthermia what can significantly affect the mineral balance of the body [12]. In experiments conducted in Australian Merino sheep, the said researchers found a correlation between high environmental temperatures and parameters of studied blood.

MATERIAL AND METHODS

The experimental animals were Polish Merino sheep, derived from the certificated national stock – breeding farm. The sheep were fed mainly with oats in a dose of 0.2 kg / capita. Water and hay were provided ad libitum. The experiment was carried out in climatic chamber. During the experiment daily microclimatic conditions, especially temperature, humidity and gas compounds (NH_3, H_2S, CO_2), were monitored. The experimental room had an autonomic ventilation system correlated with the thermostat (Simens, Germany). Climatic chamber was equipped with a split type air conditioners (Nanhai, China) and heating fan heaters (Master ERA, USA). Thermo – humidity parameters were controlled by the steady thermohygrometer (DG-E/2, Poland) and mobile electronic thermohygrometer (TED, Taiwan). The measurement of air movement was made by the rotating anemometer (Huger, Switzerland). Environmental parameters were monitored by the Scada Pro Software (MicroB, Poland). The heart rate and respiratory rate of the animals were measured as well as the internal (rectal) and skin temperature. The biochemical and hormonal analysis of blood were also performed. Blood samples were collected from the internal jugular vein (*vena jugularis*) into tubes containing EDTA as well as to the tubes Serum Z (Monovette® Sarstedt, Germany). Blood analysis were performed by the devices ABX VET, Pentra – 400 (HORIBA ABX, Canada) and the SYNERGY (Biotek, Winooski, USA). Examination of hormone levels was determined by enzyme – linked immunosorbent assay (ELISA). Laboratory analysis took place in the biochemical laboratory at the Department of Environmental Hygiene and Animal Welfare, Wroclaw University of Environmental and Life Sciences, certified by RIQAS. Statistical analysis of collected data were made by the Statistica software ver. 5.0. The bands of specific parameters were calculated including the arithmetic means with the standard deviations. To compare mean scores in the experimental and control groups the univariate variance analysis (ANOVA) was performed The significance of differences between experimental groups was evaluated by the Tukey

test. Statistical differences were considered significant at a confidence level $p < 0.05$ and for highly significant at a confidence level $p < 0.01$.

RESULTS

The aim of the study was to examine the response of animals kept in 17 °C (±1 °C) to thermal stress in 30 °C (±1 °C) and 50 °C (±1 °C) including physiological and blood parameters. It was found that at an ambient temperature of 50 °C the average heart rate was 86.63 per minute, compared with the control group maintained at 17 °C (90.34/min.) demonstrated no statistically significant difference ($p < 0.05$), but in comparison with experiment at a temperature of 30 °C determined statistically highly significant differences ($p < 0.001$). The average number of breaths per minute was 126.1, compared with the control group (57.91/min.) was considered highly statistically significant difference ($p < 0.001$). It should be noted that the experiment revealed the visible deepening of breaths in sheep at the expense of reducing their frequency. The average rectal temperature was 39.9 °C and was highly statistically significant difference ($p < 0.001$) when compared with 30 °C (39.46 °C) and the control group (39.05 °C). Statistical difference was also found in the skin temperature measured at the thermostable points. The measurements of skin temperature in thermostable areas showed that the measuring point on the back of sheep characterized by a mean of 36.71 °C, which was a highly significant difference ($p < 0.001$) in comparison with the measurements in the control group (35.79 °C). There was no statistical difference between the group discussed and the experiment at 30 ° C ($p < 0.05$). The average temperature of the skin in the groin was 36.97 °C. It was found that this ratio showed no statistical differences ($p > 0.05$) compared to the control group (36.72 °C), while significant differences were found in the comparison to the previous 30 °C examination (36.5 °C) at $p < 0.01$. There were no statistically significant differences in the thermolabile points (left leg, head). The biochemical analysis showed the blood glucose downward trend and the AST and CK level were without significant differences. In carried out hormonal sheep's blood tests were found that the average level cortisol 16.97 ng/ml, compared with the control group (1.77 ng/ml) were considered highly statistically significant difference ($p < 0.001$), but in comparison with the first study group – heat stress at 30 °C (11.86 ng/ml) the differences were statistically significant ($p < 0.05$). The level of adrenocorticotropic hormone (ACTH) was formed from 0.10 to up to 190 ng/ml, and the average value was 25.45 ng/ml, with reference to previous studies showed no statistically significant differences ($p > 0.05$). The average content of adrenaline was 7.42 ng/ml, no statistically significant difference ($p > 0.05$) was determined. The average level of noradrenaline was 24.71 ng/ml and compared with the control group (9.91 ng/ml) was a statistically significant difference ($p < 0.01$). Similar results were compared with the previous study group (11.49 ng/ml), what was considered as a statistically significant difference ($p < 0.01$).

DISCUSSION

The phenomenon of mammalian sensitivity to heat and related stress are quite widely reported in the literature [6]. A crucial source of stress factors are microclimate, especially thermal factors [3, 6]. An important role is played by the heart rate, which is one of the first reaction, reflects the level of homeostasis, and its growth is a direct response to the stressor factors, including elevated ambient temperature [7]. This creates the possibility of increased emission of absorbed heat by convection, conduction and radiation. In our study we found that average heart rate in Polish Merino was 90.34 beats per minute. Comparing the results with those of other authors can put forward the hypothesis that small variations in heart rate in sheep examinations are the result of breed variation, or due to statistical error [4]. The frequency of respiration may be one of the first signs of the heat stress, which is also confirmed [7]. According to these researchers, this issue may be related to the fact of losing by sheep up to 60% of heat due to lung ventilation. There was noted that the increase in temperature give rise to increase the amount of breaths [2]. It was also found that the high temperature causes the increase in the number of sheep breaths even to 400 per minute, but when the suppression of evaporation by pulmonary comes even to reduce the frequency of double – breaths, the breathing is becoming deeper [2]. Skin temperature is one of the supporting elements for assessing the vulnerability of animal heat stress. The assessment of skin temperature should take into account the heat absorbed by the sheep, whose donation to the environment is especially difficult in animals with a dense fleece [7]. These researchers argue that the ears are also very good indicator of heat transfer through the skin of these animals as well as the legs and the scrotum. The representative measurement of animal health is also a rectal (internal) temperature, which over the years has gained acceptance by many authors [7]. In homoethermic animals, like sheep, rectal temperature in termoneutral conditions fluctuates between 38.3 – 39.9 °C and is directly dependent on the breed what was suggested [4, 9]. In studies of heat stress, there is also a need to control both immunological parameters, as well as the level of hormones, particularly cortisol [3, 9]. A similar view presents also other authors claiming that the conditions associated with the seasonal thermal environment, particular in the periodicity of the seasons, may directly alter the morphological and biochemical parameters of blood, as well as fluctuations in cortisol levels [1, 8]. In our study we found that cortisol level increased in direct proportion to the increasing environmental temperature. There was also noted that the level of cortisol in studies of Nazifi et al [8] maintained an upward trend towards an increase of temperature to 40 °C, what resulted in doubling value of the hormone. In the present study the cortisol level in sheep increased nearly

10 times of temperature 50 °C comparing with the control group. In Indian ewes higher cortisol levels are not strictly correlated to the presence at elevated temperature [1]. The researchers point out on the role of norepinephrine (noradrenaline) in response to stress. It was stated that the fluctuations in the concentration of noradrenaline in blood have an impact not only on the secretion of adrenocorticotropin (ACTH), but also on the experience of stress, by reducing the sensitivity of nerve [10]. Unfortunately, in our study, despite clearly increasing trend of average values from the analysis of these hormones, the assumptions of these authors was not confirmed statistically, what indicates the need of further research to fully elucidate the mechanisms of thermal stress

CONCLUSIONS

- Thermal stress is a strong, empirically validated stressor in sheep, reducing the level of animal welfare.
- The pulse and respiration rates are an important elements in detecting the initial phase of stress reaction.
- The level of stress hormones in the blood, particularly cortisol is an important parameter of internal body response to the thermal stimuli.

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IDENTIFICATION AND VALUATION OF FACTORS AFFECTING THE WELFARE OF DAIRY CATTLE IN URUGUAY

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SUMMARY

Milk production in Uruguay is of great importance since it has constituted one of the most stable activities of our economy in the last few years. It is from the knowledge of the health problems affecting animals due to technological advances, the intensification in production and the daily increase in the demands regarding quantity and quality of the milk produced that it is of interest to evaluate the condition of our dairy farms and of the dairy cattle population as regards Animal Welfare. This evaluation was carried out by measuring various parameters associated with the animal (physical ailments, behaviour, relationship with the environment), the environmental conditions, the facilities, the roads and the handling of the animals.

We visited 15 dairy farms in Uruguay between October 2007 and March 2008. The information was collected using survey-like forms which were specially designed, and the data was presented in a descriptive way.

The information was analysed using electronic spreadsheets with numeric categorical variables. Descriptive statistics based on percentages and frequencies was used. Stata Statistical [10] software was used for the analysis of cortisol levels.

INTRODUCTION

Nowadays, Animal Welfare plays an important role in productive species [3]. It is known that to achieve food safe for consumption, it must be obtained from healthy animals, which requires monitoring of welfare from the very beginning in the fields [6]. There is an increasing number of consumers demanding not only health and safety in dairy products but also that they are obtained from animals that have been processed according to the rules and standards of Animal Welfare [4]. It is clear that the main goal of producers is to increase milk production by focusing on increasing its profitability, leaving aside, in many cases, animal welfare, generating, among other things, a high level of stress.

The main objective is to describe animal welfare in the Milk Production Region in Uruguay and to devise an animal welfare evaluation system by measuring the parameters related to the animals and their environment.

MATERIAL AND METHODS

We selected 15 dairy farms which were visited only once, in the period between October 2007 and March 2008. The data were collected without disturbing the milking routine or altering the staff, observing the complete milking process at each establishment visited. The information was collected always by the same people so as to maintain the same approach. Measurements were made of the potential welfare indicators grouped as follows:

Measures based on facilities

Holding pen in good condition (one who had anti-slip flooring, curved boundaries without saliency and where animals did not slip), regular (one with anti-slip flooring with broken areas, angles, and where the animals slipped) and bad (lack of anti-slip flooring, right angles and inadequate maintenance.) - Drinking troughs in good condition (easy access, adequate size, good hygiene, clean and fresh water), regular condition (easy access, adequate size, inadequate hygiene, but the water is acceptable) and bad condition (poor access, inadequate size, clear debris, poor water quality).

Milking rooms: satisfactory rooms (simple, functional, on one level, clean), regular rooms (simple, lack of cleanliness, not very functional, with no significant slopes) and unsatisfactory rooms (not functional). - Roads covered: good (smooth surface, good drainage and with no obstacles), regular (had irregular surface, drainage acceptable and obstacles) and bad (completely irregular surface, major obstacles and poor drainage.) During the tour we evaluated the herding of animals, observing whether it was done on horseback with dogs, on foot, bike, etc. In this way it was possible to assess whether this was done in a slow or rapid way, if batons or whips were used or if shouting took place.

Measures based on cattle handling inside the milking room

We proceeded to the observation of activities related to the actual milking, if this was normal / complete, incomplete or if there was overmilking.

Measures based on animals

We recorded the behaviour of animals, noting if they were excited (refusal to go into the room, vocalization, defecation, urination, kicking) or quiet (making rumination, normal respiratory rate) as well as if they were comfortable or uncomfortable showing changes in support, kicking, etc. It was also observed if the animals slipped.

We assessed the condition of the nipples through observation and palpation, following the benchmarks established by the Teat Club International [7].

At the exit of the milking room, the way a representative number of animals walked was assessed, which were classified on a scale from 0 to 2 in which 0 is indicated for animals not lame (time and weight of support are the same in all four limbs), 1 for lame animals (walking is irregular and time and weight of support is not the same in all four limbs) and 2 for those who had severe lame (or reduced support of the affected limbs) [9] [11].

At the same time, the physical condition was evaluated. Based on scale of 1 to 5 [2], it was considered acceptable for condition from 3 to 4 and not acceptable for less than 3.

We conducted a study to compare the levels of cortisol in the blood in a problem group (animals with hoof diseases) and in a control group (healthy animals).

The extraction of blood in both groups of animals was carried out under the same handling conditions and location. After treating each animal, we extracted a sample of blood from the coccygeal vein and collected in *Vacutainer* tubes. Cortisol concentrations were determined by radioimmunoassay (RIA) in solid phase using DPC kits (Diagnostic Product Co., Los Angeles, CA, USA).

RESULTS

The data presented correspond to the description of facilities and animal populations as well as to handle them.

Within a sample of 1007 animals, 74.58% showed an acceptable physical condition, 6% showed a lame and 0.6% a severe lame.

Out of a total of 2527 nipples valued, the prevailing characteristics were normal skin (63.99%), smooth sphincter (76.53%) and thickened nipple base (15.39%).

When evaluating the milking rooms in all the farms visited, 46.67% were in good conditions, 46.67% in regular condition and 6.67% in bad condition; 93.33% had right angles, steps or slopes, although it was observed that in 73.33% of the cases the animal flow was good.

With respect to the holding pens 60% were bad.

The roads covered by the cows towards the milking room were good only in 33.33% of the cases and the average distance covered per day was of 4.5 km (min. 2 km; max. 10 km).

80% of the farms provided an average supplementary ration of 2.18 kg during milking (min. 1kg; max. 4 kg). 1, máx. 4 Kg.).

Collective milking time was 163 minutes on average (min. 75, max. 300 minutes) and the average time each cow remained in the milking room was 8.2 minutes (min. 4.8, max. 11.8 minutes).

In 93.33% of the 15 establishments visited, the animals were quiet during milking with only 6.67% of animals excited.

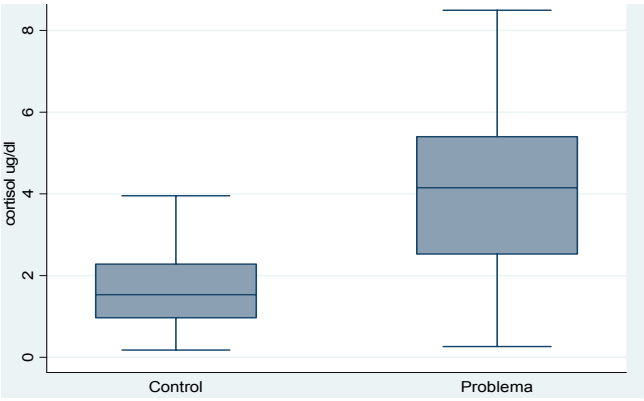

Figure 1: Distribution of the cortisol levels in the control group and problem group. The average for the control group was 1.66 ± 0.13 and for the problem group was 3.95 ± 0.35 ($p < 0.001$). The problem group scored higher levels of cortisol.

DISCUSSION

The result of the facilities in general reflect inadequate construction because the percentages obtained in the category "good" does not stand out from what is considered "regular" and "bad". Both holding pens and milking rooms are important facilities in relation to animal welfare and the animal must enter twice a day throughout the lactation period [5]. This, related to the results obtained, shows that these are inadequate.

The results of the facilities should be related to time spent by the animals inside them. The average collective milking time is not consistent with the literature [5], which recommends a maximum of 2 hours (120 minutes). Therefore, in our terms of animal production, a cow may remain up to 5 hours in unsatisfactory pens, altering welfare. The flow of animals within the room was agile in a 73.33% in spite of obstacles found in most of them.

Because the animals must travel the roads of access to the milking room at least twice a day, the condition they are in is of vital importance. If they are in poor condition and in turn the animal travels a great distance, the effort itself is even greater. The average distance was 4.5 kilometres (min. 2, max. 10 km.), so an animal can get to walk up to 10 kilometres a day on inadequate roads. Although there was a large proportion of regular and bad roads, the percentage of animals with lameness was low (6.6%).

In a majority of the facilities assessed, an acceptable physical condition was found.

The study of cortisol in this work was a good indicator for assessing stress and welfare of the animal in accordance with Radostits et al. (2002) and Zaldivar (2007). Comparing the obtained means of cortisol levels between the problem group and the control group, we confirmed a remarkable rise in these levels in the problem group. The obtained average cortisol levels for the control group differ from the values set as normal as Radostits et al. (2002). This difference can be attributed to several factors such as: entry into the trap, moves for blood extraction, direct sunlight and lack of water, as observed on the day of sampling.

CONCLUSIONS

On the sample at the time of visits, we conclude that the health of animals observed was good and that their handling was also acceptable. However, with respect to the environment, there were some shortcomings, specifically in areas outside the milking room, such as roads, troughs, shade areas, among others. With respect to the milking rooms, it is concluded that most were considered acceptable.

It is now possible to create an animal welfare valuation system based on the various parameters proposed and this study also established the foundation for future research on the subject.

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WELFARE ASSESSMENT OF PREPARTUM AND POSTPARTUM DAIRY COWS

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SUMMARY

The aim of this study was to validate a welfare assessment system for dairy cows, based on direct on-farm evaluation, haematological and hormonal parameters. Ten prepartum dairy cows and ten postpartum dairy cows were assessed on the same day using an on-farm welfare scoring system comprising twelve indicators. Blood samples were collected from all cows during the following morning and the complete blood count, cortisol and thyroxine were determined for each cow. Postpartum cows had higher total welfare scores, but lower erythrocyte counts, leukocyte counts, cortisol and thyroxine than prepartum cows, but the differences were not statistically significant. However, significant positive correlations were found in postpartum cows, but not in prepartum cows, between cortisol and total welfare scores, cortisol and neutrophil counts, thyroxine and total leukocyte counts and thyroxine and neutrophil counts. Results indicate that the proposed welfare assessment system is a useful tool for postpartum cows, allowing an accurate evaluation of the marked stress response that follows parturition. In prepartum cows, which exhibit a lengthier and more gradual stress response, the proposed welfare assessment tool needs further refinement.

INTRODUCTION

Welfare assessment of dairy cows is particularly important during late gestation and early postpartum period, when cows are exposed to several significant changes in their routine which may act as stressors (e.g. changes in social group, feeding, environment and handling routines). The aim of this study was to validate a welfare assessment system in prepartum and postpartum dairy cows, based on direct on-farm evaluation, haematological and hormonal parameters.

MATERIALS AND METHODS

Twenty Red Holstein cross dairy cows, tied in an indoor pen, were included in this study and divided in two groups: ten prepartum cows (ninth gestation month) and ten postpartum cows (5 to 22 days following parturition). The study was performed during the month of December.

On-farm welfare assessment

A welfare scoring system was devised based on twelve indicators that have been demonstrated to reflect welfare in dairy cows: rising behaviour; collisions; lameness; cleanliness; injuries; nasal, ocular and vulvar discharge; hampered respiration; avoidance distance; abnormal behaviour and body condition score /2, 3, 5, 7, 8, 11/. All cows were assessed on the same day using the welfare scoring system and each indicator was attributed a numerical score. The scoring system is described in detail below.

In tied dairy cows, lying and rising up are high priority behaviours and any changes in these behaviour patterns may reflect an increased risk for lameness. During the welfare assessment, only interrupted or abnormal *rising behavioural sequences* were scored and *collision* with stall partition during rising up was recorded as present or absent. *The avoidance distance test* was performed immediately after rising up, at a distance of 2 m in front of the cow. The animal was approached at a speed of one step per second with the arm held over head at an angle of approximately 45° from the body. The cow's behavioural response was categorised on a scale from 1 to 5: 1 - the cow sits still and allows touching; 2 - the cow sits still but does not allow touching; 3 - the cow sits still but moves backwards when hand is stretched; 4 - the cow moves backwards before the approaching assessor has stopped; 5 - the cow avoids assessor completely /7, 11/.

Lameness constitutes a major welfare issue in cattle, causing pain and alteration to normal behavior. Because the cows were tied, it was not possible to perform any locomotion scoring. Therefore we used a *stall lameness score* as decribed by *Sprecher et al* /8/ which is based upon the following indicators: *resting* (resting one leg more than the others), *standing* (standing on the edge of a step – to avoid bearing weight on one leg/part of leg), *stepping* (frequent weight shifting between legs, or repeated movements of the same leg) and *reluctance* (reluctance to bear weight on one leg when moving). Firstly the cow was observed in standing position undisturbed and then it was moved to the left and right, observing how it shifts weight from leg to leg. Finally the cows were scored as *not lame* (cow showing none of the indicators listed above) and *lame* (cow showing at least one of the four indicators listed above).

Cleanliness of cattle reflects the environment in which they are kept and has implications for health and welfare. The lower hind legs (including the hock) and the hind quarters (upper hind leg, flank and rear view including tail and the udder) were inspected from each side and from behind. Cleanliness was categorized on a scale from 1 to 3 as follows: 1 – the cow is clean or had minor splashing; 2 – more than three dirty areas, bigger than 10cm in diameter, are noticed; 3 – areas of old manure are present on more than one third of the applicable body parts /5/.

Injuries (integument alterations) included hairless patches and lesions, classed on a scale from 1 to 3: 1 – no injuries or small hairless patches; 2 – one half-hand sized injury or several injuries which together are larger than one-two hands; 3 – one injury larger than one hand or several injuries larger than two hands. *Nasal discharge*, *ocular discharge*, *vulvar discharge*, *hampered respiration* and *abnormal behavior* (stereotypies) were marked as absent or present at the time visit and scored with 0 or 2 /3/.

Body condition has important implications for health and welfare and reflects the body fat content and thus the nutritional status of an animal, as indicated by its body reserves. Cows were assessed with a three category system of *too thin*, *acceptable* and *too fat* with regard to four body regions: 1 - cavity around tail head, 2 - loin, 3 - vertebrae, 4 - tail head, hipbones, spine and ribs /2/.

Laboratory determinations of haematological and hormonal parameters

The complete blood count was determined for all cows on the same day and this served both as a health screening method and as a stress assessment method, by means of stress leukograms /6/. Serum cortisol is a validated measurement of stress in cattle and it increases with acute stress, although it lacks specificity for certain categories of stressors /1, 6/. Serum thyroxine increases under metabolic stress, as caused by low temperatures /9, 10/. As our study took place in December, we included both cortisol and thyroxine as hormonal indicators of stress.

Blood samples were collected from the jugular vein from all cows during the following morning in EDTA tubes and serum tubes. Red blood cell concentration, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, leucocyte concentration and their fractions (neutrophils, lymphocytes, monocytes, eosynophils, and basophils) and platelet concentration were determined using a Cell-Dyn 3700 system. One EDTA sample from one postpartum cow has clotted and the blood count was not performed. Two blood smears were examined for each cow. The serum cortisol and thyroxine were measured for each cow using solid phase, chemiluminesence enzyme immunoassays, with an Immulite 1000 system.

Statistical analysis was performed using Minitab 16 for Windows.

RESULTS

Final scores for the welfare assessments ranged from 8 to 10 in prepartum cows and from 8 to 13 in postpartum cows. Postpartum cows had higher total welfare scores (mean=9.6), compared to prepartum cows (mean=8.7), reflecting a more pronounced stress response (table 1). The rising behaviour appeared normal for all cows and only one collision with the housing equipment was noted. Three postpartum cows and two postpartum cows displayed mild lameness. There was no evidence of nasal, ocular or vulvar discharge in any of the cows and only one small injury was noted. Only one cow, which had very high cortisol and total thyroxine, displayed one type of abnormal behaviour, namely tongue rolling.

Prepartum cows had slightly higher mean erythrocyte counts, total leukocyte counts, neutrophil counts and lymphocytes counts than postpartum cows. Only one postpartum cow had mild anemia, with a mild decrease in erythrocyte count and haemoglobin. The same cow had quite marked lymohopenia. Three other postpartum cows and four other prepartum cows had mild to moderate lymphopenia. Two of the prepartum cows that were lymphopenic had concurrent mild neutrophilia. Prepartum cows also had slightly higher mean cortisol and thyroxine values (table 1).

There was no significant difference between the total welfare scores, leukocyte counts, cortisol and thyroxine levels in prepartum and postpartum cows (Mann-Whitney test, all p-values >0.07). Significant positive correlations were found in postpartum cows between cortisol levels and total welfare scores ($p=0.005$, $corr=0.802$), cortisol levels and neutrophil counts ($p=0.026$, $corr=0.739$), total thyroxine and total leukocyte counts ($p=0.029$, $corr=0.720$) and total thyroxine and neutrophil counts ($p=0.05$, $corr=0.664$). These correlations were not found in prepartum cows.

DISCUSSION

Previous research has demonstrated that leukocyte counts, cortisol and thyroxine represent useful welfare indicators in dairy cows /4, 9, 10/. Our study investigated whether these laboratory indicators correlate well with the results of a complex welfare scoring system for dairy cows, based on twelve welfare indicators that have been explored separately in previous studies.

High total welfare scores in postpartum cows were associated with high levels of cortisol, which is a validated measurement of stress /1, 6/. However, statistical analysis failed to reveal a similar correlation in prepartum cows. This indicates that the proposed welfare assessment system may represent a useful tool for postpartum cows, allowing an accurate evaluation of the marked stress

response that follows parturition. For prepartum cows, which exhibit a lengthier and more gradual stress response, this on-farm welfare assessment tool may need further refinement in order to demonstrate good correlation with cortisol levels.

Stress leukograms, characterized by neutrophilia and lymphopenia /6/, occurred infrequently in the study groups, namely in two of the prepartum cows only. Higher levels of cortisol and thyroxine were generally associated with inflammatory leukograms, indicating that subclinical inflammation may be an important factor which influences the welfare of dairy cows in late gestation and early parturition periods /1/. Thyroxine levels were significantly correlated with leukocyte counts in postpartum cows, possibly suggesting a relationship between intense metabolic stress and subclinical inflammation following parturition. This relationship was not demonstrated in prepartum cows.

CONCLUSIONS

The proposed welfare assessment system is a useful tool for postpartum cows, allowing an accurate evaluation of the marked stress response that follows parturition. In prepartum cows, which exhibit a lengthier and more gradual stress response, this welfare assessment tool needs further refinement.

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Table 1: Mean values of welfare scores, hematological and hormonal parameters in prepartum and postpartum dairy cows

THE WELFARE ASSESSMENT OF TIED AND FREE STALL DAIRY COWS – PRELIMINARY NOTE

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SUMMARY

In order to evaluate the dairy cows welfare, research was conducted on two farms with different systems of accommodation, in tie stall and free. Observed parameters were body condition score (BCS), body hygiene (cleanliness), leg injuries and qualitative behaviour assessment.

Assessing the welfare of dairy cows accommodates in tie stall and free we have found that free housing system provides significantly greater opportunities for expression of behavioral and socio-physiological needs.

INTRODUCTION

The environment in which dairy cows are housed has a big impact on their health and welfare. Comfortable and clean accommodation with sufficiently sized bed, although initially requires a substantial investment, is an important factor in health and a long exploitation of dairy cows on the farm [8].

Welfare is a multidimensional concept that includes physical and mental health, the absence of hunger and thirst, and provides a manifestation of the typical behaviour for that species [8].

Many authors have developed methods for estimating the welfare of cattle on farms [1,2,5,6].

Most methods for welfare assessment include: animal-related parameters, such as behaviour, body condition score (BCS), body cleanliness, lameness, skin lesions, injuries and swellings.

Therefore, in order to evaluate the welfare, research was conducted on two farms with different systems of accommodation, in tie stall and free.

Observed parameters were body condition score (BCS), body hygiene (cleanliness), leg injuries and qualitative behaviour assessment.

MATERIAL AND METHODS

It was assessed 40 cows housed in tie-stalls and 80 cows housed free. Tied cows were between the ages of 5-6 years, with an average yield of milk 4000-5000 liters of milk, a cow in a free system were aged 3-4 years with an average lactation 6000 -7000 liter of milk.

Observed parameters were determined through specific methods, described in Assessment protocol for cattle (Welfare Quality, 2009.). Body condition score was scored with regard to 4 criteria and levelled from 0 – 2. 0 represent regular body condition, 2 – very fat. Body hygiene was assessed by cleanliness of udder, flank and lower legs. Scale was from 0 (no dirt) to 2 (very dirt). Leg injuries were scaled from no changes (0) to very pronounced changes (2). Behaviour was assessed by cows observation.

RESULTS

Table 1. Assessment of observed body parts cleanliness

cleanliness indicators	tied cows n=40	loose housed animals n=80
udder	0-28 2-12	0-79 2-1
flank	0-32 2-8	0-76 2-4
lower legs	0-20 2-20	0-77 2-3

Udder:
0- no dirt or minor splashing, other than on teats
2-distinct plaques of dirt on udder or any dirt on and around the teats

Flank:
0-no dirt or minor splashing
2-separate or continuous plaques of dirt
Lower legs:
0- no dirt or minor splashing
2-separate or continuous plaques of dirt above the coronary band

Table 2. Assessment of leg injuries (lameness)

	injuries indicators
tied cows n=40	0-8 2-32
loose housed animals n=80	0-32 2-48

Tied cows:
0-not lame: cow showing none of the indicators listed above
2-lame: cow showing at least one of the indicators (resting a foot, standing on edge, stepping)

Loose housed animals:
0- not lame-timing of steps and weight-bearing equal on all four feet
2-severely lame-strong reluctance to bear weight on one limb, or more than one limb affected, joint injuries, wounds

DISCUSSION

Objects and systems for dairy cattle accommodation should be designed and constructed to enable achievement of five freedoms: freedom from hunger and thirst, freedom from uncomfortable, from pain, injury and disease freedom of expression of normal behavior and freedom from fear and harassment.
Whether the animals are bound or loose housed on deep litter, in order to maximize production and achieve the standards of welfare, accommodation has to meet their basic needs. Housing system unless they have to meet their needs as far as health and welfare should satisfy even the basic legal regulations. The study and observation of behavior and the animals themselves have become an important tool in identifying situations where their welfare is compromised [3, 9].
Our studie have shown that expression of the basic physiological behaviors such as movement, territoriality, social contact, relaxation, research, lie down, ingestion, body hygiene, parental behavior, is partially or totally disabled in cows kept tied permanently or occasionally. This fact significantly affect their well-being [6,7]. Most of the behavior indicators point to a lack of tie stall housing versus free range.
The results obtained through the assessment of the 120 cows showed that cows housed in tie stall are much thinner that cows housed freely. Tied cows have had deep cavity around tail head, as well as hip bones (tuber coxae) and spine and ribs very prominent.
The cows kept in tie stall, 50% of cows had score 2 for the cleanliness of the rear leg, 30% for the udder and 20% for the flank. Free kept cows were mostly clean (97%). These results are completely contrary to the claims of [4]. Lower leg zone contamination will indicate the amount of manure that the cows have to walk through in alleyways and exercise areas. The upper leg and flank zone will reflect contamination from lying in manure on the rear of stalls and in wet unhygienic dirt lots.
Legs injuries were in the tied cows very prominent (70% of cows had laminitis). Free-reared cows had joint injuries and wounds (60%). Regula et al. (2004) determined that herds with a high prevalence of lameness were also more likely to have a high prevalence of injuries of the skin around the hock joints.

CONCLUSIONS

Assessing the welfare of dairy cows accommodates in tie stall and free we have found that free housing system provides significantly greater opportunities for expression of behavioral and socio-physiological needs.

Further researches will determine the long-term impact of stable design on the welfare and health of dairy cows.

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WELFARE EXAMINATION OF HORSES EXPLOITED IN ,,BOUND SYSTEM"

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ABSTRACT

The introduction of criteria estimation the animals' welfare was at the basis of making this study. A team of specialists from Institute of Diagnosis and Animal Health together with specialists from the Ministry of Interior intended to realize this theme, emphasizing the study on the horse, for centuries, a companion of man in different activities.

10 (ten) pedigree horses - Thoroughbred, Gidran, Romanian Sport Horse, Metis, were examined. They are used for the Police Force Department and a clinical mental conditions.

examination was made. In order to make hematological and biochemical tests were drawn.

Simultaneously, determinations of microclimatic parameter were made - carbon-dioxide concentration, ammonia, hydrogen sulphide, the intensity of noise and measurement of aero-micro flora.

The first results are promising being possible that starting from some standardized tests to be able to estimate the ,,*animals' wealth"* and their reactions to different environ

RELATIONSHIP BETWEEN BODY TEMPERATURE AND COPING STYLE IN POST-WEANED PIGLETS

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SUMMARY

The aim of this study was to investigate in post-weaned piglets the relationship between the reactivity to the Backtest (BT) and the body temperature during a restrain stress occurred at weighing. On the basis of the BT carried out at 10 and 17 days of age, 96 "high-resisters" (HR) and 96 "low-resisters" (LR) piglets were identified. At the post-weaning 4 groups of 48 subjects balanced for BT were examined. Each group was allocated in 12 post-weaned boxes, 4 with only HR piglets, 4 with only LR piglets and 4 mixed (2 LR and 2 HR piglets). During the weighing occurred at 36, 44 and 52 d of age, dorsal and ventral Infrared Thermography (IRT) images for each piglet were taken. At the same time, rectal temperature was recorded and room Thermal-Humidity Index (THI) was calculated. The type of box (only HR, only LR and mixed) affected significatively the rectal temperature which was higher for HR boxes ($P<0.05$). There was not a significant effect of BT reactivity on skin and rectal temperatures. Skin temperatures on dorsal and ventral positions were significantly influenced by THI and live weight, respectively.

INTRODUCTION

In pigs, individual differences in stress coping style could have relevant consequences on performances, health and welfare. In the last years, tests simulated an acute stress like immobilization were developed in order to identity individual reaction patterns related to different coping strategies. The Backtest (BT) is commonly used to measure behavioural response of piglets to an imposed stressful situation [3]. In the BT a piglet is immobilized in supine position for one minute and categorized as "high-resisters" (HR) or "low-resisters" (LR) on the basis of the number of escape attempts. Differences between HR and LR pigs have been detected in behavioural and physiological responses to several stressors [2]. It is well know that stress can induce vascular changes in different parts of the body which can modify skin and rectal temperatures [1]. Variations in body surface temperature related to the stress could be measured by Infrared Thermography (IRT), without contact with the subject and with a precision of less than 0.1°C. The aim of this study was to investigate in post-weaned piglets the relationship between the body temperature and the reactivity to the BT during a restrain stress occurred at weighing.

MATERIAL AND METHODS

On the basis of the BT carried out at 10 and 17 d of age, 96 HR and 96 LR piglets balanced for sex were identified. They were weaned at 22 ± 2 d of age and 15 days later were transported to the experimental piggery of the Department. From May to September 2009 they were examined in 4 groups of 48 subjects balanced for BT reaction (24 HR and 24 LR). Each group was allocated in 12 post-weaning boxes containing 4 piglets each and arranged as follow: 4 boxes with only HR subjects, 4 with only LR subjects and 4 with two LR and two HR piglets (mixed). During the individual weighing occurred at 37, 44 and 52 d of age, dorsal and ventral IRT images were recorded by a thermo-camera Flir P640 (Flir System, Milan) placed from at distance of m 2.0 and settled with an emissivity of 0.98. Images were processed using the software Therma Cam Pro 2.9 Researcher (Flir System, Milan) (Figure 1). The minimum, maximum and mean values of temperatures were recorded for each dorsal and ventral IRT image. Rectal temperature was recorded also. Room temperature and relative humidity were measured using a data logger (Pro v2 logger, HOBO, USA) and then Thermal-Humidity Index (THI) was calculated [5]. Environmental conditions recorded at each weighing for each group are reported in Table 1. Data were analyzed by a mixed model for repeated measures including group (1-4), weighing within group (1-12), BT reactivity (HR, LR), type of box (only HR, only LR, mixed), sex (M, F) as fixed effects, piglet within group as a random effect, and THI and live weight within weighing as covariates.

RESULTS

The results of the analysis of variance (F values) are reported in Table 2. The group showed a significant effect on all measures of body temperatures as consequences of the different environmental conditions at the moment of weighing (Table 1). The effect of weighing within group was significant only on the ventral skin measurements and

on rectal temperature. The BT reactivity and the type of box did not show any effects on dorsal and ventral skin temperatures. The type of box affected significatively the rectal temperature ($P<0.05$) which was higher for HR boxes (Table 3). The sex did not show any significant effect excepted for the minimum value of IRT measurements taken on the belly ($P<0.01$). Skin temperatures on dorsal and ventral positions were significantly influenced by THI and live weight, respectively. The Table 3 shows the effects of BT reaction and type of box on skin and rectal temperatures. The values of dorsal and ventral skin temperatures were very similar between HR and LR piglets as well as between the different types of box. A significant difference ($P<0.05$) was observed between the types of box for rectal temperature which was higher in average 0.1 °C in the piglets from HR boxes than those belonging to the mixed and LR boxes. A similar difference in rectal temperature was observed between HR and LR piglets even if it did not reach the statistical significance ($P<0.07$).

DISCUSSION

The skin temperatures assessed by IRT were strongly influenced by slight variations in environmental condition at the weighing. That confirms the role of environmental temperature as a main source of variability of skin temperature. The increase of rectal temperature in HR boxes may indicate a higher reaction to a stressful treatment, such as weighing, according with the literature [4]. If individually considered, HR and LR subjects showed littlest differences in rectal temperature. Very little differences were found between minimum, maximum and mean values of skin temperatures detected by IRT. This result suggests that the restrain applied at weighing could be a treatment not so stressful to lead a relevant variation in skin temperature of HR and LR piglets. It is interesting to note that minimum temperatures showed higher values of standard error which is probably attributable to the variability of the thermal image reading, caused by the presence of dirty (i.e. low temperature area) on the skin at the time of shooting.

CONCLUSIONS

Relationship between reactivity to Backtest and rectal temperature seems to be detected at the level of piglets group. The absence of individual differences in skin temperature between HR and LR piglets suggests that the restrain applied at weighing could be a treatment not so stressful to lead a detectable variation of this parameter.

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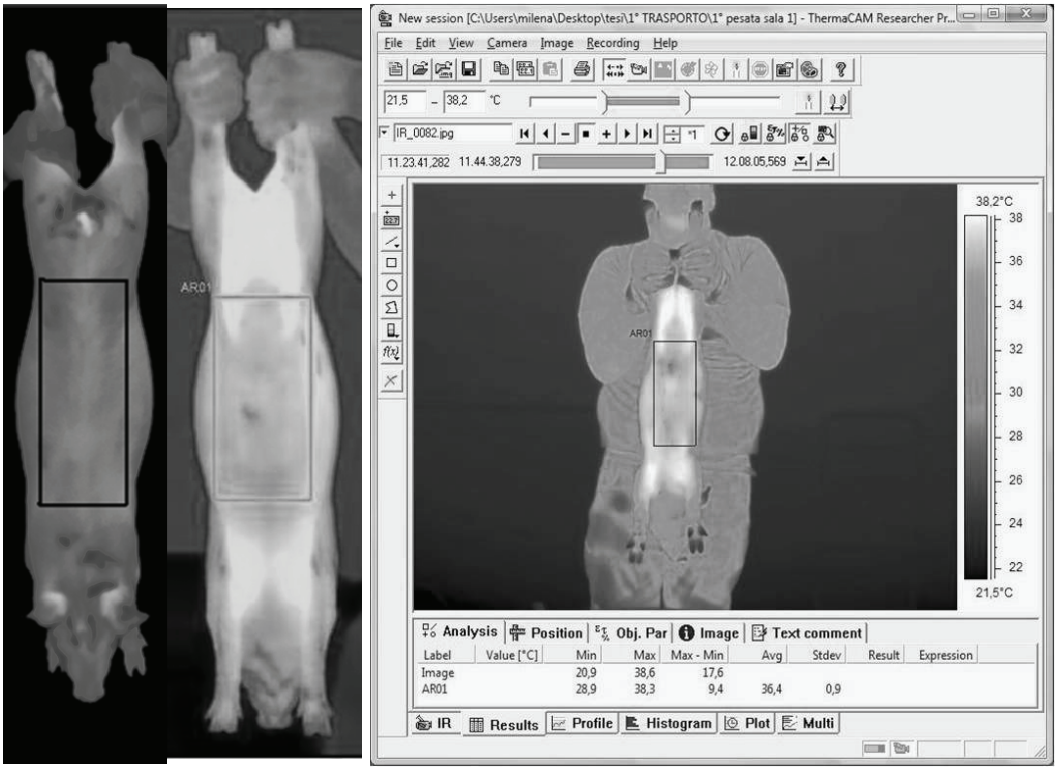

Figure 1. IRT images of dorsal and ventral area used to measure skin temperatures.

Table 1. Environmental condition at weighing for each group.

Weighing		Group			
		1 (May)	2 (June)	3 (July)	4 (September)
37 d	T (°C)	23.9	23.0	26.2	23.6
	U.R. (%)	51.7	46.0	57.0	50.0
	THI	70.5	68.8	74.2	69.9
44 d	T (°C)	26.1	26.7	28.3	21.4
	U.R. (%)	62.0	63.0	61.5	75.5
	THI	74.7	75.6	77.7	68.9
52 d	T (°C)	27.0	24.7	27.2	22.2
	U.R. (%)	47.0	66.0	59.5	76.1
	THI	74.0	73.1	75.9	70.1

Table 2. F values of the analysis of variance.

Factors	Dorsal skin Temperature			Ventral skin temperature			Rectal temperature
	Max	Mean	Min	Max	Mean	Min	
Group	57.49***	19.17***	12.44***	40.44***	23.28***	2.66*	12.44***
Weighing within group	1.71	1.90	1.85	1.86	2.68**	4.14***	7.78***
BT reactivity	0.35	0.10	0.40	0.76	0.09	0.07	2.97
Type of box	0.93	0.96	0.95	0.08	0.62	0.16	2.75*
Sex	1.97	2.91	0.79	0.01	1.50	6.74**	1.17
THI	13.37***	7.11**	0.50	0.51	0.33	0.09	0.39
Live weight within weighing	1.47	0.54	1.11	5.70***	10.85***	4.82**	0.43

***: P<0.001; **: P<0.01; *: P<0.05.

Table 3. Effects of BT reactivity and type of box on dorsal and ventral skin and rectal temperatures (°C) (Least squares means).

Factors	Dorsal skin Temperature			Ventral skin temperature			Rectal temperature
	Max	Mean	Min	Max	Mean	Min	
BT reactivity							
HR	37.18	35.84	32.51	38.73	36.43	32.94	39.42
LR	37.16	35.80	32.51	38.64	36.46	33.03	39.51
SEM	0.05	0.08	0.18	0.07	0.08	0.17	0.03
Type of Box							
HR	37.22	35.90	32.66	38.68	36.50	33.18	39.53[a]
LR	37.17	35.80	32.43	38.68	36.37	32.95	39.42[b]
Mixed	37.12	35.76	32.43	38.70	36.46	32.83	39.43[b]
SEM	0.06	0.08	0.19	0.06	0.08	0.18	0.04

a, b: P<0.05. *SEM*: Standard Error of Mean.

RISK ASSESSMENT OF WELFARE DEPRECIATION IN HORSES DURING TRANSPORT

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SUMMARY

The study aimed to identify the specific factors, which determined welfare depreciation in horses during short-term transport (1.5-6h), by measuring the physiological and behavioural responses induced by transport stress.

We measured the heart rate, plasma cortisol levels and behavioural displays in sport horses (A, n: 11) used to being transported as well as in horses transported to be slaughtered B, (n: 34) which travelled for the first time.

The results have shown an increase in heart rate and plasma cortisol levels in horses transported to the slaughterhouse during loading and unloading ($P<0.001$) compared to sport horses (A), where the increase was insignificant ($P>0.05$), as transport was a chronic stress factor with the latter. An important factor was the caretaker's experience in horse loading and unloading, factor which determined significant increases in heart rate as well as behavioural modifications in both horse groups, with some very sharp increases during loading time (90-360s).

Horses exposure to transport, loading method and caretaker's experience respectively were the most important factors to play a role in the depreciation of animals' welfare.

INTRODUCTION

Compared to other species, horses are the most often transported animals and destinations vary greatly: slaughter house, training centres, competitions, leisure and touristic areas, etc. There are sufficient scientific arguments up until the present to testify that long transport periods have a higher impact on animals' welfare than short term transport conducted under the same conditions, due to the obvious influence of extended time as well as of the higher number of stress factors. Recent research has shown that although horses adapt to transport conditions much better than other animals, transport is generally associated with modifications of some physiological indicators of stress, temporary decrease of athletic performance, increased disease incidence and even lower reproductive rates. The heart rate has been shown to significantly increase during loading/unloading time and the caretaker's experience plays a very important role to minimize the behavioural reluctance of horses during transport [5].

However, there are few studies that have analysed the effect of loding method, caretaker's experience and horses' experience on transport related stress, in comparison with the research conducted on stress levels during travel time [3]. Road transport both on long and short distances supresses the horses' feeding behaviour, increases their heart rate and leads to fatigue and weight loss due to the uncomfortable position on the means of transportation. Loading density increases the rate of injuries and incidence of aggressive displays [1,7].

MATERIAL AND METHODS

Our research has been conducted over the period of April through August 2010, and monitored eight transports with a travel time of 1.5 to 6h over distances ranging from 70 to 285 km. There were 45 horses investigated, females and males, grouped in 2 lots depending on their experince with transport: the A lot A (n:11) sport horses that had already been transported for various purposes for at least 3 times and the B lot (n:34) horses that were destined to slaughtering and had never been transported before. The transport was performed in specialised vehicles with two places (A lot) and four or six places in trucks (B lot). All transport manoeuvres were carried out in accordance with Regulation CE 1/2005, and best practices guides.

The heart rate was measured by means of a non-invasive method, using a heart monitor (Polar Electro Oy, Finland). The electrodes, transmitter and recording device were placed under a girth around the animal's body. The recordings were done 2 hours prior to transport, during transport, and 2 hours following transport. Blood samples were drawn by puncturing the jugular vein, 30 minutes prior to loading and 30 minutes following unloading in 1,3 Lithium-Heparin vacutainers (LH/1,3m; Vacutainer System). The samples were kept in ice before they were processed (centrifuged at 2000 rpm for 15 minutes) according to working protocols and analysed then in the laboratory by Elisa. The handling was conducted in the presence of the caretaker, which greatly reduced the animals' loading/unloading stress. The behavioural

manifestations of the horses (calm, slightly nervous, nervous, very nervous) were monitored by direct observation while loading (20-360s), during each transport.

Data obtained through recordings of heart rate were downloaded on a computer and processed by means of the software installed (Polar Equine SW). The statistical analysis of data related to plasma cortisol and behavioural manifestations included the t test to compare the two horse groups participants in the research.

RESULTS

The recorded heart rate (Figure 1) has shown an obvious increase in horses loaded for the first time (B lot), and less significat increase with the horses that had been transported before (A lot) where transport constituted a chronic stress factor. The heart rate dropped between the loading and unloading moments, which is indicative of the fact that animals adapted fairly quickly to the new conditions offered by the transport vehicle.

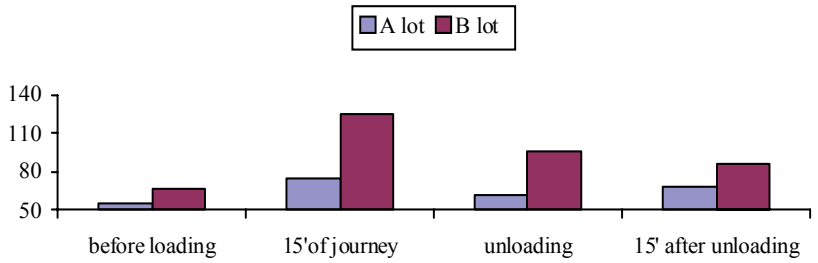

Figure 1. Heart rate variation (bpm) during horse transportation

Loading B lot horses onto the vehicles has led to a significant increase in their heart rate as a result of the methods and horse movements during this process, and the animals showed fear, nervousness and agitation. The heart rate increase in A lot was associated with competition which was the reason for their display of nervousness.

Behavioural displays of agitation in A lot horses used to loading (Figure 2) were reduced (20%) which led to a relatively short leading time of 25-35s. The horses in the B lot manifested fear, agitation and the recorded loading time was up to 360s. The behavioural responses of the latter appeared as a result of loading related physiological stress, lack of exposure to transport means and methods used. Many of the horses in the B lot have manifested agitation from the moment they noticed the vehicle (73%), and the agitation levels increased and stayed high until loading onto the vehicle (in 31% of the horses). The agitation displayed by the B lot horses was due however to loading onto the transportation means and less to the journey itself.

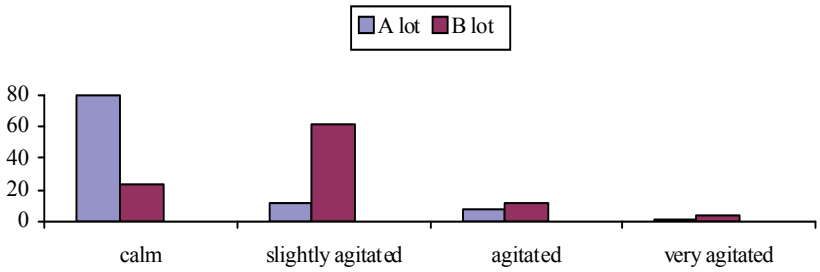

Figure 2. Percentage of behavioural display (%) in horses during loading

Plasma cortisol levels measured during transport recorded an increase in concentration for B lots ($P<0.001$) than A lot (Figure 3). The differences in plasma cortisol level with the A lot were due to the caretaker, loading method, and low values were recorded with those horses for which loading did not constitute a stress factor.

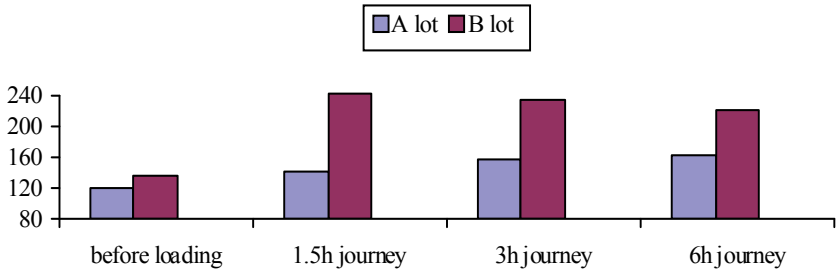

Figure 3. Cortisol level (nmol/l) of horse before and after transport at different hours of the journey

The high level of plasma cortisol did not vary with travel time, so the horses' effort to adapt to transport conditions must have been the same as a result of the relatively short travel time (1.5-6h).

DISCUSSION

Comparing the results obtained in this study with published data do not indicate largest differences in heart rate and cortisol levels.

Hyperpnea and a decrease of vegetative nervous system control on the heart rate during transport may result in a prevalence of increased heart rate and its variation. The human factor has a major effect on horses' heart rate during transport, especially at the time of their loading onto the vehicle. There is research which has shown that a person entering or exiting the horses' stable causes an increased heart rate. By contrast, stroking the horses on their favourite brushing spots greatly decreases their heart rate [2, 7]. Measuring the heart rate immediately after unloading may be an indicator of their physical fitness, performance and general health condition. Stress, fear and anxiety visibly modify animals' behaviour upon loading, regardless of their previous exposure and experience [3, 5].

Horses behaviour may be used in order to assess their welfare levels during transport regardless of its duration.

The cortisol – a hormone secreted by the adrenal gland – is involved in the body's response to stress factors and displays circadian variations, while the change in this evolution is tightly connected to these stress factors. This aspect has led to an increase in the levels of plasma cortisol for horses taken out of the environment they had been raised in [3, 6]. The increased concentration of cortisol even after unloading the animals confirms the fact that this is a stress indicator, which was signalled by other studies as well [3, 4].

CONCLUSIONS

Measurement of heart rate is a non-invasive, efficient method to monitor horses' stress during transport, as they displayed an immediate response to intensity and duration of stress factors.

Animals' behaviour may be a useful instrument in assessing the horses' welfare during transport, as it offers immediate information depending on the animal's decisions to react to various stimuli. In situations when animals had a good relation with their caretaker loading manoeuvres did not constitute a stress factor and heart rate, plasma cortisol levels and behavioural displays have recorded insignificant increases.

Plasma cortisol levels recorded during transport were high in all horses, and more obvious in the aniamls that had not been loaded before and less obvious in the animals that had been used to this manoeuvre.

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THE EFFECT OF SOUND EMMISSION ON SHEEP WELFARE

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SUMMARY

Welfare is defined as the state of physical and mental health of the animals which is reasonably achievable under conditions of complete harmony between the organism and the surrounding environment. The aim of the study was to use the electroencephalography as an estimation method of animal welfare in sheep kept in the conditions of high comfort and acoustic stressors. The experimental animals were Polish Merino sheep. Based on electroencephalography studies, the physiological state of the animal brains was monitored using the Holter EEG devices. Simultaneously with EEG in sheep, physiological tests were conducted (heart and respiratory rate, rectal temperature), the composition of chosen blood parameters was determined. It was found that the level of 90 dB directly affected the nerve cells, what has been confirmed during the analysis of electroencephalographic studies. There was also noted that the level of 90 dB resulted in a clear, statistically proven reaction of sheep, manifested by increased pulse rate and breathing rate.

INTRODUCTION

Welfare is defined as the state of physical and mental health of the animals which is reasonably achievable under conditions of complete harmony between the organism and the surrounding environment. Status of this balance may be perturbed by harmful or noxious environmental factors acting on the animals, for example by the noise. According to recent studies, stress is the most common sign of reduced welfare [7, 13]. The authors argue that the best source of information determining the level of stress and its intensity is manifested in behavioral and physiological mechanisms when body is avoiding or adapting to the existing stressful factor by changing the internal balance. Present literature [10] draws attention to a noise as an uncommon stressful factor. This agent begins to be perceived not only in terms of annoying the source sound, but also as a strong „environmental pollution". This factor is also considered as a serious problem of the modern world as a painful result of urbanization and industrialization. It is also a significant threat to the quality of life of many organisms. According to livestock, the noise is an ubiquitous aspect due to the highly developed computerization and mechanization of agriculture, not only in rural areas, but especially in large farms involved in livestock production. A highly important aspect of the hearing organ exposure to the adverse effects of sound waves with high intensities are mainly anthropogenic factors, mainly road transport, railway, electrification and mechanization [15]. These determinants often cause disruption of homeostasis of the body, causing many side effects, like stress, which is a result of discomfort and irritation, hormonal disorders, or high blood pressure, total damage of the organ of hearing, confirmed also by other researchers in this field [4]. The invasiveness of acoustic phenomena, especially the noise, depends on the intensity, duration of exposure and the individual sensitivity of animals. The most common theme in literature is to divide the symptoms of sound waves in correlation with the degree of their intensity. It is estimated that noise exceeding 30 dB may cause a specific mental reactions, from 65 dB causes psychic reactions with vegetative ones, from 90 dB directly affects the nerve cells, causing permanent hearing loss. The higher intensity of sound in excess of 110 dB causes hearing damage [6].

MATERIAL AND METHODS

The experimental animals were Polish Merino sheep, derived from the certificated national stock – breeding farm. The sheep were fed mainly with oats in a dose of 0.2 kg/capita. Water and hay were provided ad libitum. During the experiment daily microclimatic conditions were monitored. Environmental parameters were monitored by the Scada Pro Software (MicroB, Poland). Studies of long – term EEG analysis of the brain took place using portable kits AURA™ type Holter EEG's (Grass Technologies, USA). The needle electrodes (Grass Technologies, USA) were implanted to sheep heads subcutaneously under general anesthesia. Locations of needle electrodes in 10 points on the animals skull were performed according to the patent application P.393853. In studies on the effects of noise on animals the experimentally emission of sound at 90 dB (± 1 dB) has been generated for 3 hours. Acoustic device type NRG (RFT, USA) generated pink noise. The sounds were enhanced by MOS power amplifier (Audio Delay Systems, USA). Sound emission measuring device was a sound analyzer SVAN (Svantek, Poland). The heart rate and respiratory rate of the animals were measured as well as the internal (rectal) temperature. The biochemical and hormonal analysis of blood were also performed. Blood samples were collected from the internal jugular vein

(*vena jugularis*) into tubes containing EDTA as well as to the tubes Serum Z (Monovette® Sarstedt, Germany). Blood analysis were performed by the devices ABX VET, Pentra – 400 (HORIBA ABX, Canada) and the SYNERGY (Biotek, Winooski, USA). Examination of hormone levels was determined by enzyme – linked immunosorbent assay (ELISA). Laboratory analysis took place in the biochemical laboratory at the Department of Environmental Hygiene and Animal Welfare, Wroclaw University of Environmental and Life Sciences, certified by RIQAS. Statistical analysis of collected data were made by the Statistica software ver. 5.0. The bands of specific parameters were calculated including the arithmetic means with the standard deviations. To compare mean scores in the experimental and control groups the univariate variance analysis (ANOVA) was performed. The significance of differences between experimental groups was evaluated by the Tukey test. Statistical differences were considered significant at a confidence level $p < 0.05$ and for highly significant at a confidence level $p < 0.01$.

RESULTS

The aim of the study was to use the electroencephalography as an estimation method of animal welfare in sheep kept in the conditions of high comfort and acoustic stressors. During the experiment the animal anxiety was observed. Animals ceased watering and feeding. The 90 dB of noise level in the short term has caused in sheep a departure from the source of the sound and accumulation a cohesive group of individuals in the prone position. It was found that in comparison to a control group carried out in a 65 dB, the increased intensity of sound emission causes a stress effect on experimental animals. A significant difference in heart rate, which increased by 10 % (to 96.8 per min.) was noticed. The statistical difference ($p \leq 0.01$) in the respiratory rate (57.91 – 65 per min.) was also observed. The emission of sound at 90 dB drew attention to the rectal temperature fluctuations, although not confirmed statistically within the research group. Highly statistical differences were found in the content of cortisol in blood. Compared with the control group (1.77 ng/ml) its level increased up to 9.29 ng/ml during the stimulation ($p \leq 0.01$). It was also observed that noradrenaline level increased till 24.71 ng/ml, what was a significant difference ($p \leq 0.01$) in comparison with the control group (9.91 ng/ml). It was found that the EEG records were characterized by varying amplitude over 120 μV. The EEG analysis showed periodically (every 1 – 2 sec.) spikes activity with the frequency up to 20 Hz and the accompanying slow waves.

DISCUSSION

There is lack of scientifically confirmed data concerning the effect of different noise levels on sheep. There is a high probability that this factor will greatly affect the level of stress in experimental animals, what was the subject of detailed analysis. Similar studies were also carried out in cats by showing that the noise level of 120 dB at a frequency of 5 kHz, causes the intensification of emotional trauma that can cause the somatic disorders [8, 9]. Broad spectrum of noise from global mechanization and electrification used in animal production is a serious, but rarely noticed problem. The issue of noise pollution is becoming a major factor in the deteriorating quality of life [10]. Based on the efficient handling of animals through breeding and agricultural tools, there is a possibility to reduce significantly the level of welfare by noise emitting. The estimation of the animal sensitivity to stressful factors undoubtedly is not easy. Scientific basis of this issue has not been fully understood yet [3]. The experiments carried out under this project were to determine the effect of sound intensity, which was the pink noise with 90 dB and the response of central nervous system. There was confirmed that sound emission at a frequency of 2 kHz (75, 85 and 95 dB) contributes to the appetite reduction of animals and causes a significant increase in blood cortisol content [12]. There is also a perception that the sound stimulation at 0.1 – 6.3 kHz and 85 or 95 dB can reduce the cortisol concentration in the blood and this factor has little effect on the cardiovascular system [11]. Place in the discussion should also be given to the authors of the experiment in rats, who stimulated them with a sounds of 105 dB intensity and frequency of up to 20 kHz [2, 5]. Research was also performed on cats, according to the individual sound waves to determine the level of stress (trauma) of these animals [14]. Cats kept in an anechoic chamber were subjected to the sound emission of medium intensity 100 dB in order to verify the potential damage to the organ of hearing [8]. Similar studies were also carried out using a 120 dB tones at a frequency of 5 kHz [9]. The experimental studies on animals on the use of acoustic factors are not universal, what may cause many difficulties in the unequivocal interpretation of the results [4]. In this paper, the noise level was strictly determined by using both the sound emitters, as well as the sound control apparatus. However, there must be admitted that the experiment has not been done in anechoic room, which was used by the authors mentioned above. The level of 90 dB directly affects the nerve cells, what has been confirmed during the analysis of electroencephalographic studies. There was also noted that the level of 90 dB resulted in a clear, statistically proven reaction of sheep, manifested by the increased pulse rate and breathing rate. There are countless publications about the effect of sound emission in the hearing organ in humans. Unfortunately, according to animals, this topic is relatively rarely discussed. The numerous authors views, as well as our study, were presented in the article. Most of the opinions say that the rules of the „animal acoustics" are really unknown so scientist should create a uniform ways to measure and simplify the sounds analysis to develop the most optimal method [4].

CONCLUSIONS

- Broadband acoustic emission intensity of 90 dB causes a stress response resulting in the increase of number of breaths, high – potentials of brain activity and the increase in the level of selected hormones in the blood.
- The use of electroencephalographic methods in sheep provide the estimation of the stressful factors on the organism and determine the level of animal welfare.

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AN INTEGRATED APPROACH TO REDUCING INJURIOUS PECKING IN LAYING HENS MAY HAVE MULTIPLE BENEFITS

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SUMMARY

As part of a wider study aimed at reducing injurious pecking in laying hens, this paper describes interventions to encourage greater use of the range area. It considers how management changes could not only reduce levels of injurious pecking but also potentially provide other health, welfare and environmental benefits.

INTRODUCTION

Injurious pecking (IP) is widespread in flocks of laying hens. This welfare problem has complex aetiology and has been extensively researched. IP includes severe feather pecking and removal, vent pecking and cannibalism. The overall aim of project is to put into practice exisiting scientific knowledge in an effort to reduce the levels of injurious pecking in UK commericial flocks of free-range hens. This paper focuses on potential multiple benefits of range use by hens. Several studies have shown a positive association between increased range use by flocks and reduced plumage damage due to pecking by other birds [1,2,3]. Hens which range well may also distribute their manure and increase pasture fertility [4] as well as control insect pests and weeds [5]. Ranging may also reduce the manure, moisture, ammonia and dust levels in the house by reducing stocking density during the day. Whilst it is possible to achieve good performance and welfare in extensive systems [6] this is not always achieved and mortality levels are often high. IP is a major contributor to overall levels of mortality in group housing systems and both may be reduced by encouraging birds to range, provided attention is paid to maintaining hygienic conditions on the range.

MATERIALS AND METHODS

From a review of over 500 papers many risk factors associated with injurious pecking were identified. Using industry experience and knowledge we subsequently identified 40 management and husbandry interventions to address these risks on UK free-range farms. These were grouped into five broad categories which each included several interventions with a similar aim. These were 1) seamless transition between rearing and laying housing and management; 2) enabling access to litter and range from placement 3) Maintaining friable litter throughout; 4) encouraging full use of the range and 5) maximising foraging opportunities both in house and on range. The study as a whole aims to assess the commercial effectiveness of the interventions. Thus 90 flocks in the UK were recruited with each farm being visited four times. Data on feather score, evidence of IP behaviour, range use, environmental measurements and other factors were collected. The farms were divided into 45 control flocks where no additional interventions were suggested and 45 treatment flocks where tailored management and husbandry advice appropriate to the individual farm circumstances was provided to encourage uptake of additional interventions. For treatment farms we examined the welfare of the previous flock before management changes were made and compared this with the treatment flock after management changes were implemented.

Here we focus on the category of interventions that were designed to encourage greater use of the free-range area by hens. The interventions in this category focus on methods that farmers can use to facilitate easier access and improve the quality and attractiveness of the range (e.g. full expression of behaviours such as foraging and dustbathing). Interventions to encourage range use include allowing access from placement, providing artificial and natural (hedges, trees, shrubs) cover, improving drainage and cleanliness outside popholes, making popholes more accessible and not running feeders during the middle of the day, which may have the effect of attracting birds in from the range.

DISCUSSION

Case studies: Some examples of altered management practice that appeared to be beneficial on individual Treatment farms are outlined. The full analysis of data collected from all 90 flocks will take place later this year and it is clearly likely that not all interventions will be associated with improvements on all farms as so many factors can influence the health and welfare of flocks.

Farms A and B changed their management by providing 4-6 simple shelters on the range and placing stones outside

popholes to improve drainage and cleanliness. On farm A this lead on to about 40% increased use of range (to an average of 25% birds ranging at any one time) and marginal improvements to already good feather cover in comparison with the control flock housed previously. On farm B mortality was reduced and plumage cover improved in comparison with the control flock housed previously.

Farm C changed its management by the introducing of 14 additional range shelters and fencing off the muddy track through the range. Changes on farm C when similarly comparing the treatment flock with the previous control flock are summarised in Table 1.

Table 1: Case study (C) showing improved litter friability, plumage condition and reduced mortality in the treatment flock

Farm C	Control flock	Treatment flock
Litter quality (% friable)	31.3	43.8
Mortality to 40 w (%)	2.91	0.47
Plumage score (0 = good to 4 = large denuded areas)	0.26	0.11

Whilst there may be advantages in good weather in encouraging hens to access a clean, well-drained range with a diversity of habitats offering cover and foraging opportunities there are also several disadvantages, particularly in wet weather. Chief among these are the health hazards associated with ingesting pathogens and parasites from land and puddles contaminated by other birds including wild birds. Predation can result in high losses from some flocks as well as initiating panic and smothering [5]. Winter gardens (verandas) offer potential solutions to many of these disadvantages while retaining the advantages of reduced stocking density in the house, exposure to natural daylight and fresh air, and increased skeletal strength due to greater activity levels.

CONCLUSIONS

As well as reducing the risk of IP, management interventions to encourage hens to fully use the range may reduce the risk of disease by reducing stocking density in the house during the day. Additional benefits include meeting hens behavioural needs for foraging and dustbathing, improving hygiene of pasture, birds, litter, eggs and air quality in the house. A more even spread of birds on range should reduce the environmental impact of droppings. Natural cover improves biodiversity.

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RESEARCHES REGARDING GOATS' WELFARE ASSESSMENT IN A FAMILY FARM FROM SOUTHERN ROMANIA

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SUMMARY

At present, assuring animal welfare for both favourites and farm species it is not only a way to improve the production, but a moral duty of human society. The importance of animal welfare issue is proved by the fact that it has entered the sphere of concerns of both non-governmental and governmental organizations: U.N., F.A.O., W.T.O., E.U, O.I.E, W.S.P.A., Eurogroup for animal welfare etc.

This study aimed to assess goats' welfare in a family farm from the southern area of Romania, based on the housing conditions and the serum biochemical panel. The researches were conducted in a house with a capacity of 150 animals, applying deep litter bedding system, wind driven natural ventilation and mixed lighting system. The goats' house was divided in two pens of 12/9 meters, sheltering 70 animals each. There were established space allowance and microclimatic factors: physical – temperature and relative humidity using electronic thermohygrometer, light intensity using LX-1102 electronic light meter, noises using SL-4012 electronic sound meter, air draughts velocity using Hill catathermometer; chemical – carbon dioxide, ammonia and hydrogen sulphide concentrations using Oldham MX2100 portable analyzer and biological factors – air particulates level by harvesting in Berzelius beaker and weighting, bacteria by plate sedimentation (Koch method). In addition, there were collected blood samples from 5 goats of 8 years old and with the Vettest 8008 analyzer it was established the serum biochemical profile.

The results show values ranging within goats' welfare standards and national legislation provisions: space allowance of 1,54 sqm/animal, temperature 15ºC, relative humidity 70%, light intensity 20 Lx, sound intensity 45 dB, draughts velocity 0,11 m/s, CO2 concentration 1800 ppm, NH3 concentration 10 ppm, H2S 5 ppm and air particulates level of 9,17 g/sqm/30 days. However, the value obtained for air microflora (aerobic plate count of 203'246 colony-forming units/m3) was close to the maximum admitted limit. Regarding the serum biochemical profile, it was noticed a slightly increase of average values for calcium, magnesium, total proteins, lactate dehydrogenase, most likely explained by haemoconcentration and muscular effort caused by blood sampling stress.

The goats housed in the assessed shelter have a good welfare level, with proper housing conditions and minor changes of serum parameters.

INTRODUCTION

Defined by Broom as "animal state as regards its attempts to cope with its environment", animal welfare is a problem of major importance, which also reflects upon the production level [2]. Often, animal welfare issue concerns the farmer only because it is a way for improving the production. This minimalist approach is wrong. Assuring good welfare standards means much more: a moral duty of human society.

At present, the animal welfare entered in the domain of activity of many non-governmental and governmental organizations: U.N., F.A.O., W.T.O., E.U, O.I.E, W.S.P.A. Eurogroup for animal welfare etc. [6].

World-wide, for some of the farm species, it was assessed the welfare level in field conditions by using integrative numerical system (e.g. Animal Need Index 35 used in Austria for cattle, pigs and hens), the method consisting in combining engineering-based parameters (details concerning shelter architecture and endowments) with animal-based parameters (physiological and ethological) as an unique result [1]. Such systems can make possible comparisons between different farms and different housing conditions regarding livestock welfare level. For small ruminants, such numerical systems are still not present at national and international level, thus studies in this direction could create the basis for improving knowledge in order to design proper methods of assessment.

MATERIAL AND METHODS

The present paper aimed to assess the welfare level of goats in a family farm from the Southern area of Romania, based on housing conditions and serum biochemical panel.

The animals are reared in a house of 14/33 m, with a capacity of 150 individuals, with deep litter bedding system and with access to an open area (paddock) of 5 m width and 33 m length. The inner space was divided in two pens, sheltering 70 animals each. It was used wind driven natural ventilation and mixed lighting system.

After measurements, it was calculated space allowance, then there were established physical microclimatic factors: temperature and relative humidity using electronic thermohygrometer, light intensity using LX-1102 electronic light meter, noises using SL-4012 electronic sound meter, air draughts velocity using Hill catathermometer; chemical factors: carbon dioxide, ammonia and hydrogen sulphide concentrations using Oldham MX2100 portable analyzer and biological microclimatic factors: air particulates level by collecting in Berzelius beaker and weighting, bacteria by plate sedimentation (Koch method). The results were compared with the national welfare standards in force.

In addition, there were collected blood samples from 5 goats of 8 years old on 11/19/2010 and 01/16/2011. After syneresis, there were established 12 serum biochemical parameters using Vettest 8008 analyzer: blood urea nitrogen (BUN), calcium (CA), magnesium (MG), total proteins (TP), albumins (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose (GLU), lactate dehydrogenase (LDH), phosphate (PHOS), gamma glutamyl transferase (GGT) and triglycerides (TRIG). The results have been compared with the references in the medical veterinary literature [4,5].

RESULTS

Concerning the usable space, dividing the pen surface (12 m x 9 m – 108 sqm) at the number of animals within, it was obtained a value of 1,54 sqm/goat. The figure is ranged within the national goats' welfare standard in force: 1,5-1,7 sqm/animal for houses with access to open area and 2,3 – 2,5 sqm/animal for houses without access to paddock.

The temperature, relative humidity and light intensity measured in three checkpoints - one close to the frontal external wall, one close to the longitudinal one and the last in the center of the goats' house - show the following values: 14.5ºC, 15 ºC and 15,5 ºC (with an average value of 15 ºC); 68%, 70% and 72% (with an average value of 70%); 20 lux, 21 lux and 19 lux (with an average value of 20 lux). The figures are within the limits of welfare recommendations for goats (temperature between 12 and 15 ºC, relative humidity between 60 and 75%, light intensity of 20 lux).

The air draughts intensity was of 0.11 m/s, smaller than the maximum admitted limits of 0,3 m/s in winter and 0,6 m/s in summer and the sound level of 45 dB, a proper value in relation with admitted limit of 50-60 dB applicable in extensive system.

The results for chemical microclimatic factors ranged also within the welfare recommendations: CO_2 concentration - 1800 ppm (while maximum admitted limit is 3000 ppm in mammalian farm species), NH_3 concentration - 10 ppm (while maximum admitted limit is 26 ppm), H_2S concentration - 5 ppm (while maximum admitted limits in air for all farm species is 10 ppm).

Air particulates level measured in two checkpoints shows values of 9,86 g/sqm/30 days, respectively 8,48 g/sqm/30 days, the average value – 9,17 g/sqm/30 days – being much smaller than the admitted limit of 17 g/sqm/30 days.
Regarding air microbial load, the values measured in three points of the house (manger area, close to the external wall and in the centre of the house) were 249'932 colony-forming units/cubic meter, 139'899 colony-forming units/cubic meter and respectively 219'909 colony-forming units/cubic meter. The average value for aerobic plate count - 203'246 colony-forming units/cubic meter) does not exceed the maximum admitted limit (250'000 colony-forming units /cubic meter), but is closed to it.

The values of goats' serum biochemical parameters are shown in the following table.

Table 1: Average lead values in air samples – Acumulatorul Area

Biochemical parameters	Goat 1 Value – 11/19/2010 Value – 01/16/2011 *Average value*	Goat 2 Value – 11/19/2010 Value – 01/16/2011 *Average value*	Goat 3 Value – 11/19/2010 Value – 01/16/2011 *Average value*	Goat 4 Value – 11/19/2010 Value – 01/16/2011 *Average value*	Goat 5 Value – 11/19/2010 Value – 01/16/2011 *Average value*	Reference values
BUN – mg/dl	19 23 *21*	20 23 *21.5*	21 23 *22*	21 10 *15.5*	18 12 *15*	10-21
CA – mg/dl	15.9 12 *13.95*	15.7 11.6 *13.65*	11.6 8.7 *10.15*	15.3 12.4 *13.85*	8.9 7.4 *8,15*	8,2-9,8
MG – mg/dl	5.1 3.02 *4.06*	5.19 3.49 *4.34*	2.9 3.17 *3.04*	3.91 3.99 *3.95*	3.99 2.49 *3.24*	2,23-2,49
TP – g/dl	9.1 11.9 *10.5*	11 11.9 *11.45*	9.5 7.9 *8.7*	11.9 7.8 *9.85*	7.5 7.2 *7.35*	6,4-7,8
ALB – g/dl	2.8 3.5 *3.15*	3 4.1 *3.55*	3.6 2.8 *3.2*	4.3 2.6 *3.45*	2.5 3.1 *2.8*	2,8-3,8
ALT – U/l	48 31 *39.5*	60 27 *43.5*	30 25 *27.5*	34 24 *29*	34 33 *33.5*	23-44
AST – U/l	167 92 *129.5*	126 304 *215*	120 191 *155.5*	183 235 *209*	111 189 *150*	122-321
GLU – mg/dl	104 65 *84.5*	64 70 *67*	76 49 *62.5*	68 79 *73.5*	55 52 53.5	54-93
LDH – U/l	1486 1282 *1384*	1643 1362 *1502.5*	1176 953 *1064.5*	1176 1410 *1293*	1632 1265 *1448.5*	811-1250
PHOS – mg/dl	7.8 5 *6.4*	6.8 5.4 *6.1*	4.3 5.2 *4.75*	4.7 4.5 *4.6*	7.4 4.1 *5.75*	4,2-7,6
GGT – U/l	124 83 *103.5*	84 104 *94*	63 76 *69.5*	111 71 *91*	80 92 *86*	60-101
TRIG – mg/dl	9 10 *9.5*	17 12 *14.5*	10 8 *9*	12 15 *13.5*	15 13 *14*	10-29

As it can be noticed from the data in the table, the dry chemistry serum profile shows minor modifications for calcium, magnesium, total proteins, and lactate dehydrogenase. These changes didn't have pathological signification, being most likely explained by haemoconcentration and muscular effort caused by blood sampling stress. The higher changes were observed in the second individual and the lower in the fifth.

Regarding body condition score (BCS), assessed on a batch of 10 goats, the values are 2 points for 4 animals (backbone visible, some ribs can be seen and there was a small amount of fat cover, a muscle mass can be felt between the skin and the spinous process, sternal fat is developed but can be grasped and lifted by the thumb and the forefinger), 3 points for 5 animals (ribs barely discernible, the spinous process of the lumbar vertebrae cannot be easily grasped because the tissue layer covering them is thick, sternal fat is wide and thick) and 4 point for one goat (backbone and ribs cannot be seen, it was impossible to grasp the spinous processes), with an average value of 2.7 points.

DISCUSSION

The housing conditions studied in the goats' family farm ranged within the welfare standards for Romania [7] or other countries [8], showing a good designing of facilities and good management system and practices. The average score for BCS was indicating that the group health state is good and there were not present management issues [9]. Goats' biochemical serum parameters have values similar to ones obtained by other authors [3, 4, 5].

CONCLUSIONS

Based on the proper housing conditions and the minor changes observed for serum biochemical panel, the goats housed in the assessed shelter have a good welfare level - compatible with good health and productive or reproductive parameters achievement.

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CHANGES IN THE PISCICULTURAL WATER THAT LEAD TO CUTANEOUS AND GILLS' LESIONS AT THE COMMON CARP CYPRINUS CARPIO
MODIFICARI ALE APEI PISCICOLE CE DETERMINA LEZIUNI CUTANATE SI BRANHIALE LA CRAPUL COMUN CYPRINUS CARPIO

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SUMMARY

As a living environment, water is a general vector for different microorganism, that can either cause or complicate lesions. Our studies were performed on two effectives of carp belonging to two different piscicultural farms and appreciated the causes and the way of emergence of the lesions, as well as the supporting factors, specific to the studied farms.

INTRODUCTION

The acquatic environment ensures adecquate conditions for the growth of fish, an activity that is connected to the changes of the physical, chemical and biological characteristics. The changes in pH, organic substance and the suspensions that exist inside the water affect the skin and the gills, determining incipient lesions of erosion, uptil necrosis and local ulceri. On the other hand, water plays the part of a general vector for diverse microorganisms that can either cause or complicate the lesions.

Our studies, made on two effectives of carp belonging to two different farms have appreciated the causes and the way of emergence of the lesions, as well as the specific factor of the studied farm.

The V. farm uses a semi-intenssive system of reproduction and uses water from the river Jijia, having a moderate piscicultural density. Meanwhile, The T. farm has a high piscicultural density and uses the river Prut for supply. The pathological estates that were registred at the level of the skin and of the gills can be classified into non-specific, bacterial and parasitory affections.

MATERIAL AND METHODS

The following species of fish were examined, inside the piscicultural farms: common carp (autochthonous) *Cyprinus carpio,* silver carp *Hypophthalmichthys molitrix,* bighead *Aristichtys nobilis,* grass carp *Ctenopharyngodon idella,* crucian carp *Carassius auratus gibelio,* European catfish *Silurus glanis.* The fish had various ages, such as, for example, the case of the common carp: carp under 1 year of age (C0), carp over 1 year of age (C1), carp of 2 years of age (C2), sire or remonted carps – ready for reproduction process (Cr).

The clinical examination was made on the field, through a clinical investigation and through the direct examination of the fish. This type of exam was made especially in the case of the evolution of pathological estates. The direct examination of the fish was made during the necessary fishings, during the controlled fishings imposed by the technology or in the moment of delivery of the fish destined for consumption.

Using the inspection, the fish close to the shore were examined with the help of the sweep net, appreciating the general aspect and the swimming movements. For each fish that was examined, there were examined the general or local changes of the body (head, body, caudal pedunculus, swimmers), as well as the hypersecretion of mucus or the presence of macroscopical parasites on the surface of the body (skin and scales) and of the gills (by the natural opening of the operculi). The aspects that were observed on the gills were the color, the integrity and the presence of the macroscopical parasites. Each gill arc was put on a glass plate and later on examined with a microscope or a magnifying glass. We also investigated the parasites or the parasitic cysts and their localization along the gill arc and also the possible macroscopical lesions.

The necropsy was made using the techniques taken from the specialized literature. At the exterior exam, we followed the lesions that resulted from traumatic actions or from infestations with macroscopical parasites (wounds, ulceri, nodules).

The gills were examined after the partial or the complete section of the operculum, following the general aspect, more specifically the color, the presence of hemorrhages resulted from the attack of parasites, the presence of an excessive quantity of mucus (leading parasitary irritations

and the selfprotection reactions of the organism) or even the phenomenon of hyperplasia of the gills. The interior examination pointed out the shape and integrity of the internal organs, such as the liver, the intestine, the kidney etc.

For the bacteriological diagnosis, there were initially made the direct bacterioscopic exam of the cutaneous ulceri and by printing the liver and kidneys, on smears that were Gram coloured, and the sowings were made from blood taken from the heart and kidney, on a TSA gelosis and a blood-gelosis.

The parasitologic examination was made through a direct microscopic exam of the cutaneous and gill scraping, through a microscopic examination of the identified parasites that were discovered inside the abdominal cavity and through the examination of the squash preps that resulted from the organs.

RESULTS

*Species of parasites that were identified at the common carp **Cyprinus carpio** inside the piscicultural farms*

No.	Parasite	Parasited Organ	Piscicultural Farm
Protozoa			
2.	*Eimeria subepitelialis*	intestine	T
3.	*Sphaerospora molnari*	Gills, skin	V, T
5.	*Myxobolus cyprini*	intestine, kidneys	T
7.	*M. basilamellaris*	gills	T, V
9.	*Thelohanellus nikolskii*	swimmers	T, V
12.	*Ichtyobodo necator*	Skin, gills	T, V
15.	*Chilodonella piscicola*	Skin, gills	T, V
16.	*Ichthyophtyrius multifiliis*	Skin, swimmers, gills	T
19.	*Epistylis lwoffi*	skin	T
20.	*Apiosoma carpelli*	Skin, swimmers, gills	T, V
22.	*Trichodina mutabilis*	Skin, swimmers, gills	T
24.	*Trichdina nobilis*	Skin, gills	T, V
25.	*Trichodina reticulata*	Gills	T
28.	*Trichodinella epizootica*	gills	T, V
Metazoa			
29.	*Dactylogyrus sp.*	gills	T, V
31.	*Sanguinicola inermis*	gills	T
38.	*Philometroides lusiana*	skin	T
39.	*Lernaea cyprinacea*	Skin, swimmers	T, V
40.	*Argulus foliaceus*	Skin, swimmers	T, V

DISCUSSION

Non-specific affections

The pH changes of the piscicultural water on values such as 6,5 or 7,9 in two bassins of the Tiganasi farms have led to the registration of minor but extended lesions of the skin (descaling, hemorrhagic erosions), and of the gills, which suffered macroscopic lesions that consisted in the hypersecretion of mucus, and microscopic lesions such as alterning areas of hyperplasia, vacuolisation and necrosis. The exact causes of these changes in the pH were not discovered, but the correlation was made between these values and the pH of the Jijia river.

Bacterial affections

Inside the microflora of the water and the microbiotis of the carp there are germs that can be considered epiphites, that become however pathogens in the case of the degradation of the water. Along these germs there are smears of *Aeromonas, Pseudomonas* and *Myxobacterium.* The clinical examination of the 2 summer old carp from the V. Farm has emphasised lesions that were gradually represented (considering the resistence of the effective and the pathogenity of the gemr) by hemorrhagic erosions uptil muscular or cutaneous hemorrhagic ulceri. For the bacteriological diagnosis, there were initially made the direct bacterioscopic exam of the cutaneous ulceri and by printing the liver and kidneys, on smears that were Gram coloured, and the sowings were made from blood taken from the heart and kidney, on a TSA gelosis and a blood-gelosis.

Parasitic affections

The parasitic invasions of the skin and of the gills have been represented by invasions with protozoas and metazoas (digeneous trematodes, cestodes and nematodes). The majority of the parasitic protozoas are epiphites of the skin and gills, and the invasional parameters grow in the case of changes in the quality of the water; the parasites from the genres *Ichtyobodo,Trichodina, Chilodonella, Apiosoma and Epistylis* have reached a severe rate of multiplication at the carp from the T. Farm. The evolution of the infestations of the gills at the common carp inside the T farm depended on the temperature. For three years, there was observed the incidence of *Dactylogyrus sp.* inside the **EC3** pond, along with the temperature reaching 2 - 14 °C. The multiplication of the parasites has evolved progressively upto temperatures of 22 °C , at the end of May, when the parasitic intensity reached high values and imposed the installment of proper treatments.

The skin and the swimmers of the carp that was older than 2 years of age from both farms have been affected by crustaceans from the genres *Lernaea* and *Argulus.*

CONCLUSIONS

The data that was obtained following the research have demonstrated that the evolution of ectoparasitory infestations with protozoas depends on the overload of organic substance in the water. The discovery of parasitic invasions with high intesity has led to the necessity of suplimentary analysis and the verification of the organic substance quantity and of the dissolved oxygen or of the biologic consumption of oxygen.

Taking into account the specialized literature, there was concluded that the rapid growth of the degree of infestation with cilliates from the genres *Apiosoma* and *Trichodina* can constitute an element of precocious diagnosis for the change of the quality of the water, as long as current pathological examinations are made.

Our studies have confirmed the causes of the growth in organic substance in the bassins of the farms: the warping of the bassins and the prolonged maintaining of water at a low level (in both farms), the growth of the piscicultural density (in the T farm), the pouring of high quantities of organic fertilizer (manure) inside the bassin for the support of the plantonic mass (both farms), excessive feeding with the degredation of the food, either through the lack of verification of the consumption or through the lack of consumption because of high temperatures during the summer. The analysis that we made have confirmed the fact that the value of dissolved oxygen drops along with the growth of the quantity of organic substance, which leads to phenomenons of constant hypoxia, as well as reducing the growth rhythm, the diminishment of the organic resistence of the fish and the growth of the receptivity to parasitic invasions.

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PRACTICAL EXPERIENCE WITH THE USE OF PERCHES AS ENVIRONMENTAL ENRICHMENT FOR MUSCOVY DUCKS

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SUMMARY

Commercial production of Muscovy ducks is commonly carried out in unstructured confined buildings on slatted floors. Concerns are rising that this barren environment may contribute to behavioural disorders such as cannibalism and other welfare problems (BULLHELLER 2006). Therefore, more research is required to develop an appropriate housing environment for the birds. This paper reports about the behaviour of Muscovy ducks in a typical production unit (2500 male ducks per fattening period) when offered perches. The results show that the ducks prefer to sit on the offered perches (four different types were tested). Type 2 (characteristic: 4 cm high, 20 cm wide) was the most accepted. More investigations are necessary to study the use of the perches at night, to improve accessibility for example by ramps and to demonstrate the possible effects on animal health and the impact on feather pecking and cannibalism.

INTRODUCTION

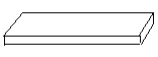
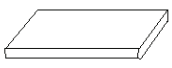
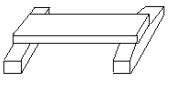
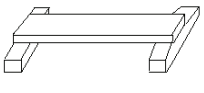
Muscovy ducks (*Cairina moschata dom.*) are one of the most common species of farmed ducks reared throughout the world. In Germany currently approximately 2.6 million ducks are kept for meat production (PINGEL 2004). For industrial meat production they are mostly housed in gender-separated groups up to 10.000 animals in poorly structured indoor conditions on perforated floor without litter. It is reported that these conditions leading frequently to feet injuries, damaged legs include broken bones and behavioural disorders like feather pecking and cannibalism (BULLHELLER 2006). Specific legal regulations for management and housing are missing presently. Only the recommendations of the Council of Europe (1999) for Muscovy ducks give some advice for environmental enrichment measures (e.g. litter, bathing water). This paper reports about the behaviour of Muscovy ducks when offered perches for retreat and relaxation during two growing periods using video analysis.

MATERIAL AND METHODS

The investigations were carried out in a duck barn housing 2500 male birds on a floor space of 425 m². Wooden perches of different length and in different heights were offered in two growing periods. In the course of the first period the birds were offered a variety of different perches and the most accepted were chosen for the second period. In the second period the ducks had (after the 14th day of fattening) the option to choose between four different types of perches (Tab. 1):

Type 1 and type 2 differ in terms of width (10 cm/20 cm) both are 4 cm high and 200 cm long. In total 6 perches of both types were offered.
Type 3 and type 4 were 18 cm high and 14 cm wide, but they varied in length (1.5m and 3m). 4 perches each of this type were offered. Altogether the ducks could use 21 m of perches.

Tab. 1: Hight, length and width of the offered perches

Type	1	2	3	4
				
Hight (cm)	4	4	18	18
Length (cm)	200	200	150	300
Width (cm)	10	20	14	14
No. perches	3	3	2	2

The required space for a male duck in the middle of the growing period sitting on a perch is approximately 18 cm, at the end of the growing period around 22 cm (own measurements, publication in preparation). When taking these figures for the presented animal density there is place for 116 ducks on the perches in the middle of the growing period and 95 ducks at the end. The use of the perches was video taped for 24 hours, in 13 different times from the middle (32th) to the end of the growing period, shortly before slaughter (74th).
Because of light restrictions video material could be taken only during the light phase from 8.30 until 18.00 o'clock. Evaluation of the material was made in the time-sampling procedure in 30-minute intervals.

RESULTS

Figure 1 shows the average frequency (ducks/meter) of use for the four offered perches during daytime between 9:00 and 18:00 o'clock. The use of the perches during the light phase is relatively constant. At 18 h for example 5.3 birds per meter can be observed on type 2, but only 0.5 animals sitting on type 3. Type 1 and type 4 were used on average just by 2 ducks.

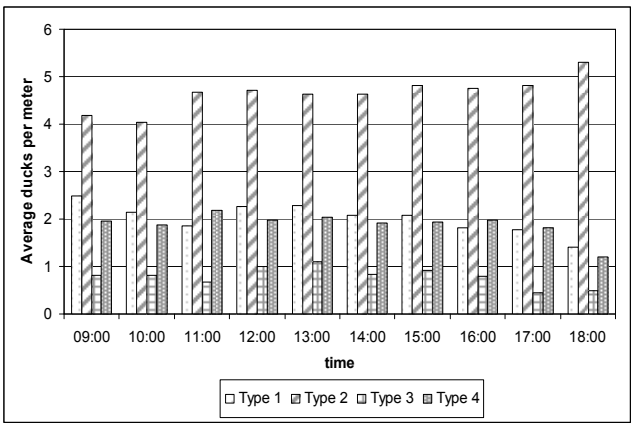


Fig. 1: Average ducks per meter on the four offered perch types during one day

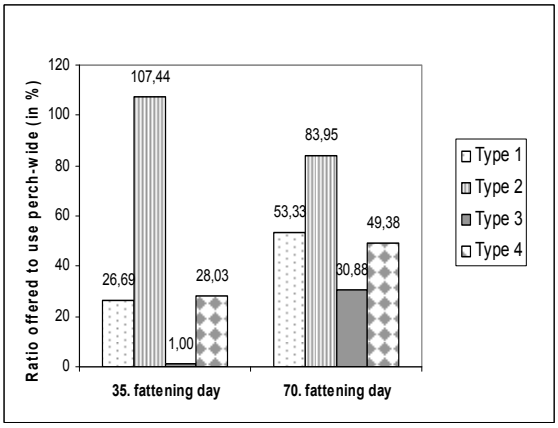


Fig. 2: Use of perches on day 35 and 70 of fattening

Figure 2 shows the average use (ducks/meter) of the perches at the 35th and the 70th day of fattening. The most preferred perch was perch type 2. At day 35 the perches were very crowded and more than 100% of the calculated space was occupied. That means that the birds preferred to sit on this perch even if they had to crouch more closely together than could be expected from normal body shape measurements. At the end of the fattening period (70th fattening day) the attractivity of this perch dropped to 84% but was still higher than the other types. In contrast, only 1% of type 3 (18 cm high, 14 cm wide) was used during the 35th fatting day. The use of types 1, 3 and 4 slightly increased during the growing period, but remained less attractive. The most preferred perches were type 2 followed by type 4, 1 and 3. It seems that longer and wider perch shapes are preferred.

DISCUSSION AND CONCLUSIONS

The results of this preliminary field study show

1. Muscovy ducks use perches under commercial conditions.
2. Length, height above the ground level and width of the perches greatly influence the acceptance and frequency of use.
3. The tested ducks preferred perches which measured 4 cm high and 20 cm wide compared to narrower and higher shapes. It seems that the ducks had problems to reach the higher perches (types 3 and 4).
4. Type 1 does not seem to be sufficiently wide to allow comfortable sitting and resting.
5. Type 2 seems to be so comfortable that the birds accept crowding on the perch, this underlines that Muscovy ducks like to sit on perches and seem to have a small individual distance in the group.
6. The use of perches during the light phase is relatively constant. This hold true for all types tested.
7. Perches seem to be a good tool for enriching the environment of Muscovy ducks in confined buildings. However, the technical installment has to be planned carefully. According to our results, a herd with 2500 birds needs perches of a length of 552.5m to allow all ducks to perch simultaneously.
6. More investigations are necessary in particular in regard to the use of perches during the night, how to install ramps for easy climbing and assessments on the impact of the perches on animal health (e.g. foot pad, breast and skin injuries) and also feather pecking and cannibalism.

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EFFECTS OF GROUP COMPOSITION ON AGONISTIC BEHAVIOUR OF PIGLETS AFTER WEANING

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SUMMARY

The objective of the present study was to investigate the effects of grouping strategy after weaning on agonistic behaviour and related injuries as well as on the daily weight gain of piglets. Following group compositions were examined: homogeneous vs. heterogeneous weight distribution within groups of 12 piglets, groups with 6 piglets from 2 different litters each vs. groups with 2 piglets from 6 different litters each and different group sizes (6 vs. 12 and 24 piglets per group). Agonistic behaviour was analysed during 72 hours after weaning and an individual rank index was calculated for each piglet. Piglets were weighed the day before weaning, 4 days later and on day 38 after weaning. Skin lesions were examined on the 4th day after mixing, using a lesion score (0 to 3) for ears, head, shoulder, flank, ham and tail. We observed significantly less fights of individuals in groups of 6 piglets compared to groups of 12 piglets. In groups of 24 piglets, there was a tendency for fewer fights but more injuries than in the smaller groups. We found a tendency for more fights and more injuries in homogeneous groups than in heterogeneous groups. In groups with 6 piglets from 2 litters each, lesion score was significantly reduced compared to groups with 2 piglets from 6 litters each. In all groups, high ranking animals fought more than low ranking animals. In groups of 12 piglets, low ranking piglets showed the fewest injuries. In groups with 6 piglets each, alpha animals showed the fewest injuries whereas low ranking animals had higher lesion scores. In both group sizes, high ranking animals were heavier at weaning. Within the first 4 days after weaning as well as during the total rearing period, alpha animals showed the highest daily weight gains.

INTRODUCTION

In commercial pig production, it is common practice to mix unfamiliar piglets in uniform weight groups after weaning assuming positive impacts on growth performance throughout the rearing period. However, every change of group composition in pigs requires the establishment of a new dominance order by fighting, leading finally to a restriction of aggressive behaviours [6]. After regrouping, pigs appear to experience social stress sufficient to cause a subsequent reduction in weight gain performance [5], and mixing is usually followed by fights resulting in more wounded animals [2]. Agonistic behaviour after weaning may be influenced by grouping strategy [1, 7]. The tendency in modern pig farming to form large groups of 40 up to 200 animals per group is associated with a combination of piglets from a high number of different litters, possibly increasing the frequency of agonistic interactions, as the number of dominance relationships to be resolved is increasing with the number of pen mates. In several studies, a positive correlation between the live weight of pigs and their social status has been demonstrated, with heavier pigs occupying high rank positions [12; 3] and with dominant pigs showing higher daily weight gains [12]. The aim of the present study was to determine whether agonistic behaviour and related injuries in weaned piglets may be influenced by different grouping strategies. Additionally, relations between rank position, skin lesions and growth performance were analysed.

ANIMALS, MATERIAL AND METHODS

A total of 868 weaner pigs were divided in groups of homogeneous weight (7.9 ± 0.65 kg) or heterogeneous weight (7.84 ± 1.74 kg), in groups with 6 piglets from 2 litters each or groups with 2 piglets from 6 litters each (12 animals per group, respectively) and in groups of 6, 12 and 24 piglets. Sex ratio was approximately equal in all groups. Except for the groups originated from 2 or 6 litters, groups were formed from as many different litters as possible. The mean initial weight of all piglets was 7.8 kg with an average weaning age of 26 days. The animals were kept without litter in partially slatted floor (0.38 m^2 per animal, animal-feeding place ratio 1.5:1) and piglets had ad libitum access to dry food and water. The number and outcome of agonistic interactions were analysed by continuous videotaping during 72 hours after weaning. Per rearing round, 2 groups were videotaped. In each round, 2 control groups were performed in order to analyse growth performance and animal health status. The number and outcome of all agonistic interactions occurred within a group were collected on an individual basis. An individual rank index was calculated for each piglet [8] allowing the attribution of the individuals to certain rank positions within a group:

$$RI = \frac{(S \cdot P_S) - (N \cdot P_N)}{(S + N) \cdot (n - 1)}$$

RI = rank index, *S* = number of wins, *N* = number of defeats, P_S = number of defeated partners, P_N = number of superior partners, *n* = number of group members

Piglets were weighed the day before weaning, the 4th day after weaning and at the beginning of the fattening period on day 38. On the 4th day after weaning, skin lesions were examined using a lesion score from 0 to 3 for ears, head, neck/shoulder, flank, ham and tail which was added up to a cumulative value for each piglet. Statistical analysis was performed using the statistical software package SAS, version 8.2 (Statistical Analysis System). Taking into account possible influences of variant (grouping strategy), rearing round, rank position and initial weight, LSQ-means were calculated for the targets (a) "number of fights", (b) "lesion score per individual" and (c) "daily weight gain".

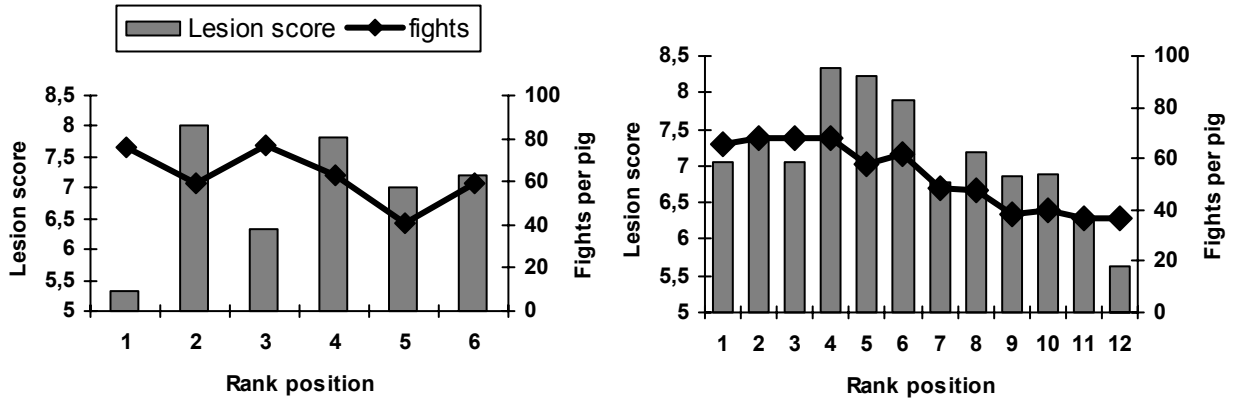
(a) $y = \mu + variant_i + round_{ij} + b\,(initial\ weight_k - \overline{initial\ weight}) + c\,(age_l - \overline{age}) + e_{ijkl}$

(b) $y = \mu + variant_i + round_{ij} + b\,(rank_k - \overline{rank}) + e_{ijk}$

(c) $y = \mu + variant_i + round_{ij} + b\,(initial\ weight_k - \overline{initial\ weight}) + e_{ijk}$

RESULTS

In groups of 6 piglets, the individuals fought significantly less (52.3 fights/72 h) than in groups of 12 piglets (63.5 fights/72 h). In groups of 24 piglets, there was a tendency for fewer fights on group level (24.4 fights/72 h) than in groups of 6 and 12 piglets. Nevertheless, piglets in groups of 24 animals had more injuries (cumulative lesion score = 8.1) than piglets in groups of 6 (7.7) and 12 animals (7.5). There was a low tendency for piglets in homogeneous groups (57.1 fights/72 h) to fight more than piglets in heterogeneous groups (51.7 fights/72 h), and there was also a tendency for more injuries in homogeneous groups (6.8 vs. 6.5). We observed only marginal differences in the number of agonistic interactions between the variations 6 piglets from 2 litters each (46.1 fights/72 h) and 2 piglets from 6 litters each (49.0 fights/72 h). In groups originated from only 2 litters, lesion score was reduced significantly compared to groups originated from 6 litters (5.5 vs. 6.7). In all groups, high ranking animals fought more than low ranking animals. In both group sizes, animals which occupied high rank positions were heavier at weaning than low ranking animals. Within the first four days after weaning as well as during the total rearing period alpha animals had the highest daily weight gains. With increasing the number of animals per group, the daily weight gain decreased within the first four days (119 g/day in groups of 6 piglets, 114 g/day in groups of 12 piglets and 105 g/day in groups of 24 piglets) as well as during the total rearing period (458 g/day; 450 g/day and 439 g/day). During the total rearing period, piglets in homogeneous groups gained 9 g/day more than piglets in heterogeneous groups, and piglets in groups from 2 origin litters had a higher average daily weight gain of 10 g/day than piglets in groups from 6 origin litters.



DISCUSSION

As a result of increasing group size from 6 to 12 animals, the individuals were confronted with more opponents to establish a rank order. This might explain why we found more fights per individual in larger groups. Nevertheless, in groups with 24 piglets we observed fewer fights than in the smaller groups. Even if the absolute space per animal remains equal, the relative space per individual increases with increasing group size. Therefore, in larger groups low ranking animals are enabled to avoid attacks of high ranking animals. However, in groups of 24 piglets there was a tendency for more injuries than in the smaller groups. Other authors observed in groups with 24 piglets less fighting than in groups of 6 or 12 piglets, but they also found longer fighting periods in groups with 24 animals compared to smaller groups [2]. Since the mean duration per fight is positively correlated to the lesion score [9], more injuries can be expected in bigger groups. A combination of piglets from only 2 litters did not significantly reduce the number of agonistic interactions, but the individual lesion score decreased significantly. This suggests that the dissolution of litter unit leads to rank order fights between littermates as well, but these interactions seem to be less violent than between piglets that are unknown to each other. The usual practice to form homogeneous rearing groups had no positive effects on agonistic behaviour, skin lesions and growth performance after mixing compared to heterogeneous groups. In all groups, there was a trend towards a decrease in rank position with decreasing weaning weight. Piglets with higher weaning weights often suckle on the sow's front teats and have already high rank positions within the litter [11]. Previous studies showed that piglets with a cranial teat position occupied high rank positions after mixing at weaning [10]. Thus, heavier piglets apparently tried to achieve high rank positions by violent fighting. Some authors also report that most attacks came from those individuals, who later achieved high rank positions [3; 4]. In groups of 6 piglets, alpha animals showed the fewest injuries despite of high fighting activity, and low ranking piglets had a high lesion score. Apparently, a large number of wins was associated with less injuries, whereas many defeats were well related to many injuries because of limited opportunities to retreat in the small groups. In groups of 12, alpha animals showed a high lesion score presumably due to a higher number of opponents, whereas low ranking animals had the fewest injuries as they had the chance to avoid attacks of dominant piglets. The highest daily weight gains were achieved by alpha animals, probably resulting from their control on important resources such as feeding place. Shortly after weaning, low ranking animals showed good growth performance as well, due to an earlier start of feed intake [5] supported by less fighting and their familiarity with solid food during the suckling period.

CONCLUSIONS

Mixing piglets from few different litters might reduce injuries and improve animal welfare, since fights for ranking order are less violent. In small weaning groups with 6 piglets each, less fighting per individual occurred compared to groups of 12 piglets. A high rank position was usually associated with good growth performance. The common practice in pig production to form homogeneous weight groups after weaning did not show any advantages neither regarding daily weight gain nor in terms of agonistic behaviour and related injuries.

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HOW MUCH FLOOR SPACE NEEDS A BROILER CHICKEN?

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SUMMARY

Stocking density is one of the most disputed criteria in broiler production. But little is known about the real floor space covered by a sitting or standing broiler. This paper gives realistic figures on the floor space covered by broilers in various body positions, age and weight in the course of fattening. The measurements were carried out by the KobaPlan colour contrast planimetric method (KobaPlan). 1550 broiler chickens (genotype Ross 308) were weighted and photographed digitally in a photo box. The birds were between 10 and 40 days old. The results showed a linear correlation between floor space covered by the broilers and bodyweight (R^2 = 0.99). The mean floor space for standing broilers with a weight of 100g was 74 cm², with 1000g 203 cm², with 2000g 320 cm² and with 2500g 372 cm². The covered floor area in sitting position was approx. 25 cm² larger. When broilers are raised for a stocking density of 42 kg/m² and a target bodyweight of 1.5 kg according to the EU Directive 2007/43/EG less than 1/3 of a square metre are not covered by broilers. The described method seems to provide reliable data for the calculation of animal density in broiler production. The figures show that the free space allowed for the birds in last days of the fattening period for behavioural activities like dust bathing or wing flapping is very limited. The results give cause for concern whether the behavioural needs of broiler chicken can be met under these conditions and for reconsidering the currently allowed high bird density at the end of fattening in the EU Directive.

INTRODUCTION

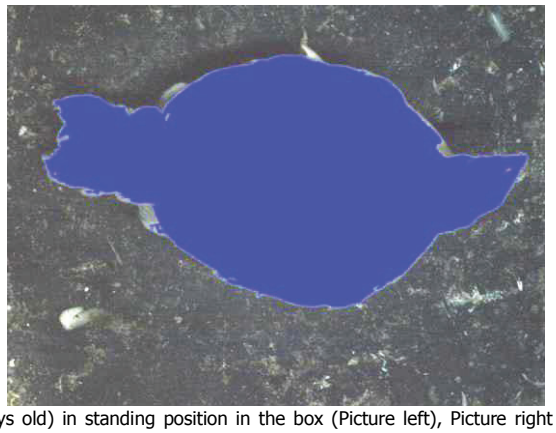
Stocking density is one of the most disputed criteria in broiler production. The EU Directive 2007/43/EG (EU-CD) [2] allows stocking densities up to 42 kg/m² when certain keeping and management conditions are met. However little is known about the real floor space covered by a sitting or standing broiler in the course of fattening. This paper describes a method how to measure the floor space covered by the body of a broiler during fattening and compares the covered area with the free space at different stocking densities (SD) and bodyweights of the birds.

ANIMALS, MATERIALS AND METHODS

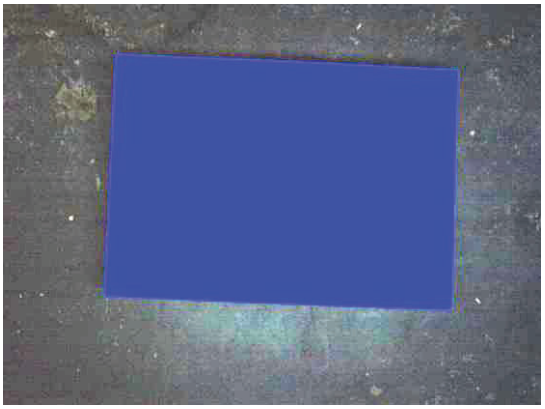
1550 broiler chickens (genotype Ross 308) were weighted, placed individually in a special photo box with a black floor (1.0m x 1.0m 0.40m) and photographed digitally in order to measure the floor space which was covered by the body of standing and sitting broilers. The white-feathered broilers gave a strong contrast to the dark floor of the box. that was designed, lightened and floored to provide high contrast pictures which were stored in a PC (**Picture 1 and 2**). The method is called KobaPlan colour contrast planimetry [1] and uses a digital camera (OLYMPUS, E-410, 17,5-45mm Objective, 10,0 Mega pixel, Co. Olympus Optical Co GmbH, D-Hamburg), mounted on a metal frame with a stationary fixed distance above the floor of the box (1.50m). All images were taken from top view (**Picture 3**). For better visualisation the program colours the recorded pixels blue (**Picture 4**). This helps to recognise defaulted photos which can be eliminated before analysis. The birds were between 10 and 40 days old. They were randomly selected from commercial herds weighted and placed in the box.

As reference area the defined surface of a sheet of paper (DIN A5 = 310.80cm² or DIN A4 = 623.70cm²) was used. This sheet of paper was photographed (once for every series of measurements) with the same adjustments as the birds in the box and stored in the PC (**Picture 5**). The program colours the recorded pixels blue (**Picture 6**). The digital images of the broilers and the reference values were analysed by the KobaPlan software which calculates the surface of the sitting and standing broilers according to the rule of proportion.


Pictures 1 and 2: Photo box in the broiler barn with metal frame for fixing the digital camera above the broilers

Pictures 3 and 4: Digital image from top view of a broiler (40 days old) in standing position in the box (Picture left), Picture right shows the same broiler after digital modelling by the KobaPlan software in blue colour

Pictures 5 and 6: Digital top view image of a reference area (paper sheet: DIN A4 = 623.70 cm²) in the box (Picture left) and after modelling by the KobaPlan software (Picture right).

RESULTS

The results show a linear correlation between floor space covered by the broilers and bodyweight (R^2 = 0.99). The mean floor space covered by standing broilers with a weight of 100g was 74 cm², with 1000g 203 cm², with 2000g 320 cm² and with 2500g 372 cm² (**Table 1**). Sitting broilers covered a floor area which was approx. 25 cm² larger.

The free space per m² which can be used by the birds for activities and movement decreases with increasing stocking densities from 33 kg/m², 39 kg/m² and up to 42 kg/m² (EU Directive 2007/43/EG). When broilers are raised for a stocking density of 42 kg/m² and a target bodyweight of 1.5 kg about 29% of a square metre are not covered by the body of the broilers. In contrast a long fattening period with a target weight of 2.5 kg at the end of fattening and a stocking density of 33 kg/m² (13 broiler/m²) results in 52% free area per m².

Table 1: Floor space covered by broilers (Ross 308) in standing and sitting position and live weights between 100g and 3500g

Live weight (g)	Floor space covered by standing broilers (cm²)			Floor space covered by sitting broilers (cm²)		
	n	Mean (Standard deviation)	Range	n	Mean (Standard deviation)	Range
100	16	73.58 (4.98)	65.85 - 80.61	0	/	/
500	89	143.21 (19.12)	107.85 - 215.96	14	142.29 (20.76)	121.88 - 193.55
1000	86	203.25 (22.40)	145.69 - 309.83	75	226.41 (43.39)	145.12 - 416.04
1500	69	254.62 (25.56)	195.11 - 309.63	64	277.60 (37.12)	180.09 - 380.18
2000	77	320.41 (35.44)	212.73 - 419.89	76	332.18 (42.49)	245.46 - 508.59
2500	56	371.65 (39.58)	289.17 - 503.21	55	400.59 (35.22)	319.91 - 498.85
3000	17	429.87 (39.27)	362.50 - 487.32	16	445.37 (35.45)	393.14 - 495.77
3500	1	507.25	/	1	506.17	/

DISCUSSION

The free floor space for broiler chickens (Ross 308) in confined buildings decreases with the growth of the broilers in the course of the fattening period. There exists a linear correlation between floor space covered by a broiler and bodyweight throughout the fattening period. The used KobaPlan method seems to be a further step forward to an objective measurement. KobaPlan based on a large random sample delivers areas covered by a broiler which are about 100 cm² lower than reported in literature earlier [3]. Reasons for these differences may be caused by different measurement practices - manually versus automatically- smaller sample size as well as genetic influences.

CONCLUSIONS

The described method seems to provide reliable data on animal density in broiler production during the course and at the end of fattening. The figures show that the space allowance for broilers in the last days of fattening is very limited for behavioural activities like dust bathing or wing flapping, in particular during short fattening in combination with the high stocking density of 42 kg/m² as allowed in the EU-Directive,. The results give cause to reconsider behavioural needs of broiler chicken and to scrutinise some of the current space allowances in the EU Directive.

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TESTES WEIGHT IN COMPARISON TO CARCASS WEIGHT AND TIME OF 2ND IMPROVAC® VACCINATION IN BOARS

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SUMMARY

Improvac® vaccination in boars is a successful and animal friendly alternative to surgical castration. This study describes the distribution of testes weight and sizes in comparison to carcass weight and the time of 2nd vaccination. Results show that testes weight and sizes correlate with carcass weight. However, if the time after second vaccination prior to slaughter is very short (less than four weeks) or very long (more than ten weeks), larger testes can be observed, independently to the carcass weight. No boar taint was observed in the vaccinated boars.

INTRODUCTION

It is well documented that vaccination of boars with Improvac® is a successful and animal friendly alternative to surgical castration. Usually, vaccination is recommended twice with the 2nd injection given four to ten weeks after the 1st injection and four to six weeks prior to slaughter [1]. The effect of the vaccination (elimination and suppression of boar taint) is guaranteed only if both of the required two vaccinations are given reliably to the animals. One possible sign for successful vaccination is the reduction of testes size [2, 3, 4]. Sometimes, this reduction is not equally distributed and some overlaps in testes weight in comparison to entire boars are to be seen [5, 6]. In few vaccinated boars the testes are larger than in entire boars but no boar taint can be observed.

The objective of this study was to evaluate testes weight and size in comparison to the carcass traits in Improvac® vaccinated boars receiving the 2nd vaccination at different times from 23 to 74 days prior to slaughter.

MATERIAL AND METHODS

A total of 253 GnRH (Improvac®) vaccinated boars of three different farms were examined at the slaughterhouse. Vaccination schemes are shown in table 1.

Table 1: Schemes of GnRH (Improvac®) vaccination in boars included in the study

		1st vaccination	2nd vaccination		age at slaughter
	number of boars	age (weeks)	age (weeks)	days prior to slaughter	(weeks)
farm 1 1st slaughter	30	10	18	46	24
farm 1 2nd slaughter	38	10	18	60	26
farm 1 3rd slaughter	20	10	18	74	28
farm 2	90	10	25	23	28
farm 3	75	10	20	46	26

Farm 1 is an Austrian farm, farm 2 and 3 are German farms. Carcass weight and lean meat were evaluated. Testes were weighted after the separation of epididymides. Testes length and width with and without epididymides were obtained with a sliding gauge. Presence of boar taint was evaluated as described in the literature [6]. Differences between the farms and slaughter groups were tested with Kruskall Wallis test and Mann Whitney test. Results with $p<0,01$ were significant. Correlations were proved with correlation coefficient after Pearson.

RESULTS

Table 2: Testes weight without epididymides and carcass weight in GnRH vaccinated boars of three different farms

	time after 2nd	testes weight g			carcass weight kg
farm/slaughter	vaccination (d)	mean ± sd	min	max	mean ± sd
farm 1, 1st	46	84 ± 31	38	170	80 ± 7
farm 1, 2nd	60	92 ± 48	29	205	80 ± 5
farm 1, 3rd	74	144 ± 70	30	238	72 ± 14
farm 2	23	192 ± 63	85	385	103 ± 9
farm 3	46	89 ± 54	24	245	90 ± 8

There was found a positive correlation between testes weight and carcass weight over all farms (table 2). By comparing every single farm an influence of the time of 2nd vaccination was found, too. Testes weight in heavy pigs (farm 2) vaccinated the 2nd time 23 days prior to slaughter was the same as in slowly growing pigs (farm 1) vaccinated the 2nd time 74 days prior to slaughter. Testes weight in pigs of farm 1 with 2nd vaccination 46 days or 60 days prior to slaughter was significantly lower than in the before mentioned pigs. Testes weight in pigs of farm 3 with 2nd vaccination 46 days prior to slaughter did not differ from farm 1 with 46 and 60 days after 2nd vaccination, though the pigs were heavier.

Table 3: Testes sizes in GnRH vaccinated boars of three different farms

farm/ slaughter	time after 2nd vaccination	testes length with epid.	testes length without epid.	testes width with epid.	testes width without epid.	testes height
	(d)	mm (mean ± sd)				
farm 1, 1st	46	104 ± 11	77 ± 10	53 ± 7	46 ± 7	39 ± 7
farm 1, 2nd	60	104 ± 15	76 ± 13	55 ± 7	48 ± 10	40 ± 9
farm 1, 3rd	74	114 ± 17	85 ± 16	63 ± 16	55 ± 13	47 ± 11
farm 2	23	135 ± 13	102 ± 10	69 ± 6	61 ± 6	59 ± 6
farm 3	46	109 ± 16	76 ± 13	55 ± 10	48 ± 9	30 ± 7

Testes sizes (table 3) show on the whole the same significant differences as testes weight. The significances, however, could not always be detected. Individual morphological differences of the ratio of length, width and height allow not such a clear result as testes weight. Therefore testes weight seams to be the more reliable parameter.

Organoleptic abnormalities (i.e. pronounced sexual odour) were not detected in any of the carcasses.

DISCUSSION

The results of the study confirm that vaccination of boars using the anti-GnRH vaccine Improvac® results in a lower testes weight in comparison to testes weight of entire boars given in the literature [1, 3, 4, 5]. The sizes and the weight of the testes depend on the age and the carcass weight of the animals as well as on the time after the 2nd vaccination prior to slaughter. Testes weight seams to be a more reliable parameter compared to testes sizes (i.e. length, width, height). Testes weight is not affected by epididymides and the weighing with calibrated scales more exactly than the measurement of the testes sizes, because linear measures are difficult to standardize and influenced by testes texture, epididymides.

The effect of the vaccination is time limited. As data show are the testes of the boars (28 weeks old) vaccinated 2nd time 74 days prior to slaughter heavier than those of the boars (24 or 26 weeks old) vaccinated 46 and 60 days prior to slaughter, although the younger animals were heavier. The recommendation of the manufacturer of Improvac® to give the 2nd vaccination four to six weeks prior to slaughter should be complied.

CONCLUSIONS

Testes weight and sizes depend on carcass weight and on the time of the 2nd Improvac® vaccination prior to slaughter. Late vaccination (less than four weeks prior to slaughter) of to heavy pigs as well as early vaccination (more than ten weeks prior to slaughter) of slowly growing pigs results in carcasses with heavy testes. However, no boar taint was observed. It is not possible to obtain the success of vaccination only by measuring testes weight, if carcass weight and time of 2nd vaccination are not considered.

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EXERCISE EFFECT ON LAMENESS PREVALENCE IN TIED DAIRY COWS

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SUMMARY

The objective of this study was to compare the lameness severity and prevalence amongst dairy cows kept in tie-stalls with exercise and without exercise. The study was realised in the winter period, in 20 tie-stall barns in Transylvania, 13 with outside access of the cows for exercise (TSE) and 7 without the possibility of the cows to exercise (TSNE). The prevalence of lameness varied widely in the investigated barns, being between 8.33% and 25.00% in TSE and between 16.00% and 28.00% in TSNE, respectively. The mean prevalence of lameness (23.20%) in those barns where animals do not benefit from exercise was significantly higher (Mann-Whitney test, $p<0.001$) than in those where animals have access to outside exercise (15.7%). The obtained results confirm the fact that the provision of exercise for dairy cows kept in tie-stalls has a beneficial effect on their locomotion, the severity and prevalence of lameness being lower in this case.

INTRODUCTION

For dairy cows lameness represents a severe welfare problem. It decreases their mobility, impairs their normal behaviour and it is common throughout Europe [11]. If dairy cows have a high motivation for locomotion, problems may arise when they are tied all year around or during the winter period. In intensive dairy systems, and especially in tie stalls, the cows have little opportunity to exercise. Phillips [7] states that today's cattle need exercise to keep healthy and productive. Most of the researches done show that daily exercise reduces the incidence of lameness [4, 5, 6, 8, 9]. There are also studies which found no association between exercise and the prevalence of lameness [1].

The objective of this study was to compare the lameness severity and prevalence amongst dairy cows kept in tie-stalls with exercise and without exercise.

MATERIAL AND METHODS

The study was realised in the winter period (2009-2010) in 20 tie-stall barns in Transylvania, 13 with outside access of the cows for daily exercise (TSE) and 7 without the possibility of the cows to exercise (TSNE). The farms were selected within a research project on dairy cows welfare. The housing conditions were similar in the investigated farms. Lameness was assessed based on the locomotion score, devised by Sprecher at al. [10], considering as lame cows those ones obtaining scores between 3 and 5. For the locomotion score, the cows were untied and led out from the barns. The locomotion score was determined by two trained observers. All the cows in each barn included in the study were assessed (1385 cows in total). The results were statistically processed with the SPSS version 17 software. Lameness prevalence in each farm and its mean prevalence in tie-stall barns with exercise and without exercise were determined. The prevalence of lameness was calculated as the proportion of cows with scores 3 or more. For the comparison of the results the non-parametric Mann-Whitney test was used.

RESULTS

The results of the cows' locomotion assessment in the 20 investigated farms are shown in table 1. It can be observed that the percentage of the cows with different lameness scores varied in the farms with exercise (TSE) and without exercise (TSNE).

Table 1: Locomotion score distribution for the investigated farms

Farm	Locomotion score				
	1	2	3	4	5
1	32	52	10	4	2
2	25	53	15	4	3
3	23	49	18	8	2
4	26	47	21	4	2
5	14	61	19	5	1
6	30	52.5	17.5	0	0
7	13.46	59.62	21.15	3.85	1.92
8	31.25	50	16.67	2.08	0
9	40.48	47.62	7.14	2.38	2.38
10	21.87	62.5	9.37	3.13	3.13
11	32	45	20	2	1
12	31.43	60	8.57	0	0
13	37.17	38.05	16.82	4.42	3.54
14	40.91	36.36	19.32	2.27	1.14
15	57.14	33.93	8.93	0	0
16	68.29	19.51	9.76	2.44	0
17	55.55	33.33	5.56	5.55	0
18	15	71.67	13.33	0	0
19	33.34	58.33	8.33	0	0
20	28.85	46.16	21.15	2.88	0.96

1-7 TSNE; 8-14 TSE

Figure 1 presents the distribution of the locomotion scores in the assessed dairy cows. In TSE (791 cows) the locomotion scores distribution was: L1=37.94%, L2=46.34%, L3=12.68%, L4=2.08%, L5=0.93%, and in TSNE (594 cows): L1=23.35%, L2=53.44%, L3=17.37%, L4=4.12%, L5=1.70%, respectively. Although certain differences were found between the two barn types for the same score, these were not statistically significant.

The prevalence of lameness varied widely in the investigated barns, being between 8.33% and 25.00% in TSE and between 16.00% and 28.00% in TSNE, respectively (figure 2). The mean prevalence of lameness (23.20%) in those barns where animals do not benefit from exercise was significantly higher (Mann-Whitney test, $p<0.001$) than in those where animals have access to outside exercise (15.7%).

DISCUSSION

The locomotion scoring system suggested by Sprecher et al. [10] was used because of the clear objective descriptions that differentiate each score. The prevalence of lameness varies in the studies done in different countries of the world.

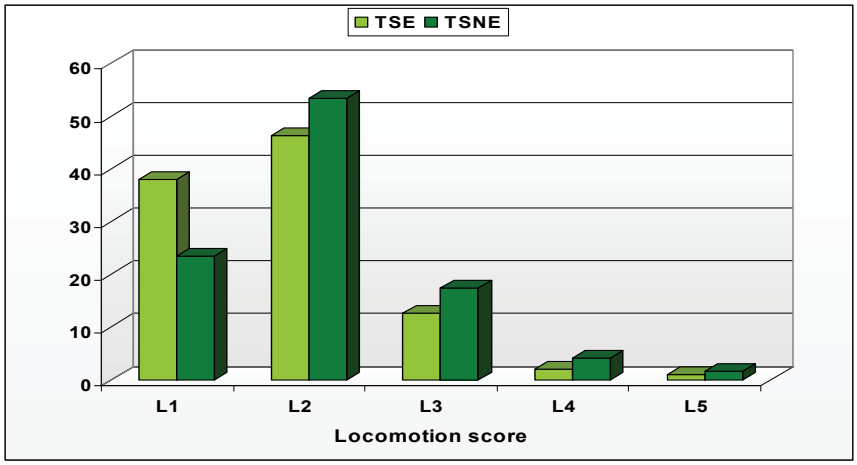

Figure 1: Locomotion score distribution in the assessed dairy cows

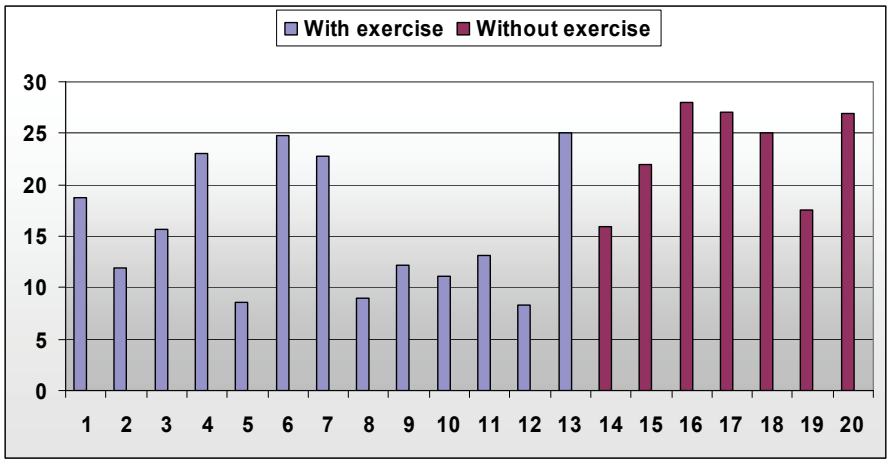

Figure 4: The mean prevalence of lameness in dairy cattle housed in 20 tie-stall barns, with and without exercise

Recent figures for the prevalence of lameness in European countries range from 22% [11] to 45% [12] for loose-housing systems and from almost 1% to 21% for systems in which cows are tied for at least part of the time [2]. The prevalence of lameness in US cows, in commercial free-stall housing is approximately 25% and in tie-stalls 21% [3], but varies greatly from farm to farm. It is possible that the great variation, both regional and national, in lameness prevalence estimation appeared due to the evaluation systems and to the observations being done by different operators. The results obtained in our study are in agreement with those of other studies. Regula and others [8] found a lameness prevalence of 21% (in 1999) and 17% (in 2000) in Swiss dairy cows kept in tie-stalls with minimal outdoor access during winter; in the same time in tie-stalls with regular outdoor exercise throughout the year the prevalence of lameness was lower. Also, Bielfeldt and others [2] observed that lameness was more frequent in cows housed in tie-stall barns without exercise (13.2%) than in tie-stall barns with exercise (9.6%). Gustafson [4], following the study he realized, concluded that the dairy cows' health in general was significantly and positively influenced by exercise, and the need for veterinary treatment was reduced. In contrast Alban [1] could not find any association between exercise (which in her study meant grazing) and the reduction of lameness. She explains that any positive influence from daily exercise was leveled out by the negative influence from, for example, small stones bruising the hooves [1]. Yet the author admits that exercise probably has a positive impact on the health of the cows' legs, but to be so, the exercise area should be in a proper condition. Gustafson [4], too, stresses the proper condition of the surface of the exercise alley as an important factor. So, given an adequate surface condition, it can be concluded that certain daily exercise has a positive effect on the health of cows.

CONCLUSIONS

The obtained results confirm the fact that the provision of exercise for dairy cows kept in tie-stalls has a beneficial effect on their locomotion, the severity and prevalence of lameness being lower in this case.

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EFFECT OF TRANSPORTATION ON EXPRESSION OF HSP90, HSP70, HSP27, AND AB-CRYSTALLIN IN THE PIG STOMACH

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SUMMARY

Twenty pigs were randomly divided into four groups based on the amount of time spent in transport (0, 1, 2, or 4 h). Pathological examination indicated that integrity of the gastric mucosa was compromised by damage occurring during the 4 h of transportation, despite the fact that gastric ulcers were not present. Variations in Hsp90, Hsp70, Hsp27, and αB-crystallin levels suggest that distinct protective functions are modulated by different Hsps in stomach tissues during transportation. Alterations in Hsp70 and αB-crystallin expression appear to be associated with protective functions, as no apparent gastric ulcers were present in pigs that underwent 4 h of transportation. Levels of HSF-1, which regulates the expression of Hsps, remained relatively stable independent of the transportation period. This observation indicates that expression of Hsps may be regulated by factors other than changes in HSF-1 expression levels in pigs during transportation.

INTRODUCTION

Transportation is severely and inherently stressful for pigs [7]. The stress can be associated with biochemical and morphological changes in the heart, liver, kidney, and other organs in transported animals [2,3,9]. Gastric ulceration in swine is a serious problem, potentially leading to bleeding and death [8]. When exposed to stressful conditions, cells increase the expression of a specific set of proteins, termed the heat shock proteins (Hsps) [1]. The aim of the present study was to investigate the relationship between histopathological changes and the levels of Hsps (Hsp90, Hsp70, Hsp27, and αB-crystallin) in the stomach tissues of pigs during short-term (<4 h) transportation.

MATERIAL AND METHODS

Twenty hybrid pigs from the Erhualian and Pietrain strains were placed into 4 groups, each group had 5 pigs. On the day of the transport trial, one group was maintained under normal housing conditions and served as the negative control group (0 h). Pigs in the remaining three groups were transported for 1, 2, or 4 h, respectively, at 30-40 kilometers per hour. The route included an equivalent mix of local roads, including town traffic, state roads, and highways. The study protocol was reviewed and approved by an animal care and use committee, and experimental procedures were undertaken following the guidelines of a regional animal ethics committee.

Following the transport period, all animals were euthanized by jugular injection with 10 mg/kg of 3% sodium pentobarbital either in the truck or in the animal house. The fundi of the stomachs were fixed in paraformaldehyde for histopathological analysis. Tissue samples to be used for subsequent evaluation of Hsp expression were placed in 1.5 ml tubes and frozen in liquid nitrogen.

RESULTS

Histopathological analysis

After 1 h of transportation, a few chief cells were detached from the crest of the mucosal folds, and the blood capillary in the lamina propria of the mucous membrane was filled with red blood cells (Fig. 1b). A similar lesion was also present in the stomach from a pig transported for 2 h (Fig. 1c). However, acute exudation was observed in the mucous lamina propria and submucosa of the stomach of all pigs transported 2 h or longer. After 4 h of transportation, no additional serious lesions were observed in the lamina propria of the mucous membrane of stomach (Fig. 1d).

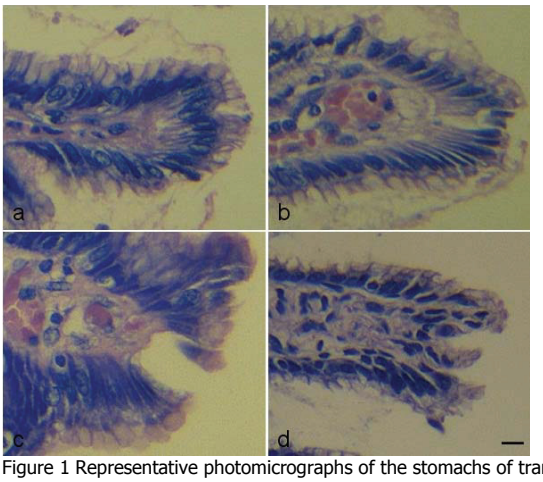

Figure 1 Representative photomicrographs of the stomachs of transported pigs

Quantitation of the levels of Hsps in the stomachs of transported pigs

As shown in Figure 2a, Hsp90 levels increased slightly after 1 h of transportation (P>0.05), followed by a decrease at 2 h of transportation (P<0.01). Hsp90 levels returned to normal levels by 4 h of transportation. In contrast, Hsp70 levels were significantly increased after 1, 2, and 4 h of transportation (P<0.05) (Figure 2b). The trend for changes in the levels of Hsp27 was similar to that of Hsp90 in transported pigs. However, the differences at the different time points were not statistically significant (P>0.05) (Figure 2c). After a reduction in expression (P>0.05) after 1 h of transportation, the levels of αB-crystallin increased persistently after 2 h (P>0.05) and 4 h (P<0.05) of transportation (Figure 2d). However, the levels of HSF remained stable after 1 and 2 h of transportation, and only decreased slightly (P>0.05) after 4 h of transportation (Figure 3).

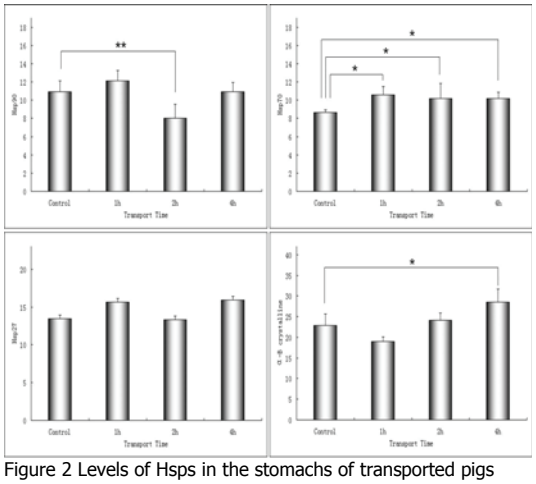

Figure 2 Levels of Hsps in the stomachs of transported pigs

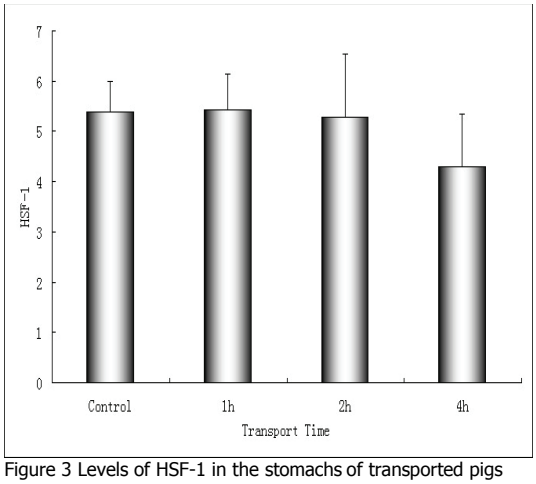

Figure 3 Levels of HSF-1 in the stomachs of transported pigs

DISCUSSION

Transportation, environmental changes and crowding were potentially stressful events or conditions that were associated with increased occurrence of par esophageal ulceration [5]. Although gastric ulcers were not observed in the present study, damages to the gastric mucous membrane were evident in transported pigs even at early time points, characterized by mucosal shedding, congestion in the lamina propria, and edema in the sub-mucosa of the stomach. These pathological changes can damage the integrity of the gastric mucous membrane and can cause corrosion to the deep mucous tissues, which may ultimately give rise to gastric ulcers.

One possible mechanism that could underlie the changes in levels of Hsp90 in our present study is that cells express Hsp90 after transport-related stress. However, under stressful conditions, consumption of Hsp90 may also increase, stimulating additional synthesis of Hsp90. It is clear that we did not observe obvious gastric ulcer during 4 h transportation. Whether the pathological changes observed in the gastric mucosa are directly or indirectly related to reduction in Hsp90 expression requires further study.

Hsp70 in gastric mucosal cells is very sensitive to transport-related stresses. The increase in levels of Hsp70 appears to correlate with its protective function in maintaining the integrity of the gastric mucosa and in reducing the number of lesions in the tissue. Hsp27 expression is critical for cytoprotection of the stomach mucosal membranes [4]. However, our results showed that levels of Hsp27 did not change significantly in transported pigs when compared to control pigs, though a similar (non-singificant) trend was observed that

paralleled same to Hsp90 levels. In contrast, levels of αB-crystallin were persistently increased after transportation and were significantly higher than that of control pigs at 4 h of transportation. Variations in Hsp90, Hsp70, Hsp27, and αB-crystallin levels suggest that distinct protective functions are modulated by different Hsps in stomach tissues during transportation.

HSF-1, is known to serve as a major mediator of Hsp gene expression in vertebrates [10]. Our results reveal that although changes in the levels of Hsp90, Hsp70, Hsp27, and αB-crystallin differ as a result of transportation, levels of HSF-1 remain stable after 1 h and 2 h of transportation, and only decrease slightly after 4 h. This implies that the expressions of Hsps may be not only regulated by the changes of HSF-1 expression levels in pigs during transportation. Nishizawa et al. [6] reported that longer ischemic treatment before reperfusion caused more-prolonged HSF activation during reperfusion.

CONCLUSIONS

Integrity of the gastric mucosa was compromised by damage occurring during the 4 h of transportation, despite the fact that gastric ulcers were not present. Distinct protective functions are modulated by different Hsps in stomach tissues during transportation. The expression of Hsps may be regulated by factors other than changes in HSF-1 expression levels in transported pigs.

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VERIFICATION OF USABILITY OF SCORING-DESCRIPTIVE METHOD FOR EVALUATION OF HORSES' WELFARE

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SUMMARY

This investigation concerned verification of usefulness the scoring-descriptive method for evaluation of horses' welfare on the base of comparing the results of evaluation conducted with the use of prepared scoring sheet to results of evaluation conducted on a base of blood indicator , behavior acts, health condition and parameters connected with breeding conditions in the stables. The scoring– descriptive evaluation results were adequate to the results obtained on the base of chosen indicators which were demanding time – consuming and labor – consuming investigations. The elaborated method also allowed to indicate, in the evaluated stables, elements which needed improvement.

INTRODUCTION

The necessity of providing farm animals high level of welfare induces conducting the investigation developing the best as possible methods for its evaluation. Conducted investigations are concerning usefulness of many different indicators belonging to the group of criteria, which conventionally are divided into: physiological, behavioral, health and productive, and complementary (technical and technological parameters of a building, microclimate) (7). It needs to be remembered that there is no single and objective indicator on the base of which it would be possible to evaluate the given breeding system in respect of the animal welfare. Every possibly used indicator from the group, concerned separately, may not be authoritative and may conduct to erroneous conclusion concerning welfare under determined conditions and management (3). Furthermore gaining data concerning many of these indicators demand expensive and labor – consuming investigation in different disciplines, therefore in practice they are useless for quick evaluation of livestock farm conditions (3, 6). Therefore in some of the countries the effort has been undertaken to elaborate the scoring - descriptive method, assuming that proper selection of criteria, conveying the complex interactions of different aspects of animal breeding, gives the possibility of quick, objective evaluation of welfare (1, 6). Results of the investigation revealed usefulness of scoring – based evaluation methods for the evaluation of cattle, swine, laying hens welfare (1,7). In the available literature there is only one publication concerning elaborating point horse welfare scoring - based evaluation method (4). The aim of this work was the comparison of the results from evaluation of horse welfare using the scoring – descriptive method elaborated by Bursztynowicz (5), to results received from the evaluation of welfare on the base of parameters concerning the breeding conditions in stables (microclimate, area-cubature indicators), chosen blood indicators, behavioral acts, and horses' health. The model method for the elaborated scoring – descriptive method was ANI 200 (8) method.

MATERIAL AND METHODS

The study was carried out during a 5 month time in autumn – winter season (November – March) in 3 stables, keeping horses on a straw bedding. Stable 1 is a building with a garret, with stall – boxes for 47 horses (every stall – box measures 7.80 m^2), with no access to paddocks. Stable 2 also has a garret, stall – boxes for 26 horses (every stall – box measures 11.05 m^2), with no access to paddocks. However stable 3 was a building without garret, there were 12 horses (every stall – box measures 12.6 m^2) with the access to paddocks. On the base of elaborated scoring sheets there were evaluated 46 parameters framed in 7 functional ranges: 1. moving conditions, 2. eating and drinking conditions, 3. social contacts conditions, 4. resting and comfort of living conditions, 5. functionality of a building, 6. zoohiginic conditions 7. health prevention and care conditions. In the same stables during a 5 month period the measurement of indicators (microclimatic (temperature, relative humidity), general number of mesophile bacterium, dust pollution, carbon dioxide and ammonia concentration, fotoclimate) and chosen blood indicators (general protein and its fractions, haptoglobin) were conducted. The evaluation of behavioral acts, and health condition of horses was also conducted.

RESULTS

Table 1. Results of the evaluation of stables using scoring – descriptive based evaluation method

Specification	Final points		
	Stable1	Stable 2	Stable 3
Range I – moving conditions	2.28	2.45	4.17
Range II – eating and drinking conditions	2.87	3.12	4.37
Range III –social contacts conditions	0.67	0.89	4.75
Range IV –resting and comfort of living conditions	0.29	1.10	4.37
Range V – functionality of a building	2.00	2.08	4.66
Range VI –zoohiginic conditions	2.25	3.15	4.20
Range VII –health prevention and care conditions	3.00	3.25	4.75
	13.36	16.04	31.38
	1.91	**2.29**	**4.48**
Level of welfare *	very low	reduced	high

Grading scale of the level of welfare: 1.00 – 1.99 points – very low level of welfare, 2.00 – 2.99 points – reduced level of welfare, 3.00 – 3.99 points – satisfactory level of welfare, 4.00 – 4.99 points – high level of welfare, 5 points – very high level of welfare.

DISCUSSION

Assessed stables received different point grade (Table 1), which indicates that there were provided distinct welfare conditions: stable 3 – 4.48 points (high level of welfare), stable 2 – 2.29 points (reduced level of welfare) stable 1 – 1.99 points (very low level of welfare). The evaluation of a single stable lasted approximately 1 hour. Comparing welfare estimation based on a 5 month lasting investigation involving blood indicators, behavioral acts, diseases and disorders, as well microclimatic conditions to scoring – descriptive method it was revealed that:

- Horses kept under conditions of very low and reduced welfare (stable 1 and 2) were characterized by significantly lower values of general protein (6.55 and 6.51 g/dl) and albumin (50.46 and 51.23%) as well as by higher values of haptoglobin (0.17 and 0.13 g/l) and alpha 2 – globulin (8.49 and 8.10 %) in blood serum comparing to horses kept under high welfare conditions (stable 3) (6.88 g/dl, 53.25%, 0.11 g/l, 7.84 %).

- In stable 3 only 4 (kicking front leg the crib, biting the stall box grille, gnawing away wood elements, oats spillage) from 11 undesirable behaviours appeared, and their intensity was significantly lower than with horses from the rest of the stables. In stables 1 and 2 all specified undesirable behaviours appeared (waving, cribiting, kicking the crib with front leg, kicking wall with hind legs, aggression towards people, aggression towards other horses, biting the stall – box grille, gnawing away wood elements, spillage of oats, excessive timidity, apathy or depression). Therefore results received with scoring method of stables 1 and 2 not entirely confirm intensity of undesirable behaviours, because in stable 2, with the higher point grade (2.29 points), in couple of cases the intensity was significantly higher than in stable 1 (1.99 point). Presumably, this pattern of behavior in this case could influence the fact that horses were exposed to a higher stress indicators such as demanding owners.

- Point evaluation confirmed results considering contribution of horses diseases and disorders during the whole period of investigation. In stable 3 providing high level of welfare lesions which reason could be improper zoohigenic conditions (respiratory and deraml diseases) did not apper. In stable 1 (very low welfare), the highest amount of respiratory diseases (25.53%) and dermal diseases (mostly mycosis) (38.29%) and mechanical injuries (12.76%) was stated. Less cases of respiratory diseases (7.60%) and dermal diseases (11.53%) appeared in stable 2, with slightly higher grading (2.29 point).

- Point evaluation of stables also reflects results of microclimatic condition indicators. More favorable microclimatic conditions in stable 3, comparing to stable 1 and 2, were confirmed by the point evaluation (4.48 points). The least favorable, especially according to air gas pollution (NH_3 – 20.14 ppm, CO_2 – 3061 ppm), dust pollution (5.23 mg/m^3) and microbiological (1150000 cfu/m^3) of air has appeared in stable 1 (1.99 point). In stable 2 (2.29 points) thermal – humidity conditions were close to conditions occurring in stable 1, but the air pollution was significantly lower.

CONCLUSIONS

Elaborated scoring – descriptive based evaluation method enables complex, quick, easy performed estimation of recreational horses' welfare. The method may be also useful in indicating the elements, which need improvement in investigated farms. Test of verification of the method usefulness conducted on 3 stables requires more tests on larger amount of buildings, furthermore it also needs a discussion about selection of evaluated criteria and their estimation.

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HOW CHICKEN'S LIVER CAN RESPOND TO CCL_4 HEPATO TOXIC EFFECT? (Abstract)

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ABSTRACT

The main objective of this work was to investigate how CCL_4 can affect chicken liver morphology and function in comparison to rat liver response to CCL_4,this was performed through biochemical and histopathological means of investigations.

The experimental design consisted of 3 experimental groups, treatments as follows:(T1) Represented as control + ve (rat treated group) received CCL_4 1ml/kg bwt ,(T2) Represented as adult chickens (hens) received CCL_4 1ml/kgbwt ,(T3) young chicks (8 day old) received CCL_4 1ml/kgbwt,after 24hours from ip injection of CCL_4 in all treated groups plasma samples were collected from 5 treated animals from each group for biochemical studies (ALT, AST, ALP, LDH,GGT ,Total protein, Albumin) then the animals were sacrificed and spicemens from liver were obtained.

Results obtained could be summarized as follows ; the CCL_4 administered dose was able to produce significant liver damage in rat liver this was reflected by marked elevation in liver enzymes and significant decrease of total proteins and albumin level this was confirmed by histo pathological examination which showed centrolobular necrosis .On the other hand adult chickens also had elevated enzymes , significant decrease of total proteins and albumin level and also had histopathological alteration while young chicks showed complete resistant pictures through normal enzyme levels and intact liver tissue with histopathological examination.

SURVIVAL OF PATHOGENIC BACTERIA IN DIFFERENT ANAEROBIC TREATMENT PROCESSES

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SUMMARY

Organic material like sewage sludge, manure and biowaste is known to contain pathogenic bacteria and other micro-organism that may turn out to be a health risk for humans and animals. The survival and reduction of pathogens is dependent on the biogas process treatment conditions e.g. temperature and time. The aim of this study was to investigate the effect of processing conditions of three different full-scale biogas plants to reduce pathogens.

INTRODUCTION

Recycling of organic waste and the development of biogas technology has increased the use of digestates as fertilisers. The producers, users and authorities need information on the quality and safety of these fertilisers. This is especially important in the case of fertilisers which are spread on fields. It is known that organic waste may contain pathogenic bacteria depending on the source and type of waste. Especially waste of animal or human origin can contain various pathogenic bacteria, parasites and viruses (Carrington 2001). Pathogenic species that are regularly present in biowaste and sewage sludge are bacteria such as *Salmonella* spp, *Listeria monocytogenes*, *E. coli*, *Campylobacter* spp, *Mycobacterium* spp. and *Clostridium* spp. (Sahlström 2003). In order to reduce pathogen risk, waste material has to be treated before use as fertiliser. Pasteurisation of biowaste at 70˚C for 1 hour is an effective way of heat treatment to reduce most pathogens (Bagge et. al. 2005).

MATERIAL AND METHODS

Samples were taken from three biogas plants processing different waste materials. All biogas plants used mesophilic anaerobic digestion. Pasteurisation was arranged in three different ways. Plants A and B had separate pasteurisation units before digestion, whereas plant C used a continuos heat drying process of 80˚C for 60 min after digestion. At plant A pasteurisation was done at 70˚C for 60 min and at plant B at 150˚C for 30 min at 5 bar. Samples were collected three times during the year from input materials and from various steps of the process, in order to get an indication of the treatment effect and the quality of the digestate that is aimed as organic fertiliser. Sample pathogens (*Salmonella* spp.) and indicator bacteria content (e. g. *Enterococcus* spp, coliforms and sulphite-reducing *Clostridia*) were analyzed by standard methods.

RESULTS AND DISCUSSION

The results showed that pasteurisation reduced the amount of indicator bacteria to undetectable levels except spore-forming bacteria (sulphite-reducing *Clostridia*) at all plants. *Salmonella* spp. was detected from raw material before treatment in two different plants and even after mesophilic digestion from one biogas plant treating e.g sewage sludge. *Salmonella* spp. was not detected at any plant after pasteurisation. Growth of spore-forming bacteria was reduced 3 $\log_{10}$ after the pasteurisation unit at plant B and inactivated after the heat drying process at plant C. At plant A, pasteurisation at 70˚C for 60 min did not have any effect on spore-forming bacteria. These results are in accordance with previous studies, which showed that pasteurisation at 70˚C does not reduce spore-forming bacteria (Bagge et. al. 2005). Instead thermal drying process seems to be an efficient method to destroy also spore-forming bacteria. Moisture loss during a drying process in high temperature has proved to inactivate many heat resistance pathogens (Romdhana et al. 2009).

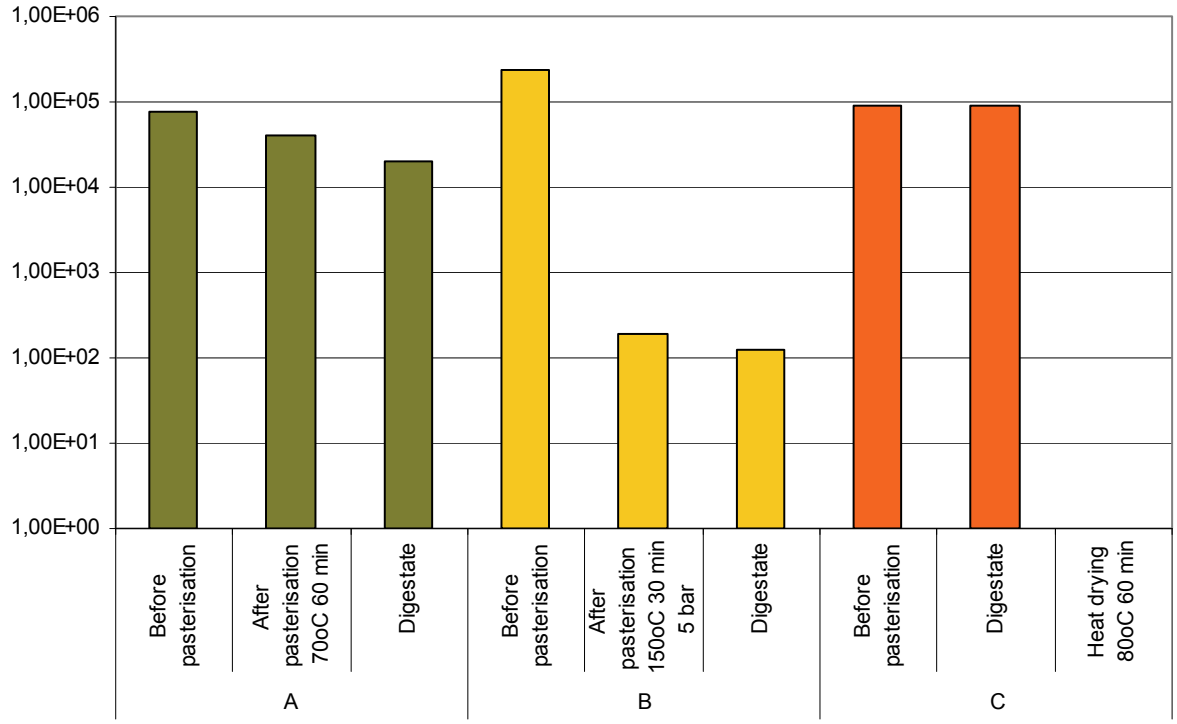

Figure 1. Medium concentration content of sulphite-reducing *Clostridia* after pasteurisation in biogas plants.

CONCLUSIONS

Results of this study indicate that it is possible to treat organic material in different types of processes and still achieve an acceptable hygienic level according to regulations criteria. However, the hygienic level of the end product is different depending on the process. Therefore when choosing a treatment process, it is also critical to consider the type of treated organic material, since the pathogen load differs between different types of materials. According to this study, the heat drying process seems to be efficient in eliminating also heat tolerant pathogens like spore-forming bacteria.

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THE FATTY ACID COMPOSITION OF SHEEP OFFAL DERIVED FROM TWO BREEDS

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SUMMARY

This paper describes the fatty acid composition of the cooked organs (brain, tongue, rumen, testicles, liver, heart, lungs, spleen, kidneys) from two sheep breeds (Dorper and Merino). Very few differences were noted in total SFA and MUFA between organs and breed. The majority of the organs had a SFA content of ~50%. Although the brain had a ~10% lipid content, the SFA content was lower (~45%) and the PUFA higher (~22%) than that of the other organs. The (n-6)/(n-3) ratio varied between the organs from the brain having a ratio of 0.43 to the kidney having a ratio of 11.44. The lungs and testicles also had favourable n-6/n-3 ratios – all below 5. Breed had little effect on the SFA or MUFA and only a few organs differed in their PUFA as pertaining to breed. Merino heart had significantly higher (7.27%) total PUFA than Dorper heart (1.78%).

INTRODUCTION

The production of lamb and mutton is an important economic activity in South Africa where there are ±29 million sheep. A by-product of the slaughter of sheep is the organs or offal. The yield of these edible by-products varies between species, gender, live weight, carcass fatness and slaughter process. For lamb it has been noted to vary between 10 to 30% [5]. For South African breeds, the yield of various organs differed between breed (eg South African Mutton Merino versus Dormer) although the effect of gender was more pronounced than that of breed [1]. Whereas the kidney is normally sold as part of the whole carcass, the other organs are predominately sold into the informal market. Surprisingly, very little knowledge exists around the nutritional and specific the fatty acid composition of these organs.

MATERIAL AND METHODS

Twelve similar aged free ranging lambs from two breeds (Dorper and Merino) were slaughtered and their organs (brain, tongue, rumen, testicles, liver, heart, lungs, spleen, kidneys) harvested, packed into individual vacuum bags and frozen prior to being transported to the laboratory for analyses. For analyses, the organs were defrosted, weighed and cooked inside a plastic bag within a water bath set at 60˚C for 60 minutes. Thereafter the whole bag contents were mixed, homogenised and analysed for fatty acid composition. A 2 g meat sample was extracted with a chloroform:methanol (2:1; v/v) solution [2]. All the extraction solvents contained 0.01% butylated hydroxytoluene (BHT) as an antioxidant. A polytron mixer (WiggenHauser, D-500 Homogenizer) was used to homogenise the sample with the extraction solvent. Heptadecanoic acid (C17:0) was used as an internal standard (catalogue number H3500, Sigma–Aldrich Inc., 3050 Spruce Street, St. Louis, MO 63103, USA) to quantify the individual fatty acids. A sub-sample of the extracted lipids was transmethylated for 2 h at 70 °C using a methanol/sulphuric acid (19:1; v/v) solution as transmethylating agent. After cooling to room temperature, the resulting fatty acid methyl esters (FAMEs) were extracted with water and hexane. The top hexane phase was transferred to a spotting tube and dried under nitrogen.

Analysis was done on a Thermo Focus GC equipped with a flame ionized detector using a BPX70 capillary column (60 m x 0.25 mm internal diameter, 0.25 µm film, SGE, Australia). Gas flow rates were 25 ml/min for hydrogen and 2–4 ml/min for the hydrogen carrier gas. Temperature programming was linear at 3.4 °C/min, with an initial temperature of 140 °C, a final temperature of 240 °C, an injector temperature of 225 °C and a detector temperature of 300 °C. The FAMEs were identified by comparing the retention times to those of a standard FAME mixture (Supelco™ 37 Component FAME Mix, 10 mg/ml in CH_2Cl_2, Catalogue Number 47885-U. Supelco, North Harrison Road, Bellefonte, PA 16823-0048, USA).

The data was analysed using an analysis of covariance which included breed (whether the sheep Dorper or Merino) and organ as main effects. This was done using the GLM model of Statistica (SAS, version 8.2) statistical software (SAS, 2002). Differences within the main effects were accepted as being significant if the probability of rejection of H_o was less than 5 % ($P < 0.05$).

RESULTS

The fatty acid profiles of the different organs are shown in table 1. With the exception of the Dorper brain, heart and kidney, no differences between organs were found for total saturated fatty acids (SFA) or total monounsaturated fatty acids (MUFA). Total polyunsaturated fatty acids (PUFA) differed between organs with the tongue having the lowest level of PUFA. Total PUFA levels ranged between organs from 2.22% to 24.01%. No differences were found between breeds for total SFA or total MUFA levels. However, total PUFA levels differed between breeds for the heart with Merino hearts having a significantly higher PUFA level (7.27%) than Dorper hearts (1.78%). The tongue was also found to have significantly lower levels of PUFA compared to the rest of the organs with Merino tongues having a PUFA value of 3.65% and Dorper tongues having a PUFA value of 2.22%.

DISCUSSION

Saturated fatty acids have long been associated with obesity, and related to a corresponding risk of the metabolic syndrome (insulin resistance), type 2 diabetes and cardiovascular disease [6]. Generally, the percentage of saturated fatty acids in the organs assessed where relatively high, and the meat cuts should be considered as high in saturated fats. The saturated fat component of total fat was found to consist of 2 main fatty acids – namely palmitic acid (C16:0) and stearic acid (C18:0). The longer chain stearic acid has been associated with favourably reducing the TC:HDL ratio when replacing carbohydrates in the diet [4]. It could be argued that the saturated fats in the offal may at the very least have little impact on serum lipids directly if consumed in the place of excess dietary carbohydrates, as the palmitic and stearic acids may counteract each other. Unsaturated fatty acids are widely considered to have a more beneficial biological effect in humans, with many arguing in favour of replacing saturated fats with unsaturated fats, rather than carbohydrates, to cause a favourable change in serum lipid profiles [3]. However, there is still debate over whether monounsaturated (MUFA's) or polyunsaturated (PUFA's) fats are a more beneficial option. This study indicates that the organ meat generally has a higher amount of MUFA's compared to PUFA's. Sheep brain and sheep testicles may be considered to have nutritional benefits for secondary prevention of heart disease based on the PUFA concentrations.

Significant differences were found for most of the fatty acids between organs. Differences were also found between breeds with Dorper hearts having lower levels of the polyunsaturated C18:2, C18:3 and C20:3 fatty acids than Merino hearts. Similarly, Merino livers had lower levels of the polyunsaturated C20:2, C20:4 and C20:5 fatty acids. Generally, differences between breeds were only in two or three fatty acids per organ, with the exception of the heart, liver and stomach.

CONCLUSIONS

The differences in fatty acid composition between the various organs would indicate that care should be taken when consumers decide which organs to consume, especially if they are in the high CVD risk group. From these findings, it is evident that although it is known that the nutritional composition of organs differs, there are also significant differences as a result of breed.

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Table 1: Fatty acid composition (%) and cholesterol content (mg/100g) of Dorper and Merino organs

Breed	Fatty Acid	Brain	Heart	Kidney	Liver	Lung	Spleen	Stomach	Testicle	Tongue
Saturated fatty acids										
Dorper	C14:0	$0.26^{b} \pm 0.06$	$1.65^{ab} \pm 0.52$	$0.83^{b} \pm 0.14$	$0.56^{b} \pm 0.24$	$0.63^{b} \pm 0.12$	$1.05^{ab} \pm 0.22$	$0.44^{b}{}_{b} \pm 0.21$	$1.71^{ab} \pm 0.63$	$3.60^{a} \pm 0.97$
	C15:0	0.22 ± 0.09	0.36 ± 0.10	0.41 ± 0.06	0.36 ± 0.07	0.51 ± 0.07	0.45 ± 0.06	$0.37_{b} \pm 0.09$	0.48 ± 0.10	0.74 ± 0.19
	C16:0	$18.13^{b} \pm 1.08$	$31.49^{a} \pm 3.55$	$23.84^{ab}{}_{a} \pm 1.23$	$20.36^{ab} \pm 4.32$	$30.69^{a} \pm 1.36$	$23.54^{ab} \pm 1.75$	$20.22^{ab} \pm 1.46$	$31.55^{a} \pm 2.93$	$30.58^{a} \pm 5.89$
	C18:0	$26.96^{ab} \pm 2.13$	$36.29^{a} \pm 4.01$	$19.70^{a} \pm 2.78$	$26.65^{ab} \pm 3.55$	$17.78^{b} \pm 0.76$	$26.85^{ab} \pm 1.17$	$28.02^{ab} \pm 1.82$	$15.45^{b}{}_{b} \pm 0.76$	$16.63^{b} \pm 6.21$
	C20:0	ND^{b}	ND^{b}	$0.20^{ab} \pm 0.08$	ND^{b}	$0.17^{ab} \pm 0.04$	$0.17^{ab} \pm 0.07$	$0.18^{ab} \pm 0.03$	$0.22^{a} \pm 0.03$	$0.10^{ab} \pm 0.03$
	C21:0	$0.12^{bcd} \pm 0.02$	$0.07^{cd} \pm 0.14$	$0.15^{abcd} \pm 0.03$	$0.18^{abc}{}_{a} \pm 0.01$	$0.25^{a} \pm 0.03$	$0.14^{abcd} \pm 0.08$	$0.23^{ab} \pm 0.03$	$0.14^{abcd} \pm 0.03$	$0.06^{d} \pm 0.02$
	C22:0	$0.97^{bcde} \pm 0.17$	$0.05^{e} \pm 0.11$	$0.62^{bcde} \pm 0.10$	$1.72^{b} \pm 0.28$	$1.28^{bc} \pm 0.14$	$1.22^{bcd} \pm 0.13$	$0.45^{cde} \pm 0.07$	$2.83^{a} \pm 0.55$	$0.12^{de} \pm 0.03$
	C24:0	$0.14^{cd} \pm 0.03$	$0.04^{d}{}_{b} \pm 0.01$	$0.78^{ab} \pm 0.17$	$1.12^{a} \pm 0.20$	$0.58^{bc} \pm 0.09$	$0.51^{bcd} \pm 0.07$	$0.25^{cd} \pm 0.04$	$0.14^{cd} \pm 0.02$	$0.07^{d} \pm 0.02$
Monounsaturated fatty acids										
	C16:1	$0.33^{c} \pm 0.04$	$0.48^{bc} \pm 0.14$	$1.03^{bc} \pm 0.26$	$0.80^{abc} \pm 0.09$	$1.07^{ab}{}_{a} \pm 0.10$	$0.73^{abc} \pm 0.08$	$0.81^{abc}{}_{b} \pm 0.15$	$1.37^{a}{}_{a} \pm 0.13$	$1.35^{a} \pm 0.43$
	C18:1n9c	25.22 ± 1.41	26.16 ± 5.46	28.68 ± 3.68	22.88 ± 6.19	24.94 ± 0.66	26.26 ± 2.10	36.21 ± 1.98	28.59 ± 2.36	41.99 ± 13.29
	C18:1n9t	$0.15^{b}{}_{b} \pm 0.02$	$1.35^{ab} \pm 0.80$	$1.81^{ab} \pm 0.40$	$0.73^{b} \pm 0.18$	$2.00^{ab} \pm 0.42$	$2.60^{ab} \pm 0.33$	$3.78^{ab}{}_{a} \pm 0.58$	$1.94^{ab} \pm 0.19$	$2.17^{ab} \pm 0.79$
	C20:1	$0.24^{b} \pm 0.03$	$0.07^{c}{}_{b} \pm 0.03$	$0.14^{bc} \pm 0.02$	$0.07^{c} \pm 0.01$	$0.52^{a} \pm 0.04$	$0.16^{bc} \pm 0.02$	$0.23^{b}{}_{a} \pm 0.02$	$0.12^{bc} \pm 0.01$	$0.08^{c} \pm 0.03$
	C22:1n9	$0.04^{ab} \pm 0.01$	$0.01^{b} \pm 0.01$	$0.10^{a} \pm 0.02$	$0.08^{ab} \pm 0.01$	$0.10^{a} \pm 0.01$	$0.09^{ab} \pm 0.03$	$0.11^{a} \pm 0.02$	$0.09^{a} \pm 0.02$	$0.05^{ab} \pm 0.02$
	C24:1	$4.05^{a} \pm 0.47$	$0.02^{c} \pm 0.03$	$0.30^{bc} \pm 0.06$	$0.21^{bc} \pm 0.03$	$1.09^{b} \pm 0.18$	$0.97^{bc}{}_{a} \pm 0.14$	$0.23^{bc} \pm 0.25$	$0.32^{bc} \pm 0.23$	$0.04^{c} \pm 0.02$
Polyunsaturated fatty acids										
	C18:2n6c	$0.72^{c} \pm 0.10$	$1.14^{c}{}_{b} \pm 0.70$	$12.58^{a} \pm 0.98$	$12.83^{a} \pm 2.13$	$6.62^{b} \pm 0.62$	$5.49^{bc} \pm 0.90$	$4.88^{bc} \pm 0.60$	$3.10^{bc} \pm 0.36$	$1.33^{c} \pm 1.11$
	C18:2n6t	$0.09^{b} \pm 0.02$	$0.18^{b}{}_{b} \pm 0.07$	$0.21^{ab} \pm 0.05$	$0.26^{ab} \pm 0.05$	$0.26^{ab} \pm 0.02$	$0.32^{ab} \pm 0.02$	$0.42^{a} \pm 0.05$	$0.24^{ab} \pm 0.02$	$0.21^{ab} \pm 0.10$
	C18:3n6	$0.04^{b} \pm 0.02$	$0.18^{b}{}_{b} \pm 0.12$	$1.53^{a} \pm 0.39$	$1.35^{a} \pm 0.34$	$0.26^{b}{}_{b} \pm 0.03$	$0.41^{b}{}_{b} \pm 0.08$	$0.76^{ab}{}_{b} \pm 0.09$	$0.35^{b} \pm 0.07$	$0.35^{b} \pm 0.08$
	C18:3n3	$2.45^{a} \pm 0.26$	$0.04^{b}{}_{b} \pm 0.01$	$0.21^{b} \pm 0.03$	$0.16^{b} \pm 0.01$	$0.48^{b} \pm 0.04$	$0.36^{b} \pm 0.03$	$0.23^{b} \pm 0.03$	$0.22^{b} \pm 0.02$	$0.09^{b} \pm 0.01$
	C20:2	$0.15^{bcd} \pm 0.03$	$0.02^{d} \pm 0.05$	$0.21^{ab} \pm 0.03$	$0.18^{abc}{}_{a} \pm 0.02$	$0.30^{a} \pm 0.03$	$0.30^{a} \pm 0.05$	$0.12^{bcd} \pm 0.02$	$0.23^{ab} \pm 0.03$	$0.05^{cd} \pm 0.02$
	C20:3n6	$5.19^{a} \pm 0.92$	$0.19^{c}{}_{b} \pm 0.12$	$4.91^{ab} \pm 0.93$	$5.62^{a} \pm 1.09$	$6.64^{a}{}_{b} \pm 0.94$	$6.61^{a} \pm 0.72$	$1.28^{bc} \pm 0.22$	$4.08^{ab} \pm 0.80$	$0.15^{c} \pm 0.04$
	C20:3n3	$0.50^{b} \pm 0.20$	$0.03^{d} \pm 0.01$	$0.33^{bc} \pm 0.06$	$0.13^{cd} \pm 0.01$	$0.92^{a} \pm 0.06$	$0.25^{cd} \pm 0.04$	$0.15^{cd} \pm 0.03$	$0.12^{cd} \pm 0.02$	$0.04^{d} \pm 0.02$
	C20:4n6	$0.80^{a} \pm 0.09$	$0.02^{c} \pm 0.04$	$0.08^{bc} \pm 0.02$	$0.11^{bc}{}_{a} \pm 0.01$	$0.24^{b} \pm 0.04$	$0.14^{bc} \pm 0.03$	$0.11^{bc} \pm 0.02$	$0.16^{bc} \pm 0.02$	$0.05^{bc} \pm 0.02$
	C20:5n3	$2.49^{a} \pm 0.32$	$0.03^{c} \pm 0.01$	$0.36^{bc} \pm 0.06$	$0.38^{bc}{}_{a} \pm 0.04$	$0.97^{b} \pm 0.10$	$0.33^{bc} \pm 0.04$	$0.11^{c} \pm 0.03$	$0.20^{c} \pm 0.04$	$0.04^{c} \pm 0.02$
	C22:2	$0.42^{bc} \pm 0.10$	$0.05^{d} \pm 0.02$	$0.25^{cd} \pm 0.04$	$0.59^{ab} \pm 0.07$	$0.71^{a} \pm 0.05$	$0.30^{cd} \pm 0.05$	$0.15^{d} \pm 0.03$	$0.09^{d} \pm 0.01$	$0.05^{d} \pm 0.02$
	C22:6n3	$10.15^{a} \pm 1.71$	$0.07^{c} \pm 0.04$	$0.77^{c} \pm 0.19$	$2.68^{bc} \pm 0.61$	$0.99^{c} \pm 0.15$	$0.76^{c} \pm 0.13$	$0.25^{c} \pm 0.05$	$6.27^{b} \pm 1.29$	$0.07^{c} \pm 0.03$
Dorper	SFA	$46.81^{b} \pm 2.69$	$69.95^{a} \pm 5.28$	$46.52^{b} \pm 2.77$	$50.95^{ab} \pm 7.37$	$51.88^{ab} \pm 1.38$	$53.93^{ab} \pm 2.04$	$50.15^{ab} \pm 2.47$	$52.52^{ab} \pm 3.52$	$51.90^{ab} \pm 12.52$
	MUFA	29.89 ± 1.25	26.75 ± 5.37	30.24 ± 3.79	24.05 ± 6.12	27.72 ± 0.71	28.21 ± 2.04	37.60 ± 2.09	30.49 ± 2.43	43.51 ± 12.97
	PUFA	$22.90^{a} \pm 3.18$	$1.78^{c}{}_{b} \pm 0.80$	$21.22^{a} \pm 0.86$	$24.01^{a} \pm 3.86$	$18.14^{ab} \pm 1.58$	$14.94^{ab} \pm 1.58$	$8.04^{bc} \pm 0.89$	$14.82^{ab} \pm 2.44$	$2.22^{c} \pm 1.14$

Table 1 (cont): Fatty acid composition (%) and cholesterol content (mg/100g) of Dorper and Merino organs

Breed	Fatty Acid	Brain	Heart	Kidney	Liver	Lung	Spleen	Stomach	Testicle	Tongue
Saturated fatty acids										
Merino	C14:0	$0.22^{b} \pm 0.06$	$2.16^{a} \pm 0.52$	$0.61^{ab} \pm 0.14$	$0.77^{ab} \pm 0.24$	$0.47^{b} \pm 0.15$	$1.03^{ab} \pm 0.22$	$1.28^{ab}{}_{a} \pm 0.19$	$0.47^{b} \pm 0.78$	$1.64^{ab} \pm 0.97$
	C15:0	$0.20^{c} \pm 0.09$	$0.66^{ab} \pm 0.10$	$0.38^{abc} \pm 0.06$	$0.36^{abc} \pm 0.07$	$0.56^{abc} \pm 0.08$	$0.46^{abc} \pm 0.06$	$0.77^{a}{}_{a} \pm 0.09$	$0.27^{bc} \pm 0.12$	$0.37^{abc} \pm 0.19$
	C16:0	17.58 ± 1.08	30.67 ± 3.55	$19.93_{b} \pm 1.23$	20.83 ± 4.32	25.80 ± 1.67	22.25 ± 1.75	23.49 ± 1.33	24.91 ± 3.59	22.06 ± 5.89
	C18:0	26.23 ± 2.13	33.98 ± 4.01	22.66 ± 2.78	23.50 ± 3.55	18.18 ± 0.93	26.68 ± 1.17	25.22 ± 1.66	$18.80_{a} \pm 0.94$	20.21 ± 6.21
	C20:0	ND^{b}	ND^{b}	$0.18^{ab} \pm 0.08$	ND^{b}	$0.24^{a} \pm 0.05$	$0.26^{a} \pm 0.07$	$0.15^{ab} \pm 0.03$	$0.18^{ab} \pm 0.03$	$0.07^{ab} \pm 0.03$
	C21:0	0.19 ± 0.02	0.28 ± 0.14	0.15 ± 0.03	$0.11_{b} \pm 0.01$	0.27 ± 0.03	0.26 ± 0.08	0.16 ± 0.02	0.14 ± 0.03	0.07 ± 0.02
	C22:0	$0.97^{bc} \pm 0.17$	$0.36^{bc} \pm 0.11$	$0.60^{bc} \pm 0.10$	$0.86^{bc} \pm 0.28$	$1.42^{b} \pm 0.17$	$0.85^{bc} \pm 0.13$	$0.31^{c} \pm 0.06$	$3.23^{a} \pm 0.67$	$0.07^{c} \pm 0.03$
	C24:0	$0.13^{bc} \pm 0.03$	$0.14^{bc}{}_{a} \pm 0.01$	$0.86^{a} \pm 0.17$	$0.73^{a} \pm 0.20$	$0.74^{a} \pm 0.10$	$0.55^{ab} \pm 0.07$	$0.16^{bc} \pm 0.03$	$0.16^{bc} \pm 0.03$	$0.04^{c} \pm 0.02$
Monounsaturated fatty acids										
	C16:1	0.36 ± 0.04	0.55 ± 0.14	0.88 ± 0.26	0.82 ± 0.09	$0.54_{b} \pm 0.12$	0.74 ± 0.08	$1.30_{a} \pm 0.13$	$0.87_{b} \pm 0.16$	1.32 ± 0.43
	C18:1n9c	26.86 ± 1.41	20.22 ± 5.46	30.26 ± 3.68	31.86 ± 6.19	26.05 ± 0.80	28.96 ± 2.10	36.90 ± 1.81	29.74 ± 2.89	49.12 ± 13.29
	C18:1n9t	$0.28^{d}{}_{a} \pm 0.02$	$2.80^{a} \pm 0.80$	$2.32^{ab} \pm 0.40$	$0.73^{cd} \pm 0.18$	$1.87^{abc} \pm 0.51$	$2.15^{abc} \pm 0.33$	$0.83^{cd}{}_{b} \pm 0.53$	$1.71^{abcd} \pm 0.24$	$0.98^{cd} \pm 0.79$
	C20:1	$0.28^{b} \pm 0.03$	$0.17^{bcd}{}_{a} \pm 0.03$	$0.12^{cd} \pm 0.02$	$0.05^{d} \pm 0.01$	$0.51^{a} \pm 0.05$	$0.18^{bc} \pm 0.02$	$0.15^{cd}{}_{b} \pm 0.02$	$0.14^{cd} \pm 0.01$	$0.06^{cd} \pm 0.03$
	C22:1n9	$0.04^{b} \pm 0.01$	$0.05^{ab} \pm 0.01$	$0.11^{ab} \pm 0.02$	$0.08^{ab} \pm 0.01$	$0.14^{a} \pm 0.02$	$0.14^{a} \pm 0.03$	$0.06^{ab} \pm 0.02$	$0.08^{ab} \pm 0.02$	$0.03^{b} \pm 0.02$
	C24:1	$4.96^{a} \pm 0.47$	$0.10^{c} \pm 0.03$	$0.37^{c} \pm 0.06$	$0.16^{c} \pm 0.03$	$1.44^{b} \pm 0.22$	$0.54^{bc}{}_{b} \pm 0.14$	$0.44^{bc} \pm 0.22$	$0.96^{bc} \pm 0.28$	$0.04^{c} \pm 0.02$
Polyunsaturated fatty acids										
	C18:2n6c	$0.67^{b} \pm 0.10$	$4.95^{ab}{}_{a} \pm 0.70$	$9.56^{a} \pm 0.98$	$9.95^{a} \pm 2.13$	$5.63^{ab} \pm 0.76$	$5.35^{ab} \pm 0.90$	$5.38^{ab} \pm 0.55$	$3.65^{b} \pm 0.44$	$2.91^{b} \pm 1.11$
	C18:2n6t	$0.11^{b} \pm 0.02$	$0.60^{a}{}_{a} \pm 0.07$	$0.34^{ab} \pm 0.05$	$0.28^{b} \pm 0.05$	$0.26^{b} \pm 0.02$	$0.34^{ab} \pm 0.02$	$0.39^{ab} \pm 0.04$	$0.27^{b} \pm 0.02$	$0.28^{b} \pm 0.10$
	C18:3n6	$0.08^{d} \pm 0.02$	$0.78^{bcd}{}_{a} \pm 0.12$	$1.60^{ab} \pm 0.39$	$1.86^{a} \pm 0.34$	$0.39^{cd}{}_{a} \pm 0.04$	$0.75^{bcd}{}_{a} \pm 0.08$	$1.13^{abc}{}_{a} \pm 0.08$	$0.51^{cd} \pm 0.08$	$0.35^{cd} \pm 0.08$
	C18:3n3	$2.55^{a} \pm 0.26$	$0.09^{cd}{}_{a} \pm 0.01$	$0.16^{cd} \pm 0.03$	$0.12^{cd} \pm 0.01$	$0.54^{b} \pm 0.05$	$0.30^{c} \pm 0.03$	$0.18^{cd} \pm 0.03$	$0.22^{cd} \pm 0.02$	$0.06^{d} \pm 0.01$
	C20:2	$0.18^{ab} \pm 0.03$	$0.11^{ab} \pm 0.05$	$0.15^{ab} \pm 0.03$	$0.10^{ab}{}_{b} \pm 0.02$	$0.26^{a} \pm 0.04$	$0.20^{ab} \pm 0.05$	$0.08^{b} \pm 0.01$	$0.19^{ab} \pm 0.04$	$0.04^{b} \pm 0.02$
	C20:3n6	$5.08^{b} \pm 0.92$	$0.87^{cd}{}_{a} \pm 0.12$	$6.78^{ab} \pm 0.93$	$4.28^{bc} \pm 1.09$	$10.22^{a}{}_{a} \pm 1.16$	$6.22^{b} \pm 0.72$	$1.08^{cd} \pm 0.20$	$6.36^{b} \pm 0.98$	$0.12^{d} \pm 0.04$
	C20:3n3	$0.91^{a} \pm 0.20$	$0.06^{b} \pm 0.01$	$0.32^{b} \pm 0.06$	$0.10^{b} \pm 0.01$	$0.87^{a} \pm 0.08$	$0.24^{b} \pm 0.04$	$0.09^{b} \pm 0.03$	$0.14^{b} \pm 0.02$	$0.04^{b} \pm 0.02$
	C20:4n6	$0.85^{a} \pm 0.09$	$0.12^{c} \pm 0.04$	$0.10^{c} \pm 0.02$	$0.07^{c}{}_{b} \pm 0.01$	$0.39^{b} \pm 0.05$	$0.17^{c} \pm 0.03$	$0.07^{c} \pm 0.02$	$0.12^{c} \pm 0.03$	$0.02^{c} \pm 0.02$
	C20:5n3	$2.64^{a} \pm 0.32$	$0.05^{c} \pm 0.01$	$0.33^{c} \pm 0.06$	$0.20^{c}{}_{b} \pm 0.04$	$1.21^{b} \pm 0.12$	$0.33^{c} \pm 0.04$	$0.11^{c} \pm 0.03$	$0.24^{c} \pm 0.05$	$0.03^{c} \pm 0.02$
	C22:2	$0.61^{a} \pm 0.10$	$0.11^{bc} \pm 0.02$	$0.26^{bc} \pm 0.04$	$0.37^{ab} \pm 0.07$	$0.55^{a} \pm 0.06$	$0.26^{bc} \pm 0.05$	$0.08^{c} \pm 0.02$	$0.09^{c} \pm 0.02$	$0.03^{c} \pm 0.02$
	C22:6n3	$7.79^{a} \pm 1.71$	$0.13^{b} \pm 0.04$	$0.96^{b} \pm 0.19$	$1.80^{b} \pm 0.61$	$1.44^{b} \pm 0.19$	$0.80^{b} \pm 0.13$	$0.19^{b} \pm 0.04$	$6.54^{a} \pm 1.58$	$0.05^{b} \pm 0.03$
Merino	SFA	45.52 ± 2.69	68.24 ± 5.28	45.37 ± 2.77	47.16 ± 7.37	47.68 ± 1.69	52.33 ± 2.04	51.54 ± 2.25	48.16 ± 4.31	44.52 ± 12.52
	MUFA	32.51 ± 1.25	21.09 ± 5.37	31.75 ± 3.79	32.97 ± 6.12	28.68 ± 0.87	30.56 ± 2.04	38.86 ± 1.91	31.79 ± 2.98	50.57 ± 12.97
	PUFA	$21.35^{a} \pm 3.18$	$7.27^{cd}{}_{a} \pm 0.80$	$20.22^{a} \pm 0.86$	$18.87^{a} \pm 3.86$	$21.50^{a} \pm 1.93$	$14.62^{abc} \pm 1.58$	$8.38^{bcd} \pm 0.82$	$18.06^{ab} \pm 2.99$	$3.65^{d} \pm 1.14$

ND = non detected

^{abcde} means between organs, within breed, with the same superscript do not differ ($P < 0.05$); $_{ab}$ means between breed, within organs, with the same subscript do not differ ($P < 0.05$)

RUMINAL KINETICS OF CRUDE PROTEIN CORN SILAGE WITH ADDED MANURE

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SUMMARY

Nonlinear rumen parameters were estimated in cattle fed with corn silage with different levels of inclusion of dairy cattle manure: T1 (corn only), T2 (corn + 15% manure) and T3 (corn + 25% manure) with crude protein content (CP) of 7.87%, 8.79 and 8.03% respectively, at different incubation periods (36, 24, 12, 8, 4 and 2 h). The variables estimated were: dry matter degradability (DMD) with the exponential equation: $p = a + b (e^{-ct})$, where: p = rate of disappearance of nutrients in a period of time, a = intercept of the solubilized portion at the beginning of incubation (time 0), b = potentially degradable fraction in the rumen, ch^{-1} = velocity or rate of degradability of fraction b, and t = incubation time. Potential degradability was obtained (pd) = a + b and effective degradability (ed) = a + b * c / (c 0003) assuming a passage rate of 3%. There was no difference in the effective degradability (P> 0.05) obtaining 15.95[a] (T1), 15.99[a] (T2) and T3 (15.17[a]). All treatments had the same rate in the degradation rate with 3.0%. It is concluded that using manure from dairy cattle in the 15% to 25% as an ingredient in corn silage for animal feed does not affect the effective degradability and ruminal passage rate of the CP.

INTRODUCTION

Silage corn (Zea mays) is a product with high energy content and low protein content, but the sudan grass silage (Sorghum sudanense) has a lower quality than corn and less desired (FEDNA, 2004). The generation of organic waste is a global environmental problem, with almost zero handling and use. These can be broadly applied from the preparation of compost, to animal feed (Uicab-Brito and Sandoval, 2003). In the last decades there has been an increase in the use of manure from some animal species due to its high potential as feed (Smith and Wheeler 1979). The incorporation of ingredients such as molasses and animal manure for forage, improves energy and protein quality of silage (Smith and Wheeler, 1979). The feces of animals can be used by ruminants as a protein source and energy (Bhattacharya and Fontenot, 1966, Calvert and King, 1977; Cobos *et al.,* 1988). The feeding value of forage is defined as the ability to promote livestock production as a result of the availability of nutrients, as well as its consumption (Beever *et al.,* 2000). The objective was to estimate the effect of including molasses and bovine feces at different ruminal incubation periods and *in situ* digestibility of dry matter and use of maize silage and sudanese forage.

METHODOLOGY

This work was done at the zootechnical post belonging to the School of Agronomy of the Autonomous University of Sinaloa, located in the municipality of Culiacan, Sinaloa, Mexico. Six silages were evaluated (Table 1), which were developed by Archer *et al.* (1991). The samples were dried at 60 □C for 48 h, for latter analysis of chemical components such as: Crude Protein (CP), hemicellulose (HEMI), Cellular Content (CC), acid detergent fiber (ADF), neutral detergent fiber (NDF) (Goering and Van Soest, 1970 AOAC, 1975). Dry matter degradability (DMD) was estimated for different periods of incubation 48, 24, 12, 8, 6, 4 and 2 h. Subsequently, the bags were washed with running water for 5 min, until they were clean. For *in situ* degradability of dry matter (DM) we applied the formula of Schneider and Flatt (1975). We used two male crossed Cebu animals cannulated in rumen of 300 kg. For each animal and treatment we introduced and 105 plastic bags of 10x20 cm (Ankom) with 5 g of ground sample (1 mm), weighed and identified by the bag, animal and period, with a pore size of 50 ± 15 mμ, and area exposure of 18 mg $cm2^{-1}$. Nonlinear rumen parameters were estimated (NLRP), DMS and NDFD (Ørskov and McDonald, 1979) with the exponential equation: $p = a + b (e^{-ct})$, where: p = rate of disappearance of nutrients in a while a = intercept of the solubilized portion at the beginning of incubation (time 0), b = potentially degradable fraction in the rumen, c = velocity or rate of degradability of fraction b, and t = incubation time. Potential degradability was obtained (pd) = a + b and effective degradability (ed) = a + b * c / (c+ 0.003) assuming a passage rate of 3% (Ørskov and McDonald, 1979). The data were analyzed using PROC NLIN of the SAS package version 9.2 (2004).

RESULTS

The treatments received a crude protein content of 7.87%, 8.79 and 8.03% (T1, T2 and T3). Nonlinear parameters of the treatments are shown in Table 1, which shows that there was no statistical differences for any variables (P<0.05). In effective degradability (ed) ruminal crude protein was obtained at 15.95%, 15.99% and 15.17% (T1, T2 and T3). At the speed of the rate of degradation (c h^{-1}) the results were 42.55%, 42.51% and 43.33% respectively.

DISCUSSION

The addition of manure at levels of 15% CP content increased compared to the control variable, but did not affect ruminal passage rate, at the level of 25% (T3). The nutritive value of silage is estimated by analyzing their chemical content (Bogdan, 1997). The use of silage feces mixed with easily fermentable forage content increases CP but does not improve the effective degradability and degradation rate (Al-Rokaya *et al.,* 1998; Rasool *et al.,* 1998, Fontenot and Jurubescu, 1980).

CONCLUSIONS

All treatments had the same rate in the degradation rate with 3.0%. We conclude that the use of manure from dairy cattle in the 15% to 25% as an ingredient in corn silage for animal feed does not affect the effective degradability and ruminal passage rate of raw protein (CP).

Table 1: Nonlinear parameters in siturminal degradability: CP

Silage	CP				
Treatment	a	b	c (h^{-1})	dp (a+b)	de
T1	3.55	39.00	0.03	42.55	15.95[a]
T2	3.51	39.00	0.03	42.51	15.99[a]
T3	4.33	39.00	0.03	43.33	15.17[a]

Values in the same row with different literal differ statistically P<0.01)

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BACTERIAL COUNTS IN PIG SLURRY AMENDED WITH ZEOLITE ADDITIVES

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SUMMARY

The aim of investigation was to determine the effect of natural zeolite, feed and slurry additives, on counts of different physiological groups of bacteria i.e. sulfide-producing, faecal and total coliform, aerobic and anaerobic mesophilic, and thermophilic bacteria in pig slurry. As slurry additive zeolite was spread daily directly over partially slatted floor in a dose of 0.4 kg/1 m^2 and as feed additive admixed to the feed in the amount of 2% by weight, during the pig fattening period. The results demonstrated that the counts of all investigated groups of bacteria were lower after addition of the zeolite in comparison to the control slurry.

INTRODUCTION

Intensive pig production, with slatted floor technology, results in the accumulation of large amounts of slurry. Its composition, a high concentration of organic matter, nutrients, trace elements, and variety of microorganisms, including pathogenic ones, is the reason for its treatment before removing to the environment. The causative agents of many infectious diseases are excreted by the faecal route and also with other excretions or secretions of the body [1]. Pathogens may persist in the slurry for a long time depending on the type of slurry, storage condition and temperature and pathogen type. They will be inactivated after exposure to the environment but may survive long enough to be of public and/or animal health concern [2]. Many scientific papers report about the possibilities of use the natural zeolites in the removal of chemical pollutants, i.e. binding of ammonium ions or trapping gaseous ammonia, from waste water including animal slurry, however the data about the effect of the zeolites on bacterial counts in the slurry are rare and were carried out *in vitro* conditions [3]. Although data are available regarding the major chemical compounds causing malodorous emissions from livestock waste, less is known about the microorganisms responsible for production of those compounds [4]. Therefore, the need for and necessity of slurry hygienization in addition to its mechanical and biologic treatment have been increasingly emphasized, especially for slurry microbiologic composition, which is considered to be potentially pathogenic. The aim of the investigation was to determine the effect of natural zeolite, feed and slurry additives, on counts of different physiological groups of bacteria in the fattening pigs' slurry.

MATERIAL AND METHODS

The investigation was conducted at the Dubravica pig-breeding farm in Croatia during winter-spring period in three equal fattening units with partially slatted floor, each with 400 pigs on an average. In the comparison with the control group in the experimental group I the zeolite, commercial preparation "ZeoClean", containing 80% of clinoptilolite, was directly spread over the partially slatted floor in a dose of 0.4 kg/1 m^2 and in the experimental group II the zeolite treatment consisted of adding 2% by weight commercial preparation "Zeofeed", with also 80% of clinoptilolite, to the feed mixture, as recommended by the supplier. Samples of the pig slurry were collected on 7 occasions, during the fattening period of 130 days, from the channels under the slatted floor. Dark, malodorous specimens were collected in sterile bottles and analyzed within 4h. For bacteria determination supernatant was used after dry solids were settled down. The number of sulfide-producing bacteria (SPB) was determined in liquid sulfate-thiosulfate medium after incubation at 35°C for 48h [5]. The number of fecal coliforms (FC) and total coliforms (TC) was determined in MacConkey broth after incubation at 44.5°C for 24h and 35°C for 48h, respectively. The numbers of heterotrophic aerobic and anaerobic mesophilic bacteria, as well as thermophilic bacteria were determined on Nutrient agar plates. After inoculation of samples, plates for determination of aerobic heterotrophic bacteria were directly incubated at 35°C for 72h. Plates for determination of anaerobic heterotrophic bacteria were placed into Anaerocult A (Merck) and incubated at 35°C for 72h. Plates for determination of thermophilic bacteria were incubated at 55°C for 24h. The number of bacteria from liquid media was determined as the most probable number (MPN) using the standard evaluation tables, while the number of bacteria grown on solid media was determined as colony-forming units (CFU) per one L of the slurry [6]. Statistical analyses were carried out using Statistica Software 9.1 (StatSoft, Tulsa, USA). The numbers of bacterial CFU were logarithmically transformed beforehand to normalize distribution and to equalize variances of the measured parameters. The comparisons between samples were done using the one-way analysis of variance (ANOVA) and subsequently the

post-hoc Duncan test was performed for the calculations concerning pair-wise comparisons. Statistical decisions were made at a significance level of $P<0.05$.

RESULTS

Results of the investigation are presented in Table 1 and Figures 1-6.

Table 1. Numbers of bacteria in control and experimental samples of pig slurry amended with ZeoClean and Zeofeed.

Bacteria	Control	ZeoClean	Zeofeed
Sulfide-producing bacteria (MPN/L)	$1.61 \pm 4.14 \times 10^{10}$	$1.55 \pm 3.46 \times 10^{9}$	$3.07 \pm 7.91 \times 10^{9}$
Faecal coliforms (MPN/L)	$1.18 \pm 1.74 \times 10^{8}$	$4.00 \pm 8.44 \times 10^{6\ A}$	$4.73 \pm 8.19 \times 10^{6\ A}$
Total coliforms (MPN/L)	$6.71 \pm 9.25 \times 10^{8}$	$2.37 \pm 3.26 \times 10^{7\ A}$	$1.20 \pm 2.78 \times 10^{8}$
Aerobic mesophiles (CFU/L)	$9.62 \pm 5.57 \times 10^{10}$	$3.26 \pm 3.81 \times 10^{10}$	$4.16 \pm 5.23 \times 10^{10}$
Anaerobic mesophiles (CFU/L)	$1.01 \pm 1.00 \times 10^{11}$	$4.36 \pm 7.07 \times 10^{10}$	$5.53 \pm 7.67 \times 10^{10}$
Thermophiles (CFU/L)	$1.60 \pm 1.35 \times 10^{7}$	$4.49 \pm 4.93 \times 10^{6\ A}$	$7.45 \pm 7.08 \times 10^{6}$

Values are expressed as mean ± SD; n = 7 *per* measurement in each group; A = significantly different than control; B = significantly different than ZeoClean.

DISCUSSION

Environmental and health problems related to the slurry microbiological composition include emanation of malodour, detrimental gases and spread of infections, so imperative of manure treatment is to imply additional procedures in order to reduce microbiological count. The zeolites have been increasingly used in various areas. In the field of veterinary medicine they are mostly used as a feed and/or slurry additives because animal feed, as well as slurry composition, has direct impact on animal health as well as public health. In this investigation the effect of natural zeolite, the feed and the slurry additives, on counts of different physiological groups of bacteria in the fattening pigs' slurry was investigated. Pig slurry naturally contains an excess of 10^{10} bacteria *per* ml, some of them are considered to be potentially pathogenic [1]. The SPB are common inhabitants of pig caecum and are excreted with faeces in the slurry, where they are responsible for the characteristic malodour [7], which originates from hydrogen sulfide, one of the most potent malodours emitted from anaerobic pig slurry channels [4].

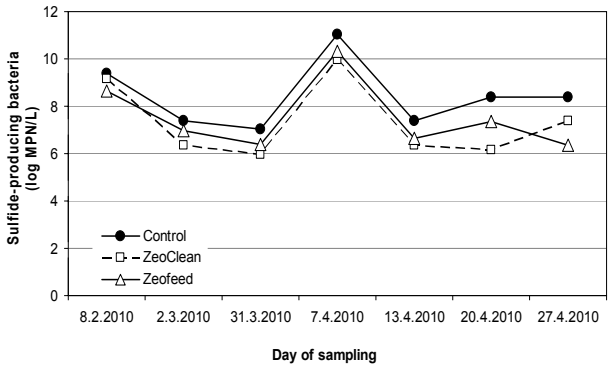

Figure 1. Counts of sulphide-producing bacteria (SPB) in control and experimental samples of pig slurry amended with ZeoClean and Zeofeed.

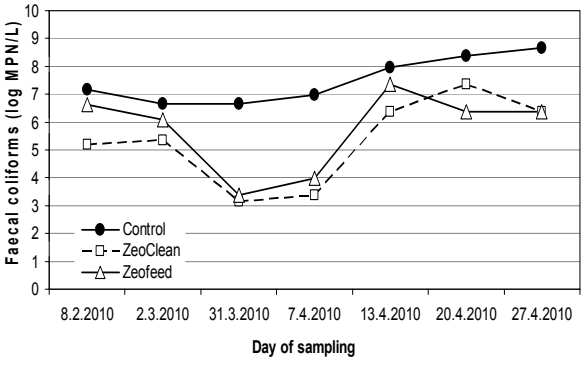

Figure 2. Counts of faecal coliforms (FC) in control and experimental samples of pig slurry amended with ZeoClean and Zeofeed.

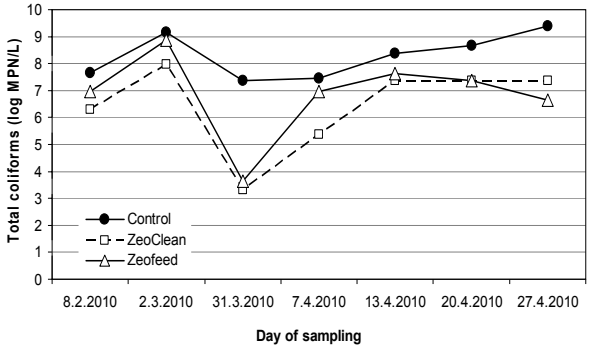

Figure 3. Counts of total coliforms (TC) in control and experimental samples of pig slurry amended with ZeoClean and Zeofeed.

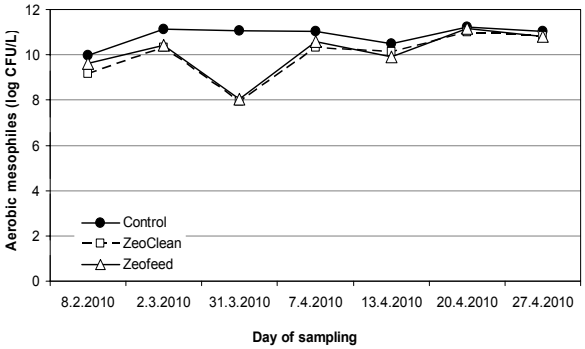

Figure 4. Counts of aerobic mesophilic bacteria in control and experimental samples of pig slurry amended with ZeoClean and Zeofeed.

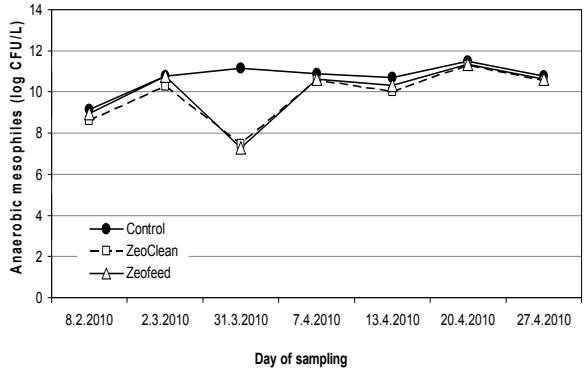

Figure 5. Counts of anaerobic mesophilic bacteria in control and experimental samples of pig slurry amended with ZeoClean and Zeofeed.

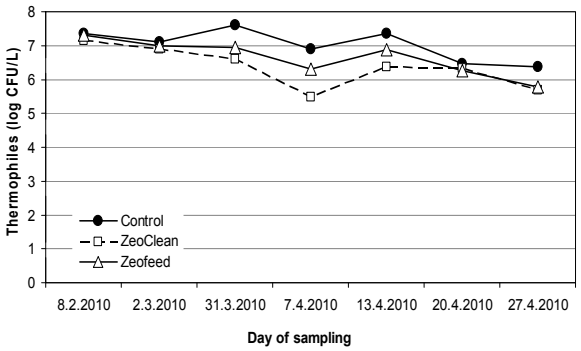

Figure 6. Counts of thermophilic bacteria in control and experimental samples of pig slurry amended with ZeoClean and Zeofeed.

The FC and TC bacteria inhabit the pig intestine and are excreted with faeces in the slurry. Low temperatures generally help the growth and survival of enteric bacteria i.e. faecal coliforms. Other heterotrophic mesophilic (aerobic and anaerobic) and thermophilic bacteria are also present in pig intestinal tract and excreted with faeces in the slurry. The use of the ZeoClean as slurry additive and Zeofeed as feed additive resulted in the lower counts of all investigated groups of bacteria when compared to the control slurry (Table 1). When assuming the results collected during the whole investigation, the statistically significant difference was found between control and ZeoClean for counts of FC and TC and thermophiles. The statistically significant difference was found between control and Zeofeed only for counts of FC. No significant difference was found between the experiments where ZeoClean and Zeofeed were used. However, the single results collected on the same date (Figures 1-6) showed lower counts of all physiological groups of bacteria in the experimental slurries when compared to the control. The highest difference between the control and experimental slurries can be seen for FC, which are considered as the most seriously potential pathogens among the investigated physiological groups of bacteria. The lower counts of bacteria in the experimental slurries when compared to the control are explained by the process of spontaneous adsorption and immobilization of bacteria on the particles of natural zeolite [8].

CONCLUSIONS

It may be concluded that the addition of the zeolite in the nutrition of pigs or used as additive on the floor resulted in reduced counts of different physiological groups of bacteria in the fattening pigs' slurry.

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OPTIMIZATION OF THE QUALITIES OF THE DIGESTATE FOR ITS USE FOR BIOLOGICAL PRODUCTION OF CROPS

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SUMMARY

Our previous researches revealed that, taking into consideration the quantity of the toxic elements, the use of the digestate for increasing the soil fertility in biological production is allowable. A mathematical model is used to estimate the ecological effectiveness, according to the criterion of mineralization level (CM) of the organic substance. An additional ecological criterion is applied into the current analysis: the reduction of the chemical necessity of oxygen in the substrate (CRO). For the biological production of forage, the allowable import of nitrogen into the soil is up to 170 kg/ha. For the quality optimization of the digestate, obtained in conditions of continuous methane fermentation, mesophilic temperature rate (34^0C) and dry matter consistency, laboratory researches were made for the following combinations of substrates: manure from dairy farms with non-litter breeding, non-replaceable litter from broiler production and pig manure. The highest ecological efficiency is provided by raw material of cattle manure - 56% mineralization and CRO reduction by 52.1%. In order to optimize the quantity of the imported nitrogen, various combinations of substrates are examined. The highest level of nitrogen was marked in the raw material of non-replaceable litter – 6, 5%, and lowest when cow compost is used – 3, 2%.

Based on 146 conducted laboratory experiments, following the limit of the quantity of imported nitrogen and the criteria of maximum ecological efficiency, combinations of the afore-mentioned substrates are suggested.

INTRODUCTION

The concept of biological production in stock-breeding includes the idea of producing holdings of the stock-breeding farm and the adjoining area, providing food for the animals. Our previous researches reveal that the digestate is not exceeding the allowable content of toxic elements and has got a degree of mineralization, that guarantees an optimal resource of biogenic elements and a proportion that combines chemical elements, such as mineral salts (that can be immediately used by the plants) with undigested organic substances that gradually digest in the biocenosis of the soil. In the biological production the quantity of nitrogen in the soil additives is limited to 170kg/ha, which requires a combination of the substrates for the production of the digestate in order to follow this standard.

MATERIAL AND METHODS

The researches were made on the following combinations of substrates: non-replaceable litter from broiler; pig manure and manure from dairy farms with non-litter breeding. As experimental devices, bioreactors with capacity of 2l, with working capacity of 1l, were used. The conditions of the experiment, were: Continuous working process; mesophille temperature of 34^oC; pH from 6.8 to 7.2 and dry remainder after hardening 7%.

In accordance, with the applied scheme for the dilution (D), in the interval of 24H, we added a fresh manure substract, meanwhile, extracting the workout digested material.

The input manure mixtures were grinded, homogenized and treated with water until reaching an suspense of 7% dry material. Here are the analyses, made on the input and outtake organic materials and the used methods and standards:

- determination of pH through electronic pH meter OP – 221/1radelkis, Budapest;
- determination of total dry remainder after hardening, and losses due to hardening (BNS 17.1.4.04-80);
- determination of bi-chromatic oxidation (BNS 17.1.4.02-77);
- determination of nitrogen content (according to BNS-EN 13342);
- determination of methane and carbon dioxide through an electronic gas analyzer from the company Dreger.

RESULTS

Experiments with 86 combinations and repetitions with a duration of 20 days were conducted. In table 1 we present the results of 8 combinations of substrates, as follows:

- Substrate 1 – 80 % swine manure and 20 % non-replaceable bedding;
- Substrate 2 – 60 and 40 % of the same components respectively;
- Substrate 3 – 40 and 60 % of the same components respectively;
- Substrate 4 – non-replaceable bedding from broiler production containing hay;
- Substrate 5 – manure from non-bedded breeding of milk cows;
- Substrate 6 – 20 % of manure from cows and 80% of non-replaceable bedding;
- Substrate 7 – 50 and 50 % of the same components;
- Substrate 8 – 80 and 20 % of the same components.

DISCUSSION

The basic technological results are presented in table 1. The accent is focused on the obtained biogas and the ecological effect of the anaerobic digestion, which is defined by the mineralization of the substrate and the reduction of COD. In order to comply with the EU directive for limiting the nitrate pollution of soil and water, some data, concerning the quantity of the nitrogen in the input of the system is presented. The technological data in Table 1 reveals that the highest production of biogas is provided by substrate No4 – non-replaceable litter from the broiler production. The quantity of nitrogen in the dry matter is also highest with this substrate. The quantity of nitrogen in the digestate is lowest when a substrate of non-litter cow-breeding is used. In this case the percentage of mineralization is highest. The quantity of nitrogen in the digested materials is lowest when a substrate of non-litter cow-breeding is used. In this case the percentage of mineralization is highest. In order to limit the quantity of nitrates in the soil we base on regulation No35 for biological production in stock-breeding. The regulated quantity of nitrogen is 170kg/ha per year, that is a foundation for developing particular technologies in the areas of the farms – holdings for producing a biologic production. In order to fulfill the idea of creating a holding for biologic production, we suggest a combination of the substrates with the objective to limit the quantity of the nitrogen to the fore-mentioned standard for biological production.

Table 1:

Substrate number	Input of dry matter (%)	Output of dry matter (%)	Input of organic matter (%)	Output of organic matter (%)	Share of mineralization (%)	Reduction of COD (input/output) (%)	Average 24 h yield (ml)	Nitrogen input (% of dry matter)
1	7.0	3.2	78.2	37.2	47.5	45.5	281.3	4.8
2	6.9	3.9	83.6	42.0	50.2	41.4	246.0	5.2
3	6.8	3.4	85.4	44.5	52.1	45.1	745.5	5.9
4	6.5	3.0	85.2	40.3	47.3	34.4	1146.6	6.5
5	7.0	3.9	78.3	43.9	56.0	52.1	260.1	3.2
6	6.8	3.5	82.1	42.1	51.2	49.4	420.2	4.1
7	6.9	3.5	84.1	42.0	49.9	48.1	820.2	5.2
8	7.0	3.4	83.6	41.2	49.7	48.3	840.5	5.8

CONCLUSIONS

The above made researches are leading us to the following conclusions:

1. Limiting the Nitrogen level into the Digestives could be achieved by combining of the substrates: non-replaceable litter from broiler production, pig manure and manure from dairy farms with non-litter breeding. Such a combination, Could achieved a limit of 170kg/ha per year.
2. Modeling the Nitrogen level in the Agricultural system; it should be accepted as the most important in the "non-replaceable bedding from broiler production", and the lowest one in the manure from dairy farms.
3. The level of the mineralization is in the region of 47.3- 56.0%, which make the inputting of the compost in the very best moment.
4. The analyses, proved the possibility to be established an ecology system for dunging the soil, that could be differentiate in view of the agricultural demands of the cultures, and their specific ability to use the favorable conditions of the environment, so to absorb and use the mineral substances, creating an increase of the harvest.

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SURVIVAL OF BACTERIA IN THE PROCESS OF SLURRY COFERMENTATION WITH MEAT AND PLANT WASTES

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SUMMARY

Slurry is a valuable natural fertilizer which, however, has to be sanitized before its agricultural use, due to the presence of many microorganisms and eggs of parasites. The optimal solution seems to be slurry co-fermentation, which allows obtaining both the safe fertilizer and renewable energy.

The aim of this study was to estimate the sanitization effectiveness of pig slurry during methane co-fermentation realized in an agricultural biogas plant in the temperature range 40-45^{o}C.

The research on the sanitization effectiveness of fermentation was carried out using pig slurry with an addition of co-substrates based on the inactivation kinetics of *Salmonella*, *E. coli* O157:H7 and faecal streptococci of D group. The culture of microorganisms was conducted according to the generally accepted methods and their number was determined using the MPN method. During the process of co-fermentation the group D faecal streptococci were characterized by the longest theoretical time of survival amounting to 171.93 hours, whereas the bacilli of *E. coli* O157:H7 showed the shortest survival, equal to 14.59 hours. The study indicated a high sanitization effectiveness of methane fermentation, since a period of about 7 days turned out to be enough to eliminate all the tested bacteria from the studied material.

INTRODUCTION

Slurry constitutes a valuable organic fertilizer, but its improper management can pose a serious sanitary and health hazard, resulting from the presence of numerous bacteria, viruses and parasite eggs. Therefore it is essential to find out and develop effective methods for slurry sanitization. The optimal solution seems to be subjecting liquid animal wastes to methane fermentation conducted in agricultural biogas plants. Facilities of this type are quite common Western Europe, and in recent years their development has also been observed in Poland [4]. Biogas production in small agricultural biogas plants will make it possible not only to manage considerable amounts of slurry but to meet the assumptions of the Renewable Energy Industry Development Strategy of 2001, in accordance to which the proportion of renewable energy in the energy balance of Poland is supposed to reach 14% in 2020.

Methane fermentation allows production of, on average, about 50-55 m^3 of biogas from 1 ton of the fresh weight of slurry [2]. The process of co-fermentation of liquid animal faeces with an addition of waste biomass (silages with bad parameters, post-harvest residues, slaughter wastes, rumen contents of cattle, pig intestines and blood) is definitely more efficient [4]. Fermented slurry constitutes a valuable fertilizer containing readily assimilable nitrogen, phosphorus and potassium compounds and the biogass produced during fermentation decreases the demand for conventional energy carriers. It is appropriate, however, to check if the post-fermentation product does not pose a sanitary and epidemiological hazard during its agricultural use.

The aim of this study was to estimate the sanitization effectiveness of pig slurry during methane co-fermentation conducted in an agricultural biogas plant within the range of temperatures 40-45^{o}C.

MATERIAL AND METHODS

The material for this study was a batch composed of 70% of pig slurry, 22% of maize silage and 8% of pig intestines and fatty esters. The effectiveness of sanitization was estimated based on the inactivation kinetics of *Salmonella*, *E. coli* O157:H7 and faecal streptococci of group D introduced into the tested material in carriers. Collection of samples and temperature measurement was made every 4 hours and in the case of streptococci every single day. The culture of microorganisms was carried out according to the commonly accepted methods and their number was determined on the basis of the MPN method. The theoretical time of survival and elimination rate was calculated for each bacteria on the basis of regression equations. The results obtained were subjected to the statistical analysis in the program SAS 9.2 PL.

RESULTS

The initial concentration of the tested bacteria amounted to 10^8 MPN/ml.

During the process of co-fermentation a gradual decrease in the count of populations of all the tested microorganisms was observed (Table 1).

Table 1: Changes in number of tested bacteria during co-fermentation and statistic parameters determined for them based on regression equations.

Sampling time [h]	Number of bacteria [MPN×ml^{-1}]	Regression equation	Elimination rate [log MPN×h^{-1}]	R^2	Theoretical survival time [h]
Enterococcus spp.					
0	9.5×10^8	y = -0.054x + 9.284	0.054^A	0.98	171.93^A
24	11.5×10^7				
48	7.5×10^6				
72	4.5×10^5				
96	2.5×10^4				
120	2.5×10^2				
Salmonella spp.					
0	7.5×10^8	y = -0.444x + 8.415	$0.444^{B,a}$	0.96	$18.95^{B,a}$
4	11.5×10^6				
8	7.5×10^3				
12	2.5×10^2				
16	4.5×10^1				
20	n.d.				
E. coli O157:H7					
0	4.5×10^8	y = -0.545x + 7.954	$0.545^{B,b}$	0.93	$14.59^{B,b}$
4	7.5×10^5				
8	11.5×10^1				
12	2.5×10^1				
16	n.d.				
20	n.d.				

A, B – highly statistically significant differences (p≤0,01)
a, b – statistically significant differences (p≤0,05)

The number of group D faecal streptococci decreased from 9.5×10^8 to 2.5×10^2 NPL×ml^{-1} for 120 hours of the experiment (Table 1). The total inactivation of these bacteria was not observed during the study (Table 1). Reduction in the number of *E. coli* O157:H7 and microorganisms of the genus *Salmonella* occurred much faster. In the case of the bacteria *E. coli* O157:H7 their population decreased from 4.5×10^8 to 2.5×10^1 NPL×ml^{-1} during only 12 hours of co-fermentation, whereas for *Salmonella* bacilli a decrease from 7.5×10^8 to 4.5×10^1 MPN×ml^{-1} was observed during 16 hours of the process (Table 1). The total elimination of bacteria *E. coli* O157:H7 and bacilli of the genus *Salmonella* was observed after 16 and 20 hours of the study, respectively (Table 1).

The theoretical survival time calculated on the basis of regression equations was the longest in the case of faecal streptococci and amounted to 171.93 hours and the shortest for *E. coli* O157:H7 and was equal to 14.59 hours (Table 1). Group D faecal streptococci were characterized by the lowest value of elimination rate (0.054 log MPN×hour^{-1}), and the bacteria *E. coli* O157:H7 by the highest (0.545 log MPN×hour^{-1}) (Table 1).

The statistical analysis of the results obtained showed highly statistically significant differences between the theoretical survival time and the elimination rate of group D faecal streptococci and values of these parameters determined for the other tested microorganisms (Table 1). The elimination rates and theoretical survival times of *Salmonella* and *E. coli* O157:H7 differed statistically significantly from each other (Table 1).

Throughout the study the temperature inside the fermenter remained within the range 42.7-44.8^{o}C.

DISCUSSION

The sanitization effectiveness of the process of methane fermentation depends largely on temperature, the composition of material introduced into the reactor, pH, concentration of volatile fatty acids, dry weight content, the time of hydraulic retention and the type of fermentation process [3]. Many reports can be found in the literature showing distinct diversification in the rate of eliminating microorganisms from fermented slurry depending on the temperature of the process. Many researchers report that the time of a decimal reduction in the anaerobic mesophylic process ranges from 1 to 2 days for *E. coli* and from 2 to 4 days in the case of *Salmonella* [6, 9]. Paluszak et al. [7] indicated that the theoretical survival time of *Salmonella* Enteritidis was 12.94 days, thus it was considerably longer than that obtained in the present experiment. This, however, probably results from

differences in the temperature of the process. According Kumar et al. [5] fermentation carried out at 18-20°C provided the total elimination of *E. coli* and *Salmonella* Typhi bacilli during 20 days, and at 35°C, during 10 days, whereas in the case of faecal streptococci they observed their total inactivation after 35 and 15 days, respectively. In respect of sanitization, the anaerobic thermophylic process is much more effective. Olsen and Larsen [6] report that DRT during thermophylic fermentation (53°C) for *Salmonella* Dublin bacilli amounts to 0.6 hours and for *Salmonella* Typhimurium 0.7 hours. In the present study, the process of co-fermentation was conducted in the range of temperatures at the border between meso- and termophylic conditions and was characterized by a relatively high sanitization effectiveness. Large effectiveness of methane fermentation in eliminating microorganisms from slurry was also indicated by Paluszak [8]. He found that in the course of the anaerobic process conducted at 50.1-51.8°C the total elimination of *E. coli* O157:H7 occurred during 3 hours, and of bacteria of the genus *Salmonella* after 4 hours, whereas a decrease in population of group D faecal streptococci by 5.15 log took place after 20 hours [8]. These tendencies were reflected in the present study. There are reports in the literature indicating that the process of sanitization proceeds faster in the case of fermentation of slurry alone than in its co-fermentation with an addition of other substrates [1]. Böhm et al. [1] indicated that the decimal elimination time of *Salmonella* Senftenberg and *Salmonella* Enteritidis amounted to 15 minutes in the case of fermentation of slurry alone and 5 hours in the process of co-fermentation.

From the present study and the reports of other authors presented above it appears that slurry co-fermentation is a process providing the effective sanitization of liquid animal faeces, and consequently, making it possible to obtain a valuable and safe fertilizer.

CONCLUSIONS

1. Co-fermentation is an effective process of treating slurry for agricultural purposes, at the same time allowing production of renewable energy.

2. Under conditions of the experiment the total elimination of tested microorganisms from slurry was obtained after about 7 days, which allows supposition that the twenty-day time of hydraulic retention, which is most often used, is sufficient to produce a safe fertilizer.

3. Sanitization effectiveness of methane fermentation turned out to be varied, depending on the tested microorganism. Faecal streptococci of D group are characterized by the longest survival rate and therefore they can be recommended as indicator bacteria.

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EFFECT OF BACTERIAL COMPOSITE ON MICROFLORA IN MUNICIPAL SEWAGE SLUDGE

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SUMMARY

The effect of bacterial composites on the development dynamics of sewage sludge microflora incubated under various conditions of aeration was evaluated. Two biopreparations were tested, with a composition based on microorganisms isolated from compost (B_k) and chicken manure (Bp). Composite Bp in conditions of constant aeration had a favourable effect on the development of the tested microflora in most samples. In conditions of reduced air access a higher number of bacteria was obtained in the control samples, which may indicate the lack of the effect of composites or their inhibition on native microflora. Incubation conditions turned out to be a significant factor stimulating the development of microorganisms. Considerably more bacteria, fungi and actinomyces were isolated from aerated sludge than from unaerated.

INTRODUCTION

An increase in the biological efficiency of the sewage treatment system may be obtained by adding properly selected bacterial strains. Biological decomposition of permanent organic pollutions by microorganisms is one of the most important and effective methods of removing these compounds from the environment. Biodegradation of organic compounds is mostly a multi-stage process, taking place with participation of many organisms which often show synergic action [2, 3]. The range and rate of biodegradation changes are determined by a series of factors, such as: the composition and activity of bacterial flora, properties and "age"of the pollution, presence of other compounds, temperature, pH value, access of oxygen, the content of nutrients and the physicochemical properties of the medium where the process occurs [5, 8].

Biopreparations are natural bacterial-enzymatic compounds, which serve to support biodegradation of organic substances and changes in non-organic compounds. Biopreparations are made on the basis of carefully selected strains of microorganisms, which are usually previously isolated from natural habitats containing a given type of xenobiotics [7]. However, using biopreparations in sewage treatment technology requires the knowledge about microorganisms contained in this types of products and about the conditions they can be active.

The aim of this study was to estimate the effect of two bacterial composites on the development dynamics of sewage sludge microflora.

MATERIAL AND METHODS

Material to analyses was municipal sewage sludge from the Municipal Sewage Treatment Plant in Toruń. Sludge with a weight of 3 kg was placed in hermetic incubation chambers with a capacity of 6 dm^3, where bacterial composites were applied. Two bacterial composites tested were obtained as a result of selection of microorganisms isolated from compost (B_k) and chicken manure (B_p). These biopreparations were developed by ITFiM of Łódź University of Technology. Bacterial suspensions B_k and B_p before the application were multiplied on TSB medium for 24 hours and shaken at 27ºC. Then the cultures were centrifuged and all the biomass after removing post-culture liquid was suspended in distilled water. Suspensions of bacterial composites were applied by spraying on the surface of sludge. The control sample was material without the applied composite. Two variants of sludge aeration were used during incubation. In the first variant, without aeration (I), containers were sealed and the access of air to the incubated material was limited. In the other variant (II) containers were aerated at two-hour intervals by pumping air under wastes for 15 minutes with a velocity of 150 dm^3h^{-1}. Sludge samples for microbiological analyses were collected on the day of the establishment of the experiment and after 5 and 14 days. The number of microorganisms was determined with the plate method following Koch, using sludge dilutions prepared in 0.9% NaCl. Standard nutritional agar was used for isolation of total bacteria, Martin medium for mould fungi and Pochon medium for actinomyces. The obtained results of cfu counts were worked out statistically (separately for samples with and without aeration) with the method of the analysis of variance and the significance of differences between means was assessed with t– Student test (P=0.95) in the program *STATISTICA*.

RESULTS

The total number of bacteria in the tested samples of sewage sludge reached the level 10^8 cfu (Table 1). The largest development of bacteria was observed in the aerated sludge with an addition of composite B_p (88 x10^8cfu/g). In the material which was not subjected to aeration the number of bacteria was definitely less and the lack of significant differences between the effect of the tested biopreparations was indicated. It should be noted that both in aerated and unaerated samples with applied composite B_k, the number of bacteria was lower in relation to the control. Also conditions of sludge incubations had a significant effect on the development dynamics of actinomyces (Table 2). Aeration stimulated their growth, and the numerical value obtained were 10 times higher than those under conditions without aeration. When comparing the effect of the tested biopreparations, significantly more actinomyces were found in the samples with added Bp (max. 48.5x10^6 cfu) than in those with B_k (max. 18x10^6 cfu).

The count of fungi in municipal sewage sludge ranged from 5 to 77.5x10^6 cfu (Table 3). In the material subjected to aeration an increase in the number of fungi occurred in 5th day both in the control and in the samples with added biopreparations, and then their decrease was observed on 14th day. No significant differences were shown between the effect of composites B_k and B_p in relation to the control, for which the highest average number of fungi from two dates was obtained (43x10^6 cfu in conditions with aeration). Stimulating effect of biopreparation Bp was observed in the 14th day in unaerated samples, where significantly more fungi were isolated than in sludge with an addition of B_k and the control. It was observed that fungi development was highly varied and depended not only on incubation conditions and the effect of biopreparations, but also on the date of analyses. At 1st date of analyses more fungi were isolated under conditions with aeration, and then their number fell, whereas under conditions of limited access of air the reverse was the case.

Table 1: Effect of bacterial composite on development of total number of bacteria in sewage sludge without (I) and with (II) aeration

Type of composite	Time of incubation, day			
	0	5	14	mean**
I - unaerated sludge (x10^8cfu)				
Control	12	19.0^{a***}	16.0^{a}	17.5^{a}
B_k*		14.0^{a}	10.5^{ab}	12.3^{ab}
B_p		14.5^{a}	5.0^{b}	9.8^{b}
II- aerated sludge (x10^8cfu)				
Control		60.0^{b}	49,5^{b}	54.8^{b}
B_k		52.0^{b}	30.0^{b}	41.0^{b}
B_p		88.0^{a}	69.5^{a}	78.8^{a}

*B_k- composite from compost, B_p- composite from manure
**mean values were calculated for two dates after 5 and 14 days,
*** numerical values in columns marked with the same letter does not differ statistically ($p<0,05$), LSD $_{T,p<0,05}$ composite x time of incubation

Table 2. Effect of bacterial composite on development dynamics of actinomycetes in sewage sludge without (I) and with (II) aeration

Type of composite	Time of incubation, day			
	0	5	14	mean
I - unaerated sludge (x10^6cfu)				
Control	6.0	1.5^{a}	2.0^{a}	1.8^{a}
B_k*		2.0^{a}	2.0^{a}	2.0^{a}
B_p		4.5^{a}	1.5^{a}	3.0^{a}
II - aerated sludge (x10^6cfu)				
Control		9.0^{a}	37.5^{a}	23.3^{a}
B_k		2.7^{b}	18.0^{b}	10.3^{b}
B_p		1.5^{b}	48.5^{a}	25.0^{a}

*for description, see Table 1

Table 3: Effect of bacterial composite on development dynamics of mould fungi in sewage sludge without (I) and with (II) aeration

Type of composite	Time of incubation, day			
	0	5	14	mean
I - unaerated sludge (x10^6 cfu)				
Control	6	26.0^{a}	20.0^{b}	23.0a
B_k*		6.0^{b}	16.0^{b}	11.0^{a}
B_p		5.0b	44.0^{a}	24.5^{a}
II - aerated sludge (x10^6cfu)				
Control		77.5^{a}	8.5^{a}	43.0^{a}
B_k		70.0^{a}	10.0^{a}	40.0^{a}
B_p		65.0^{a}	12.5^{a}	33.7^{a}

*for description, see Table 1

DISCUSSION

On the basis of the conducted study it was found that adding biopreparations to sewage sludge had a varied effect on the development dynamics of microorganisms. Both growth and decrease in the number of microbes was observed in samples with applied biopreparations. When comparing the effect of the tested bacterial composites, it was proved that composite Bp made on the basis of microorganisms isolated form chicken manure, had a significant effect on an increase in the number of bacteria as compared with biopreparation B_k. The aeration system turned out to be a very important factor. The number of bacteria and actinomyces was definitely higher in aerated sludge, as compared with unaerated. For fungi these differences were even larger. An increased access of oxygen stimulates the development of aerobic microorganisms, which directly increases the intensity of organic matter decomposition processes in aerobic conditions [1]. In the study by Wierzba and Nabrdalik [9] it was shown that the introduced biopreparation made the process of organic compounds mineralization in wastes more than two times faster in relation to the autochtonous microflora. The highest reduction of proteins and carbohydrates was observed after the first 30 days of composting. Reduction of organic compounds was accompanied by a considerable growth in the count of proteolytic, lypolytic and cellulolytic bacteria [9]. In the case of actinomyces, the thesis was confirmed that they belong to the group of dominating organisms in decomposed wastes with time [1]. After 14 days of incubation significantly more of them were isolated than in the samples in 5th day. In the course of waste decomposition processes both bacterial cultures dominating in individual stages and changes and their quantitative composition are subject to changes. At the initial stages, non-sporeforming bacteria and moulds, and with time sporeforming bacteria and actinomyces predominate [6] . Biopreparations introduced into the environment as biological strains enable the effective development of active microflora, considerably support and sometimes even determinate the processes of contamination elimination [4, 5].

CONCLUSION

Tested bacterial composites showed varied effect on the development of microflora in municipal sewage sludge. The stimulating effect of biopreparation was observed particularly in the composite which was based on microorganisms from chicken manure. The cases were also found where composites limited the growth of bacteria and fungi. Incubation conditions turned out to be a significant factor stimulating the development of microorganisms. The system of aeration applied improved aerobic conditions in the sludge, which contributed to an increase in count of all the tested groups of microorganisms.

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NITROGEN LOSS DURING COMPOSTING OF POULTRY LITTER

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SUMMARY

The principal objectives of composting of poultry litter is to reduce volume, odour and pathogens and to improve its quality as a soil amendment. Optimum degradation of organic C and retention of N through microbial biomass formation is achieved by addition of organic material, e.g., sawdust. The disadvantages of composting of animal wastes include loss of nitrogen and other nutrients. About 35 kg of the initial N (65 % of the initial N) was lost during composting, which indicates that composting reduced the value of the poultry litter as N fertilizer. Composting of poultry litter converted the soluble nutrients to more stable organic forms, thereby reducing their bioavailability and susceptibility to loss when applied to crop fields.

INTRODUCTION

Poultry manure is a good fertilizer due to its high content of C and N and the low C:N ratio. Fresh poultry manure is difficult to handle because of its higher water content and cannot be applied to crops due to caustic effects on foliage [3].One method of litter treatment that will enhance raw litter quality and reduce the environmental impact of land application is composting. Composting is a biological process in which organic wastes are stabilised and converted, under controlled conditions, into a product to be used as a soil conditioner and organic fertilizer [5]. The process improves the storage and handling characteristics of the manure by reducing its volume and weight, kills pathogens and weed seeds, minimizes the production of phytotoxic substances, reduces unpleasant odours, and stabilizes the nutrients and organic matter. However, one of the most negative effects of composting animal manures is the loss of N through ammonia (NH_3) volatilization which reduces the fertilizer value of the manure, and constitutes an important economic loss. Hence, composting changes the nature of the waste and can affect its usefulness as a soil amendment [6]. Ammonia emissions are produced by aerobic and anaerobic bacterial activities in the manure. They are responsible for N losses between 47% and 62% of the initial total N during composting of poultry manure. Organic material, e.g., sawdust, is added to increase the C:N ratio to achieve optimum degradation of organic C and retention of N through microbial biomass formation. However, the relative biodegradabilities of the organic material in poultry litter and the amendment are usually not known. Furthermore, it is assumed that as microorganisms metabolize organic compounds and produce CO_2, they increase biomass and, therefore, retain N [2].

Of all organic wastes poultry manure is the richest source of nitrogen and carbon and exhibits low C:N ratio. Availability of nitrogen is affected by various losses in the form of emissions to the atmosphere, denitrification, immobilization, mineralization and leaching. Mineralization is most intensive after application of poultry excrements into soil [1]. This is the reason why this substrate should be subjected to treatment and stabilization, the processes which also decrease both odourous emissions and environmental problems arising from NH_3 losses. The final product has higher dry matter content and lower total volume which facilitates its handling.

The aim of the present study was to investigate the processes during composting of chicken manure and sawdust with focus on nitrogen losses.

MATERIAL AND METHODS

The study was carried out on poultry excrements collected from a layer house. The total quantity used in the experiment was 300 m^3 and was produced on the same day. The experiment was conducted in winter in a pilot-plant open composting facility under a roof. The substrate for composting was prepared from poultry manure and cut straw mixed at a ratio of 1:1.63 by volume. The experiment lasted 114 days with turning of the substrate on days 9, 21 and 94 of composting. The basic physical-chemical properties of the substrate (pH, dry matter DM, organic matter OM, inorganic matter IM, total nitrogen N_t, ammonia nitrogen N_{NH4+}, total carbon C, total phosphorus P, C:N ratio) were determined at the beginning of composting and throughout the process until the end of the experiment. The temperature in the pile was monitored continuously. The methods used corresponded to the STN 465 735. The C content was calculated according to the content of organic matter by the method of [5]. Results are reported per dry weight.

RESULTS

Poultry manure and straw used in the experiment were examined for the basic physical and chemical properties (Tab. 1). Mean of three replicates from each sampling are shown. The OM content in both components, fresh poultry manure and cut straw was high (96.2 % and 91.1 % in DM, resp.), but the N_t content in fresh poultry manure was 10-fold higher compared to that in straw (45.5 and 4.2 $g.kg^{-1}$ DM, resp.).

Table 1: Physical and chemical properties of the basic materials

	Straw	Solid poultry manure
pH	7.01	7.02
DM (%)	95	28.6
OM (%)	96.2	91.1
IM (%)	3.8	8.9
N_{NH4+} ($g.kg^{-1}$ DM)	0.1	1.96
N_t ($g.kg^{-1}$ DM)	4.2	45.5
C:N ratio	117:1	10:1

The initial pH values ranged between 8.4 and 8.6 and increased during composting due to release of ammonia and rising temperature which reached 61.7°C on day 10 of composting. The N_{NH4+} level in the substrate decreased most during the first 26 days of composting, the total losses amounting to 72 % of the original level. The losses of total nitrogen, which were largely attributed to volatilization of ammonia (NH_3), were the highest within the first 26 days when the pile temperatures were above 39 °C and pH value reached 8.4. The moisture loss increased with increasing C:N ratio, the water content dropping from initial 67% to 59 % at the end of composting. The total N decreased from the initial 53.7 $g.kg^{-1}$ DM to 18.7 $g.kg^{-1}$ DM, i.e. by 65.2%. We observed also decrease in total carbon by 22.8 % compared to the initial level. The decreases observed were related to water losses during composting but also to the losses of organic matter. Composting processes are associated with mineralization of carbon and nitrogen and release of the produced volatile compounds (CO_2, NH_3). The initial carbon to nitrogen ratio (C:N = 10:1) increased throughout the composting in relation to compost turning and reached 15:1 on day 10, 18:1 on day 26 and 22:1 on day 114 of the experiment (Tab. 2).

Table 2: Changes in chemical properties of the composted material

Days	pH	Temp. °C	OM $g.kg^{-1}$	N_{NH4+} $g.kg^{-1}$	N_t $g.kg^{-1}$	Total C $g.kg^{-1}$	C:N
0	8.4	7.3	967	13.8	53.7	531	10:1
10	8.6	61.7	909	10.8	41.2	505	15:1
26	8.4	39.8	865	6.2	30.6	481	18:1
95	8.7	15	826	4.8	22.7	460	21:1
114	8.6	14	750	3.9	18.7	417	22:1

DISCUSSION

Nitrogen loss is considered the major problem during composting of poultry litter. About 35 kg of the initial N (65.2 %) was lost during composting which indicated that composting reduced the value of poultry litter as N fertilizer. Total nitrogen includes both nitrogen forms inorganic (nitrate and ammonia nitrogen) and organic, the latter unavailable to plants without previous microbial decomposition. Nitrogen usable by plants is determined as a sum of available nitrogen (particularly ammonia N) and portion of organic N converted to inorganic forms by decomposition processes. Although nitrogen losses in the form of emissions decrease the total content of N the usable nitrogen is the best source of available nitrogen in the first year following application of manure to the soil [6]. The losses depend predominantly on the way of application. The application of manure is not unlimited and one must consider the vegetative period, weather, manipulation and storage of manure, etc. Application is spring before sowing provides best protection against surface runoff and penetration of nutrients into surface and ground water [2]. Nutrient losses include ammonia emissions into atmosphere and during microbiological decomposition in soil N-losses in the form of nitrates which are highly mobile and are easily leached into ground water. Phosphorus is less mobile but raises problems in surface water after application of manure to soil and due to surface runoffs. Transformation of nitrogen in the first stage of composting of material rich in nitrogen is characterized by high rates of ammonification processes. The highest losses occur in the form of gas emissions of NH_3. High losses of ammonia nitrogen decrease the agronomic value of the final product and the losses themselves contribute to environmental pollution. Due to the mentioned changes we record considerable decrease of nitrogen that can come up to 50%, the higher losses occurring in the initial stage. NH_3. Mondini et al, observed that during 65-day composting of poultry litter mixed with sawdust the level of N decreased to 56.1 % and of C to 82.9 % of the initial level of these nutrients [3]. Nitrogen loss was a major problem during composting of poultry litter, even when the piles were not turned under the forced-aeration system. About 18 kg of the initial N (58 %) was lost during composting reducing the value of poultry litter as N fertilizer [7]. Poultry compost is used as

a valuable manure in agriculture because of high content of nutrients, higher in comparison with compost prepared from manure of other farm animals. The main limiting factor of application of composted poultry excrements to soil are nitrogen losses in the form of ammonia [8]. To study the feasibility of co-composting poultry manure with low quantities of high-value, carbon-rich materials experiments to characterize three pilot-scale piles were carried out. Poultry manure and straw showed the lowest loss in nitrogen content (88.9%) and produced the final compost with the highest C/N ratio (14.7) [9]. The C:N ratio of the initial composting material is the most widely used parameter in composting; high initial C:N ratio will cause a slower beginning of the process and the required composting time to be longer than usual while low initial C:N ratio results in high emission of NH_3 [6].

CONCLUSIONS

Nitrogen loss is the major problem during composting of poultry litter. Most of the loss observed in our study occurred in the form of ammonia during the first 26 days of composting when the temperatures were high and pH level was well above 8. This reduced the value of poultry litter as N fertilizer but a more stabilized organic matter was produced compared to the uncomposted litter.

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MICROBIAL CHARACTERIZATION OF THE WASTE WATER FROM A MAJOR ABATTOIR AND IT'S RECEIVING SURFACE WATER IN ABEOKUTA, NIGERIA

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SUMMARY

This study was conducted to assess the different methods of waste disposal at the Lafenwa abattoir and the environmental and public health implication. In addition, the microbial status of the effluent and its receiving surface water were investigated to determine total viable and coliform counts using surface plating and multiple tube test techniques, respectively. Bacterial and fungal contaminants were isolated and identified by standard microbiological procedures. Waste disposal in the abattoir was by open dumping of solid wastes while effluent was discharged into the nearby Ogun River, which is also used by butchers for meat processing. The mean Total Bacteria Count (TBC) and Total Coliform Count (TCC) for waste water during and after slaughtering were $5.2x10^7$, $4.9x10^7$ and $4.26x10^7$, $3.06x10^7$ cfu/ml respectively while the receiving surface water had mean TBC and TCC of $4.15x10^7$, $3.83x10^7$, and $3.89x10^7$, $2.87x10^7$ cfu/ml respectively. Bacterial organisms isolated from the effluent include *Enterobacter aerogens*, *Hafnia alvei, Erwinia mallotivora, Edwardsiella ictaluri, Enterobacter amnigenus* and *Escherichia coli* O157strains while *Proteus miriabilis*, *Staphylococcus spp*, *Pseudomonas aeruginosa*, *Enterobacter intermedius , Yersinia aleksiciae, Serratia odorifera, Enterobacter cloacae, Enterobacter aerogens* and *Eschericia coli* O157 strains were isolated from the surface water. The fungal species isolated were *Trichoderma spp, Trichophyton spp, Aspergillus spp, Scedosporium spp* and *Coccidiodes spp*.

Keywords: Wastewater disposal, abattoir, surface water, food safety, Public Health, Nigeria.

INTRODUCTION

The abattoir is a specialized facility approved and registered by the regulatory authority for inspection of animals, hygienic slaughtering, processing and effective preservation and storage of meat products for human consumption (Alonge 2001). In addition, appropriate facilities to ensure safe disposal of abattoir wastes in a manner that will not constitute a potential hazard to public health, animal health and the environment is considered very essential. Most abattoirs in Nigeria have no facilities for waste treatment; wastes are either disposed on open dumps or are discharged into nearby streams, hence constituting an environmental menace (Adeyemo *et al.*, 2002).

Waste water or effluent generated from the abattoir is characterized by the presence of a high concentration of whole blood of slaughtered food animals and suspended particles of semi-digested and undigested feeds within the stomach and intestine of slaughtered and dressed food animals (Coker *et al.*, 2001). In addition, there may also be the presence of pathogenic microorganisms, such as *Salmonella, Eschericia coli* (including serotype O157:H7), *Shigella*, parasite eggs and amoebic cysts (Bull *et al.*, 2001) which are of public health importance. Recent studies have shown that zoonoses from abattoir wastes are yet to be fully controlled in more than 80% public abattoirs in Nigeria (Cadmus *et al.*, 1999). Also, several pathogenic bacteria and fungi species has been isolated from abattoir wastewater and surface water; including Staphylococcus, *Escherichia coli*, *Streptococcus*, *Salmonella*, *Aspergillus*, *Muccor*, *Saccarhomyces .spp* and *Penicillium spps* (Adebowale et al ., 2010; Coker *et al.*, 2001 and Adesomoye *et al.*, 2006).

These pathogens isolated might threaten public health by migrating into ground water or surface water, wind or vectors like animals, birds and arthropods which can help to transmit diseases (Gauri, 2004). The risk of epidemics, water contamination and pollution, annihilation of biotic life, global warming and soil degradation by waste materials are real problems confronting developing countries where issues concerning waste management have been grossly neglected (Adedipe, 2002; Adeyemi and Adeyemo, 2007). In Nigeria, adequate abattoir waste management is lacking in all public abattoirs such that large solid wastes and untreated effluents are common sites (Adeyemo, 2002).

MATERIALS AND METHODS

Waste water samples from the major channel for effluent outflow and water samples from the receiving surface water, (which is also used for meat processing) were collected for microbial investigation using sterilized 250ml bottles. Sampling was carried out during and post slaughtering once a week for a period of 3 months (November 2009 to January 2010). Samples were transported to the laboratory in isothermal boxes with ice for microbial analyses.

Total Bacteria and Total Coliform Counts were determined using sterile Nutrient and MacConkey agar plates which were inoculated aseptically in duplicates with 1 ml aliquot of 10^{-7} and 10^{-8} of 1 in 10 serially diluted on samples using surface plate technique. The inoculated plates were incubated at 30°C for 24 hours (Boulter *et al*., 2002).

Pure isolates were subjected to colonial characterization, Gram staining and microscopy as well as biochemical tests according to Cappucino and Sherma 1998. Isolates characteristics were interpreted with an online bacteria identification software system (ABIS 7.0) to determine the isolates.

For detection and identification of *E. coli* O157, samples were inoculated on Sorbitol MacConkey-BCIG Agar (Oxoid) supplemented with cefixime-tellurite selective supplement (Oxoid). Straw coloured colonies suspected to be *E. coli* O157 were subjected to biochemical test for *E. coli* identification. Isolates with characteristics consistent with those of *E. coli* were further confirmed *E. coli* O157 using dryspot *Eschericia coli* O157 test kits (Oxoid).

Fungal isolation and identification were carried out by inoculation of samples onto Potato dextrose agar (PDA) plates supplemented by Streptomycin (100µg/ml) and inoculated with 1 ml aliquot of dilution 10^{-4} after a tenfold serial dilution of the effluent and water samples using the surface plate technique. Inoculated plates were incubated at 30^{o}C for 72 hours (Adesemoye and Adedire, 2005). Fungal colonies were studied morphologically. Smears were made from each colony and stained with Lactophenol Cotton Blue for microscopy at x40 magnification.

RESULTS

Solid wastes generated included ruminal contents, horns, hooves, fat, meat trimmings and animal faeces which are disposed as open dumps around the abattoir. Liquid wastes including blood, urine and water used for various activities in the abattoir were discharged without pre-treatment; into the Ogun River which is also used for meat processing for human consumption.

The mean TBC and TCC for waste water during and after slaughtering were $5.2x10^7$, $4.9x10^7$ and $4.26x10^7$, $3.06x10^7$ cfu/ml respectively while the contaminated receiving surface water had mean TBC and TCC of $4.15x10^7$, $3.83x10^7$, and $3.89x10^7$, $2.87x10^7$ cfu/ml respectively (P>0.05). *Escherichia coli* O157 was detected in 8 (16%) of all 50 samples examined (Table 1). Other bacterial organisms identified in the samples include *Bacillus spp, Staphylococcus spp, Pseudomonas spp, Enterobacter spp, Hafnia alvei, E. coli, Erwinia spp, Proteus spp and Klebsiella spp* (Table 2) while the fungi are *Trichoderma spp, Trichophyton spp, Aspergillus spp, Scedosporium spp* and *Coccidioides spp* (Table 3).

Table 1: Frequency of isolation of *E coli* O157 isolates.

Sample	Frequency of *E coli* O157 isolates (%)
Effluent	4(8.0)
Contaminated Surface water	4(8.0)

Table 2: Bacteria isolated from Abattoir Effluent and Contaminated Surface Water.

ORGANISM	ABATTOIR EFFLUENT	SURFACE WATER
BACTERIA	*Bacillus spp*	*Enterobacter intermedius*
	Staphylococcus spp	*Erwinia carotovora*
	Pseudomonas spp	*Erwinia chrysanthesis*
	Enterobacter aerogens	*Proteus miriabilis*
	Enterobacter cloacae	*Klebsiella oxytoca*
	Hafnia alvei	*Klebsiella plantocele*
	Escherichia coli	*Klebsiella pneumoniae subsp. pneumoniae*
		Escherichia coli
		Bacillus spp
		Staphylococcus spp
		Pseudomonas spp

Table 3: Fungi isolates from the abattoir effluent and contaminated surface water.

Organism	Abattoir effluent	Contaminated surface water
Fungi	*Trichoderma spp*	*Aspergillus spp*
	Trichophyton spp	*Coccidioides spp*
	Aspergillus spp	*Trichophyton spp*
	Scedosporium spp	*Scedosporium spp*

DISSCUSSION

The study revealed that the waste disposal methods at the Lafenwa abattoir are open dumps and effluent discharge into the nearby Ogun River. Most, if not all abattoirs in Nigeria uses these methods of disposal (Adeyemo 2002). These methods are currently prohibited in most developed countries because it provides no safeguard against risks to human health and the environment and is unlikely to foster public confidence in waste management. Unfortunately this is still a common practice in most developing countries due to lapses in the governmental policies and programmes in these countries. The primary food safety risk associated with these methods of disposal is the potential for pathogen, chemical contaminants being transferred to humans directly or through other animals (Ekugo 1998).

The microbial analyses of the waste water and receiving surface water showed that the mean total bacteria and total coliform counts exceeded the Federal Environmental Protection Agency (FEPA1999) and World Health Organization (W.H.O 2004) . A similar study carried out by Adebowale *et al* (2010) on the surface water revealed that the total bacteria (cfu/ml) and *E coli* counts/100ml exceeded the recommended limits hence making this source of water unfit for meat processing. These serve as legible indicators of the extent of the pollution of the water body used for meat processing at the abattoir. The presence of *Eschericia coli O157 strains* in both waste water and receiving water body as well as other pathogens (especially the Enterobacteraecae, many of which are associated with gastroenteritis in humans) indicate the need for waste water treatment before discharge into water bodies after complying with international limits. The reported relatively high prevalence of *E coli* O157 strains (8%) from the waste water and the contaminated river (8%) in this study poses a major concern as other studies by various authors in Nigeria , reported low prevalence of 0.5 -2% (Luga 2006., and Agbogu *et al*., 2005).

CONCLUSION

The results obtained from the investigation showed that the use of this contaminated water for meat processing by butchers portends a serious public health risk to consumers who purchase their meat from this abattoir. There is therefore an urgent need to discourage the use this water for meat processing by butchers so as to safe guard the health of the populace. Untreated animal wastes discharge into surface waters should be totally prohibited and proper animal waste management should be enforced so that these unacceptable practices do not constitute persisting environmental, animal and human health hazard.

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EMISSIONS OF HAZARDOUS GASES FROM PIG HOUSING DURING WINTER AND SUMMER SEASON

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SUMMARY

The aim of this investigation was to determine the concentrations and emissions of hazardous gases in pig housing facilities with full slatted floor. The concentrations of ammonia and greenhouse gases (CO_2, CH_4, N_2O, H_2O), airflow rate and temperature were monitored during two fattening cycles (summer, winter).

The significant differences in concentrations of all observed gases and in emissions of NH_3, CO_2 and N_2O between winter and summer cycle were found ($P<0.001$).

In winter cycle the total emissions of NH_3, CO_2, N_2O were 1.6, 1.4 and 1.7 times higher than in summer cycle, respectively. The total emissions of CH_4 and H_2O showed approximately stable trends in both fattening cycles. The CH_4 emission was only slightly higher in summer cycle and H_2O emission in winter cycle.

The emission factors of NH_3, CO_2 and N_2O were 1.6, 1.4 and 1.7 times higher in winter cycle than in summer cycle, respectively. The emission factors of CH_4 and H_2O showed approximately the same value. They were only slightly higher in summer cycle.

INTRODUCTION

Agricultural production is closely connected with the production of toxic ammonia (NH_3) and greenhouse gases (CH_4, N_2O, CO_2, H_2O). Ammonia on the one hand affects the health of animals and service staff and on the other hand it damages the environment, participating in the formation of acid rain and eutrophication of soil and water systems. Greenhouse gases contribute to the greenhouse effect, when the long wave (infrared) radiation, which is emitted by the Earth's surface, is fixed by these gases and partially re-radiated to the ground and therefore the ground atmosphere is warming.

Due to environmental protection and transformation of the EC norms into national laws it was necessary to determine exactly the emissions of harmful gases from farm animals, mainly from intensive pig and poultry farms. It was needed to define their relationship to the housing microclimate, technology and techniques of farming, storage, handling and application of excreta.

MATERIAL AND METHODS

The experiment was carried out in intensive pig breeding facilities during two fattening cycles (summer-348 animals, 105 fattening days; winter-352 animals, 121 fattening days; weight 25-110 $kg.head^{-1}$). Pigs were housed on a slatted floor. Slurry was stored in pits under slatted floor. Ventilation was provided by three exhaust ceiling fans and inlet flaps located along the sidewalls of the building.

To calculate the gas emissions it was necessary to determine their concentrations ($mg.m^{-3}$) in the housing area and to identify the airflow rate (m^3). The gas concentrations were measured using 1312 Photo acoustic Multi-Gas Monitor with multi-channel sampling and dosing gas analyser Multipoint Sampler 1309 (Innova Air Tech Instruments, Denmark). The airflow rate was monitored using measuring fans, and air temperature was recorded by thermocouples. Sampling tubes installed at measuring points (indoor: 3 ceiling fans, animal zone; outdoor) transported air from the measuring points to the analyzer. Thermocouple probes scanning the air temperature were also placed at these points and measuring fans were fitted at three ceiling fans. Air suction was ensured by compressors. The data measured were registered in a database at 12 min intervals (gases) or three times per hour (temperature, airflow rate) during both fattening cycles. To evaluate each cycle they were divided into three equally long time fattening phases (FP).

RESULTS

In the summer cycle the concentrations and daily emissions of all observed gases showed upward trend from I^{st} to III^{rd} FP. In the winter cycle (in contrast to summer cycle) the concentrations and daily emissions of CO_2, N_2O and CH_4 recorded downward trend from I^{st} to III^{rd} FP (exception CH_4 emissions - about stable trend in I^{st}, II^{nd} FP). In this cycle NH_3 concentrations and daily emissions showed decreasing or stable trend, respectively (from I^{st} to II^{nd} FP) a then increasing trend (from II^{nd} to III^{rd} FP). It was in contrast with H_2O emissions. H_2O

concentrations and daily emissions increased from Ist to IInd FP and then decreased from IInd to IIIrd FP.

Concentrations of NH_3, CO_2, N_2O, CH_4 and H_2O were 2.4, 2.1, 2.6, 1.5 and 1.6 times higher in winter cycle than in summer cycle, respectively. Daily emissions of NH_3, CO_2 and N_2O were 1.6, 1.4 and 1.7 times higher in winter cycle than in summer cycle, respectively. Daily CH_4 and H_2O emissions have about the same value in the winter cycle as in the summer cycle and therefore the ratios were 0.96 and 1.00, respectively.

Between winter and summer cycle the significant differences of NH_3, CO_2, N_2O, CH_4 and H_2O concentrations were found ($P<0.001$). Also the significant differences of NH_3, CO_2 and N_2O daily emissions between winter and summer were identified ($P<0.001$). Differences of CH_4 and H_2O daily emissions between winter and summer were not significant ($P>0.05$).

In the summer cycle the housing temperature recorded about the stable trend. In the winter cycle temperature decreased from Ist to IInd FP and then increased from IInd to IIIrd FP. Between winter and summer cycle the significant differences of housing temperature were registered ($P<0.001$).

In the summer cycle the average hourly airflow rate showed increasing trend from Ist to IInd FP and decreasing trend from IInd to IIIrd FP. In the winter cycle the average hourly airflow rate increased from Ist to IIIrd FP. The average hourly airflow rate was 1.5 times higher in summer cycle than in winter cycle. Between winter and summer cycle the significant differences of average hourly airflow rate were identified ($P<0.001$).

The total emissions of NH_3, CO_2 and N_2O, were 1.6, 1.4, 1.7 times higher in winter cycle than in summer cycle, respectively. The same was true for emission factors of NH_3, CO_2 and N_2O, which were 1.6, 1.4 and 2 times higher in winter cycle than in summer cycle. The total emissions and emission factors of CH_4 and H_2O recorded about the same value in winter cycle as in summer cycle. Therefore their ratios were 0.97 and 1.0; 0.96 and 0.99, respectively (tab. 1).

Tab. 1: Total emissions and emission factors of NH_3, CO_2, N_2O, CH_4 and H_2O during two fattening cycles

Gas	Total emission (kg)				Emission factor (kg.head^{-1}.year^{-1})		
	summer 105 days	winter 121 days	winter 105 days	Ratio w/s	summer	winter	Ratio w/s
NH_3	159	295	256	1.6	1.6	2.5	1.6
CO_2	60 168	94 962	82 405	1.4	601	814	1.4
N_2O	13.4	26.2	22.7	1.7	0.1	0.2	2
CH_4	1 162	1 294	1 122	0.97	11.6	11.1	0.96
H_2O	110 124	127 669	110 787	1.0	1 100	1 094	0.99

DISCUSSION

Emissions of monitored gases were directly dependent on their concentrations in housing area and emitted airflow rate. Emitted airflow rate was associated with ventilation intensity, which was affected by temperature of housing area. The gas concentrations were associated with degradation of animal excreta (degradation and storage conditions CO_2. In case of CO_2 and H_2O their concentrations are also related with oxidative demands of animals.

In summer cycle NH_3 concentrations and daily emissions increased from Ist to IIIrd FP. It was due to the filling of slurry pits and growth of animals. This was in conformity with study of Osada [10], who found that a larger slurry amount has a larger NH_3 emission potential. Also distance of slurry surface from slates plays here specific role. The same was observed by another scientists [1,3,10], who discovered a direct correlation between the growth of animal and the amount of NH_3 emissions. This characteristic was also applied for N_2O and CH_4 emissions, the main source of which was slurry degradation. Daily emissions of CO_2 a H_2O increased from Ist to IIIrd FP, reproducing upward trend of their concentrations. This was conditioned mainly by the growth of animals and thus higher demands for the body oxidation per livestock unit (LU). It was confirmed by the study of Nicks [9], who found that the higher CO_2 and H_2O productions were the result of higher oxidative requirements of pigs (per LU). Because in this cycle the temperature had about the stable trend and airflow rate decreased from IIst to IIIrd FP they could not affect positively the emissions of monitored gases.

In winter cycle NH_3 daily emissions were the same in Ist and IInd FP, and NH_3 concentrations decreased from Ist to IInd FP. It was induced by discharge of slurry pits in IInd FP, what was confirmed in another study too [10]. Ventilation capacity in these FP (Ist-IInd) increased and therefore NH_3 daily emission in IInd FP was the same as in Ist FP. Later NH_3 daily emissions increased (from IInd to IIIr^{d} FP) due to increasing of NH_3 concentrations (refilling of slurry pits), growth of animal [6] and increasing of ventilation capacity. Also the temperature could affect enzymatic processes in slurry and thus NH_3 release from its [2,5,4,7]. From Ist to IInd FP the temperature decrease and NH_3 concentration decreased also. Later from IInd-to IIIrd FP the temperature increased, what was reproduced by increase of NH_3 concentration and emission. In this cycle N_2O and CH_4 concentrations and N_2O daily emissions decreased (Is^{t}-IIIrd FP) and CH_4 daily emissions had about the same trend (Ist, IInd FP) and then decreased (IInd-IIIrd FP). This was in contrast to NH_3 concentrations

and daily emissions, which increased from IInd to IIIrd FP. It could be induced by increase of ventilation capacity, which supports aerobic degradation. In this cycle CO_2 daily emissions decreased from Ist to IIIrd FP, what was in accordance with decrease of their concentrations. In these FP (from Ist to IIIrd) the ventilation capacity increased, but this increase did not affect emission decrease from Ist to IIIrd FP. CO_2 daily emission was the greatest in Ist FP that was associated with heating of housing area (winter cycle, small pigs), when CO_2 was produced by the burning of natural gas. This affected CO_2 concentration and daily emission in Ist FP [8]. Daily H_2O emissions increased from Ist to IInd FP a then decreased from IInd to IIIrd FP, replicating their concentrations. This situation was very hard to explain. The greatest H_2O daily emission was in IInd FP, although the best condition for the greatest emission was in Ist FP (higher temperature and therefore higher evaporation of H_2O from slurry, residual water from disinfection before starting of fattening cycle could also be present). It was not possible to apply higher oxidation requirements of animals (higher CO_2 and H_2O production) per livestock unit [9], because these would be the greatest in IIIrd FP (the highest body weight of pigs). Just in IIIrd FP the lowest H_2O emission was recorded.

CONCLUSIONS

Concentrations of all observed gases and daily emissions of NH_3, CO_2 and N_2O showed significant differences between winter and summer cycle ($P<0.001$). Only differences of CH_4 and H_2O daily emissions between winter and summer cycle were not significant $P>0.05$). Therefore for objective determination of emissions and emission factors of hazardous gases (mainly NH_3, CO_2 and N_2O) one-year measurement period is necessary.

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ODOUR NUISANCE AT PIG FARM

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SUMMARY

The aim of the investigation was to determine odour concentration and to identify volatile odour compounds in the air of pig farm. Air samples were collected at commercial pig farm, in the building with slatted floor (3 sections) where 180 weaner pigs were housed. Sampling of volatile odorous compounds in each section of the building was performed with headspace solid phase microextraction following GC-MS. The tentative identification of odour compounds based on comparative analysis of determined mass spectrum and mass spectrum of commercial library NIST. Odour concentration was determined by dynamic olfactometer. Based on determined results emission indicators were calculated.

On the basis of obtained results, high odour nuisance of examined air in each of 3 sections of the pig rearing building was approved. It was determined at the level of 3472,67 ou_E/m^3, which gives 19,29 ou_E/head. The VOC's identification in the tested building, that was carried out parallel, enabled to determine 10 volatile compounds, including indole, being one of the most nuisance volatile compounds, recognized as the key odour component at pig farm.

INTRODUCTION

Large-scale farms have bad influence on the comfort of people living in their neighbourhood.

As a result of farming structures and residential areas that were manufactured through the years, minimal distance are often not kept and people living in the vicinity of stock-raising farms are complaining about their nuisance more and more often. However, new livestock buildings or expanding production plants may be permitted only by use of far-reaching means to reduce the emission. On the basis of researches from the last years as well as analyses of residents complaints, carried out by the Institute for Environmental Protection, rise of odour emission was noted and its main sources were defined. The biggest contribution to the odour generation has production (43%). Animal breeding causes 17% of whole odorous emission. From among breeding animals most gases are emitted during poultry and pigs breeding, respectively 39% and 35%. The smallest participation in the emission have animals valued for their fur 9%, livestock, beef and dairy cattle 5% [10]. The rise of odour emission is a big problem for people. Their particular properties cause that human olfactory sense is able to detect and recognize odorous compounds under the threshold of detection, typical of technical devices. It is supposed that human nose is able to detect the gas existence by already 10^8 - 10^9 molecules of fragrant substance, on the contrary to the measuring instrument which detection ability starts by 10^{16} (1 g of ethyl mercaptan in the air contains 10^{16} molecules of its gas). It is $10^7 – 10^8$ times more than the human threshold of detection. The talent for smelling offensive fragrances depends on individual features as well as on environmental factors [1, 2, 11].

Currently, a number of studies on their identification and classification is carried out. Jugowar [8] has identified until now 136 compounds generated during the animal breeding, from among them about 23-24 were recognized. Hartung [6] has also classified in the gas mixture composed of 136 compounds only 22 of them. Mostly appearing compounds are: ammonia, hydrogen sulphide as well as thiols, sulfides, fenoles, ketones, aldehydes, aliphatic acids, esters, aliphatic amines, heterocyclic compounds containing sulphur, nitrogen, aliphatic alcohols. Additionally, the occurring of aromatic hydrocarbons such as toluene and xylene was proved. Of the greatest importance from among all the substances are ammonia and hydrogen sulphide, because of their occurrence in high concentration and standardization of their level [4, 10, 12].

The extension of gases created as a result of animal production depends on many factors. Their variability causes that precise estimation of the odour concentration nearby the livestock buildings is very time-consuming and demands use of many measuring instruments, therefore, it is very costly and difficult. Factors having influence on the gas extension are following: equilibrium state of the atmosphere, wind speed, emission points level, ground roughness, temperature inversion and coefficient of atmospheric diffusion. An important factor is also the distance form livestock building, because the rarefaction of gases in the air takes place in conjunction with the distance growth [8].

The aim of the investigation was to determine odour concentration and to identify volatile odour compounds in the air of pig farm.

MATERIAL AND METHODS

Air samples were collected at commercial pig farm, in the building with slatted floor (3 sections) where 180 weaner pigs were housed. Odour compounds were extracted (sampling time=60 min.) from headspace of each sections in the temperature of 25^0C. The extraction of analyzed substances was carried out by the SPME technique, manual SPME holder with StableFlex 50/30 µm divinylbenzene/carboxen/polidimethylsiloxane DVB/CAR/PDMS fibres was used (Supelco, USA). The injection port operated at 240^0C for SPME desorption (time of desorption - 7 min.). Before each sampling fibres were conditioned for 1 hour in the injection port at 270^0C. Chromatographic separation was performed using Restec column (5% diphenyl; 30m x 250µm x 0,25µm) in Thermo Electron, Finnigan Focus Polaris Q type gas chromatograph coupled with mass spectrometer (GC/MS method). The identification of odorous compounds was based on comparative analysis of determined mass spectrum and MS from spectrum commercial library NIST. Odour concentration was determined by dynamic olfactometer according to PN-EN 13725 directives. On the basis of determined results emission indicators were calculated.

RESULTS AND DISCUSSION

On the basis of obtained results, high odour nuisance of examined air in each of 3 sections of the pig rearing building was approved. It was determined at the level of 3472,67 ou_E/m^3, which gives 19,29 ou_E/head (Table 1).

Calculated emission coefficients were on average by 29 ou_E/s/head. The values demonstrate quite high odour nuisance of the estimated pigs rearing sector. It is also an evidence of data published in the report of UE Odour Impacts and Odour Emission Control Measures for Intensive Agriculture [13], where for example in Netherlands the emission coefficient in this stage of porker rearing amounts to 16,6 ou_E/s/head maximal. Additionally, the report informs about recommended optimal value of the emission coefficient per head in this production stage which amounts to 6 ou_E/s/head. Slightly lower odour emission rate per animal was obtained by Hayes et all [7] who have estimated fragrant nuisance of pig farms in Ireland. The emission coefficient of examined pig weaner sections oscillated between 3,7 and 4,6 ou_E/s/head.

Table 1: *Odour nuisance in pig weaner section in 3 repetitions for each section*

Section	Odour nuisance [ou_E]	Odour nuisance [ou_E/head]	Odour emission rate [ou_E/s/head]	Average olfactory nuisance [ou_E]	Average olfactory nuisance [ou_E/head]
I	3597	19,98	30,21	3471,67	19,29
II	2896	16,08	24,32		
III	3922	21,78	32,68		

The VOC's identification in the tested building, that was carried out parallel, enabled to determine 10 volatile compounds (Fig. 1.).

Among substances identified during own studies also occur characteristic VOC'S like: heptanes-1-ol, heptan-2-on, 1,4 dichlorobenzene, nonane-4-on as well as indole. The researches of Cai et all [3], Schiffman et all [14] confirm above results. Pointing at indole as key odorous component is well-grounded. It is confirmed by above quoted researches as well as the studies of Kai and Schafer [9] that also indicate i.a. indole as characteristic odorous air component in pig weaner buildings. On the other hand, Hanajima et all [5] points as key odorous components derived from pig feaces NH_3, methyl mercaptan and dimethyl disulphide.

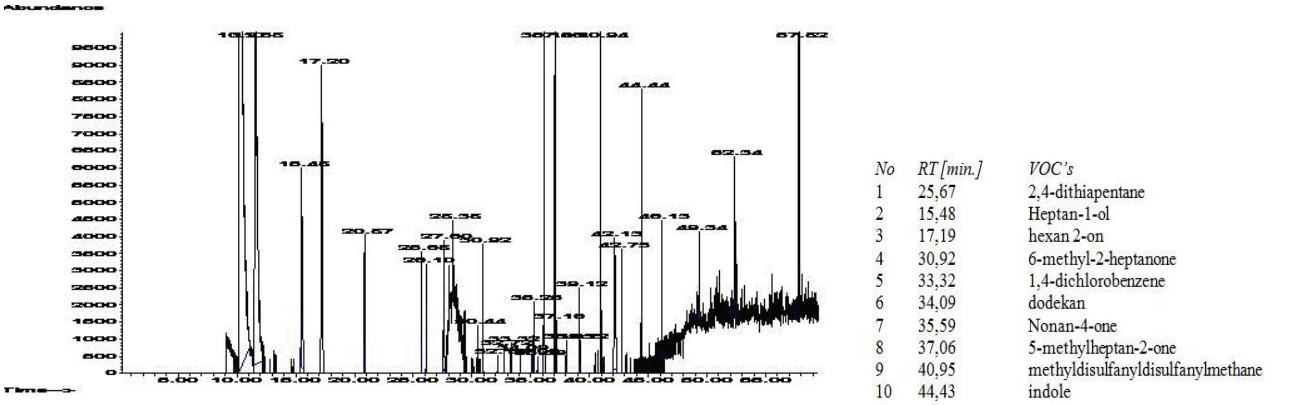

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Figure 1: Chromatogram of gas sample collected from weaner pig section.

CONCLUSIONS

The investigated building at commercial pig farm was characterized by high odour nuisance (19,29 ou_E/head) and indole was determined as the key odour component.

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EVALUATION OF BLOOD LEAD AND CADMIUM STATUS IN SHEEP GRAZING ON STREET GARBAGE

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SUMMARY

The goal of the present study is to evaluate blood lead and cadmium status in sheep grazing on street garbage. A total number of 30 ewes (2 – 3 years old) were subjected to study. Animals were classified into two groups, the first group (No.= 20) included animals grazed on street garbage in Egypt, the second group (No.= 10) were selected from sheep lived indoor at the Veterinary Teaching Hospital, Assiut University, Egypt. Blood lead concentration was 1.44 ± 0.16 (ppm) in the street garbage group and was 0.38 ± 0.17 (ppm) in the Indoor group. Blood cadmium concentration was 0.16 ± 0.02 (ppm) in the street Garbage group and was 0.008 ± 0.004 (ppm) in the Indoor group. Statistical comparison of the two groups revealed that street garbage group had a higher blood lead and cadmium concentrations than the indoor group. In conclusion, the increased blood lead and cadmium in sheep grazing on street garbage indicated the continuous exposure to the two elements and bear significant health hazard on the health condition of animals and on human health.

Keywords: sheep, lead, cadmium, street, garbage

INTRODUCTION

Industrial and agricultural processes have resulted in the release of many toxic metals into the environment, although relatively high concentrations can also occur naturally. Cadmium, lead, arsenic, and mercury are the elements that have probably caused the most concern. This is because they are readily transferred through food chains and can pose a potential health risk to animals and humans [20]. Lead is considered one of the major environmental pollutants and has been incriminated as a cause of accidental poisoning in domestic animals more than any other substance, particularly in cattle, sheep and horses [14]. Cadmium is recognized as one of the most toxic elements to man and animals. This metal is naturally present in the environment, including sea and fresh water, soils, sediment and air [23]. It is used in many industrial processes and it is a contaminant in some fertilizers, especially in partially acidulated phosphate fertilizers [17] and in urban sewage sludge to fertilize pastures or crops [9, 13, 15]. This widespread distribution and the industrial fallout have resulted in all food being exposed to and containing cadmium [19]. Sheep naturally grazing on street garbage is exposed to a wide variety of health hazard materials, that originate from the exposure to environmental pollutants and from eating garbage that contains hidden toxicants, the evaluation of blood lead and cadmium status in sheep naturally grazing on street garbage is the aim of the present study.

MATERIAL AND METHODS

Animals

A total number of 30 ewes (2–3 years old) were subjected to study. Animals were classified into two groups, the first group (No. = 20) included animals naturally grazed on street garbage in Assiut City, Assiut Governorate, Egypt. The second group (No.=10) was selected from sheep live indoor at the Veterinary Teaching Hospital, Assiut University, Egypt.

Samples

Blood samples (5 ml) were collected from the jugular vein in Vacutainer tubes containing heparin, and kept in deep freeze (-20 ºC) for chemical analysis.

Digestion of samples

Blood samples were [27], briefly, to each 1 ml whole blood sample, 2 ml of digestion mixture (equal volume of concentrated nitric acid and 72% perchloric acid) was added in a 50 ml Teflon beaker and left to react over 24 hours at room temperature. The mixture was then heated on a hot plate at 100ºC until the sample became colorless.

The samples were then diluted with bidistalled water up to 20 ml.

Analytical methods

Lead and cadmium concentrations were determined in digested whole blood using Atomic Absorption Spectrophotometry (Atomic absorption 906, GBC, Australia). Certified standard solutions of the elements were used for the preparation of the elements standard working solutions.

Statistical analysis

Statistical analysis was conducted using SPSS 16.0 for windows (SPSS, Chicago, USA) and were carried out using one way ANOVA. Data were expressed as Mean ± SD.

RESULTS

Blood lead concentration was 1.44 ± 0.16 (ppm) in the street Garbage group and was 0.38 ± 0.17 (ppm) in the Indoor group.

Blood cadmium concentration was 0.16 ± 0.02 (ppm) in the street Garbage group and was 0.008 ± 0.004 (ppm) in the Indoor group.

Statistical comparison of the two groups revealed that street garbage group had a higher blood lead and cadmium concentrations than the indoor group. Results are summarized in table 1.

Table 1. Blood lead and cadmium concentrations in sheep

	Street garbage group (No.=20)	Indoor group (No.=10)
Lead (ppm)	1.44 ± 0.16**	0.38 ± 0.17
Cadmium (ppm)	0.16 ± 0.02**	0.008 ± 0.004

DISCUSSION

Increasing biological interest in minerals has led to the search for reliable methods to quantify body levels of trace elements and toxic metals. A number of studies have reviewed specific aspects of lead toxicity in man [10, 26] and animals [1, 2, 6, 11, 12, 16, 21, 24]. Various specimens, such as blood (8, 18, 25] may be used to assess element status in man and animals. In some areas of Assiut City (Assiut, Egypt), sheep are grazing on street garbage, which may carry health hazard. Significant increase in blood lead concentration ($p<0.01$) was observed in sheep grazing on street garbage (1.44 ± 0.16 ppm) when compared with the indoor group (0.38 ± 0.17 ppm). Sheep grazing in the street are daily exposed to lead, which originates from various industrial operations and automobile exhausts [7]. After combustion, the tetraethyl lead contained in petrol settles as lead oxide or chloride on the vegetation by roadsides [4]. Blood lead observed in the current study is higher than the permissible limit in the blood of ruminant. Whole blood lead level in normal ruminants is usually below 0.05 – 0.25 ppm [22].

Blood cadmium concentration was significantly higher in street grazing group (0.16 ± 0.02 ppm) than the indoor group (0.008 ± 0.004 ppm). The higher level of cadmium in the grazing group may be attributed to the ingestion of incinerated solid wastes during grazing on garbage [3], or may be due to the intake of foods contaminated with cadmium from manmade sources [5].

CONCLUSIONS

The increased blood lead and cadmium in sheep grazing on street garbage indicated the continuous exposure to the two elements, which may affect the reproduction and production of the animals and bear significant health hazard to human.

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RESEARCHES ON LEAD POLLUTION AND ITS INFLUENCE UPON THE ANIMALS IN THE EASTERN AREA OF BUCHAREST

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SUMMARY

Lead is one of the pollutant elements emitted in the eastern part of Bucharest. It has a high binding capacity of various proteins and minerals from organs, its toxicity being obvious at vascular, neurological and kidney levels.

In the area of influence of "Acumulatorul" industrial unit lead was measured in air, water samples, forages, soil and organs (liver and kidney). Determination of lead was achieved by atomic absorption spectrometry, and the interpretation was based on the regulations in force. For animals, analysis methods consisted of clinical and histopathologic examinations.

Lead concentrations exceeded maximum admitted limits in "Acumulatorul" area both in air samples and in the soil, water, forage and organs ones, the values decreasing with the distance from the pollution source. High doses of lead established from samples collected were correlated with symptoms of neural and gastrointestinal disorders and lesions located in the filtering organs (liver and kidney).

Correlation of lead concentrations in air, soil, water, plants and organs with clinical signs as well as the anatomopathological changes confirms the status of chronic lead toxicosis in the eastern part of Bucharest.

INTRODUCTION

In the areas nearby the polluting units, neglecting the environmental protection measures can often cause air, water and soil degradation with severe consequences on plants and animals [8]. Among other elements produced by "Acumulatorul" plant (the main polluting agent in the Eastern area of Bucharest), lead is also present.

Lead has no proved physiological role in organism and it is considered having a toxic activity at vascular, neurological and renal levels due to its capacity of binding different proteins and minerals [2, 6].

The present study is meant to be an alert signal due to the fact that pollution has reached such a level that could affect humans, animals and their environment [5].

MATERIAL AND METHODS

In the Eastern area of Bucharest, near "Acumulatorul" plant there were collected samples of water, soil, forage and organs (liver and kidneys) both from dead and slaughtered animals in case of necessity.

From each sample there was established lead concentration. There was also measured lead in air samples, considering that this heavy metal has a harmful effect by massive inhibition of the Delta-aminolevulinic acid dehydratase.

The analyze method consisted in atomic absorption spectrophomometry. Results interpretation was made according to the provisions in force: Regulation 1881/2006, Government Resolution (G.R.) 128/2002, Law 311/2004, Order 161/2006 of Environment and Water Management Ministry.

Soil samples were collected from different distances from the main pollution source: 200, 500, 1000, 2000 meters and different depths (between 0 and 100 cm).

For animals, there were run clinical examinations and anatomopathological and histopathological exams for the organs.

RESULTS

The average lead concentration levels in air are shown in table no. 1 for three directions from the pollution source: East, South and West, at three distances: 50 m, 500 m, respectively 1500 m. Analyzing the data in the table, it can be noticed that the highest overvalues in relation with the maximum limit have been recorded for the samples collected from the East of the polluting source, at 50 m distance. Lead exceeding in the air samples, collected at 50 m distance of "Acumulatorul" plant was: East – by 16 times, South – by 2.5 times and West – by 9.3 times; while for the samples collected at 500 m distance, it was: East – by 5.56 times, South – by 2.58 times and West by 1.8 times.

Table 1: Average lead values in air samples – Acumulatorul Area

Sampling point	The distance from the pollution source		
	50 m	500 m	1500 m
East	7,96 mg/m^3	2,78 mg/m^3	0,52 mg/m^3
South	1,27 mg/m^3	1,29 mg/m^3	0,01 mg/m^3
West	4,56 mg/m^3	0,9 mg/m^3	0,02 mg/m^3
The maximum admitted limit GR no. 128/2002	0,5 mg/m^3		

It is noticed that the higher the distance from the pollution source the lower lead concentration values. Thus in South and West, at 1500 m distance, the obtained values ranged within the normal limits.

Table 2: Average lead values in soil samples - Acumulatorul Area

Sampling area	Sampling depth (cm)	Obtained values depending on the distance from the pollution source (ppm)			
		200 m	500 m	1000 m	2000 m
In the East of the polluting source	0-5	750	250	450	200
	5-10	1000	-	-	-
	10-20	75000	-	-	-
	20-50	150	-	-	-
	50-100	150	-	-	-
In the West of the polluting source	0-5	700	450	200	150
	5-10	400	-	-	-
	10-20	1800	-	-	-
	20-50	300	-	-	-
	50-100	250	-	-	-
The admitted limit (GR no. 128/2002)	100 ppm				

Soil analysis (table no. 2) in the neighborhood of the industrial area confirmed lead presence in surface soil (0 – 5 cm), with values exceeding by 2.5 to 7 times the admitted limit. The concentrations decrease with the distance from the polluting source.

The presence of lead in the soil samples in concentrations that exceed both the warning and intervention limits implies long and continuous soil pollution with this metal.

In table no. 3 there are presented the values of pH and lead concentrations in drinking and surface waters.

Table 3: Average lead values in water samples - Acumulatorul Area

Source type	Sampling point	No. of samples	Obtained values mg/l	Water pH
Surface water	Lake 1	10	0,004	7,9
	Lake 2	12	0,039	7,9
	Lake 3	12	0,038	7,7
The maximum admitted limit: 161/2006 Order	0,05 mg/l			-
Drinking water	Well 1	12	0,09	7,6
	Well 2	10	0,027	8,0
	Well 3	12	0,031	7,8
The maximum admitted limit: 311/2004 Law	0,01 mg/l			6,5-7,4

Analyzing the results, it can be noticed that lead concentrations in the surface waters did not exceed the maximum admitted limit in any sample, while in drinking water there were recorded overvalues in all sampling points, by 3 to 9 times. Regarding water pH, it is slightly alkaline.

Lead's high concentrations detected in the soil and water had a negative effect upon the cultivated plants in this area, used as forages. By radicular absorption, the plants stored lead in concentrations that exceed the maximum limit by 2.4 times in the alfalfa hay and by 5.48 times in the fodder flour (table no. 4).

Table 5: Average lead values in forage samples - Acumulatorul Area

Sample type	No. of samples	Lead level mg/kg	The maximum admitted limit Reg. 1881/2006 mg/kg
Alfalfa hay	10	94,65	40
Wheat flour	12	54,81	10

Lead concentration in animal organs (table no. 5) recorded exceeding of the maximum admitted limits by 16 times in liver and by 24 times in kidneys.

Table 5: Average lead values in dead or slaughtered animals - Acumulatorul Area

Sample type	No. of samples	Lead values mg/kg	The maximum admitted limit Reg. 1881/2006 mg/kg
Liver	11	8,18	0,5
Kidney	11	11,86	0,5

The clinical examination of the animals in "Acumulatorul" area revealed loss of appetite, incoordination and equilibrium disorders, violent psychomotor signs, digestive troubles with saturnine colic, head against the manger posture, dromomania.

The histopathological examination revealed: hepatic cells degeneration caused by fat infiltration in the peripheral areas of the hepatic lobules, bile duct hyperplasia, degenerative kidney modifications, glomerulo-nephritis.

The high lead concentrations in air, water, soil, plants and organs samples were correlated with the clinical symptomatology of the animals, dominated by psychomotor and digestive signs and with severe hepatic and renal lesions.

DISCUSSION

In relation with the results obtained by other authors [1, 4, 7], the study shows alarming values which point a major lead pollution in the area near Bucharest. The pollution level is close to that in the well-known critical point in Romania – Copsa Mica [3], the lead values being similar for water, soil, fodder concentrations and just a little lower (by 1.4-1.5 times) for liver and kidney concentrations.

CONCLUSIONS

1) Lead concentrations exceed the maximum admitted level by 2,5-16 times in air samples collected from the eastside of "Acumulatorul" area, at 50 m distances. It was observed a decreasing of obtained values with the distance from the polluting source.

2) The average lead content in "Acumulatorul" area show overvalues of maximum admitted limits by 2-7.5 times in water samples, by 2-5 times in soil and by 16-24 times in organs.

3) The high lead concentrations found in air, water, soil, plants and organs, are correlated with clinical signs and pathological changes, which confirm the fact that there is present a chronic lead poisoning in the East side of Bucharest.

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THE PROPOLIS AS A BIOINDICATOR OF ENVIRONMENTAL HEAVY METALS POLLUTION

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SUMMARY

The aim of the study was to determine the extent of chosen toxic elements (Zn, Cu, Pb, As and Cd) bioaccumulation in propolis collected in Opole area and determination of propolis usefulness as a bioindicator of environmental contamination. The research material were samples of propolis originated from 5 bee colonies in 30 apiaries (n=5x30=150). Quantitative analysis of studied elements were conducted using Varian ICP-AES plasma spectrometer with mass detection controlled, and CETAC-5000 AT ultrasonic nebulizer. The presence of toxic elements was determined in an examined biological materials. The sequence of accumulation level of studied elements in propolis was as follows: Zn>Cu>Pb>As>Cd. An average concentration of zinc, copper, lead, arsenic and cadmium amounted to 56.28, 7.12, 6.91, 0.745, 0.218 $mg \cdot kg^{-1}$, respectively.

INTRODUCTION

The industry and motorization development as well as intensive agriculture based on the chemicalization contributed to a massive increase of environment pollution. Among many pollutants there are elements of toxic properties, which natural content in soil and the atmosphere is insignificant [2, 5, 20]. Nevertheless, they are widely used in the human economy, therefore they are common environmental pollutants. Even at low concentrations they may cause a lot of diseases and abnormalities in the functioning of the human and animal organisms.

Honey bee (*Apis mellifera* L.) is inextricably linked with the external environment, from which it derives not only air and water, but food as well. The products gathered by the bee colony are kind of pooled samples derived from a large area. The pollutants occurring in a given area can also be accumulated in the raw material collected by bees, and in bees itself [9, 17, 18]. Therefore, bees and bee products can be a valuable indicator material in the investigation of environmental contamination [1, 3, 15, 19].

During the processing of raw materials to propolis by worker bees its purification does not take place. Therefore, it contains many minerals and trace elements which concentration reflects the given region abundance with these chemical elements [6, 15]. There is a close correlation between the level of heavy metals accumulation in soil and plants and their content in bee products [11, 15, 19].

MATERIAL AND METHODS

The research material for the investigation were samples of propolis originated from stationary apiaries situated in Opole area. Material was collected from May to August 2009. Propolis samples originated from 5 bee colonies in 30 apiaries (n=5x30=150). The individual samples were combined into one pooled sample weighting about 50 g representative for the particular apiary. Propolis was collected directly from the hives by scraping down with a sharp instrument from their wooden elements (walls and frames) to a clean plastic containers. The received samples were homogenized by freezing, fragmentation and mixing. The 1000 mg of material from each sample was weighted (with precision of 0.1 mg) and diluted with 20 ml of concentrated, spectrally pure, nitric acid solution produced by Merck company. Next, samples were mineralized using the microwave technique at an elevated pressure in the chip-type MD-2000 station manufactured by CEM-USA. Quantitative analysis of studied elements were conducted using Varian ICP-AES plasma spectrometer with mass detection controlled by P-3202 computer cooperating with Philips Scientific analytical combine (PU-7000 model), and CETAC-5000 AT ultrasonic nebulizer. Quantitative analysis were conducted in Analytical Laboratory of Wroclaw University of Environmental and Life Science (Poland). The one-way analysis of variance (ANOVA) was performed to evaluate differences among groups. Mean concentrations of elements, standard deviations and correlations between elements were calculated. Significance level was taken as $P \leq 0.01$.

RESULTS

Toxicological status of propolis fully reflects the harmful compounds and toxic elements contamination of raw materials and the environment. The study results are presented in Table 1. Very high level in this product was observed in the case of zinc, which averaged 56.28 mg·kg^{-1} and in individual samples ranged from 11.49 to 118.32 mg·kg^{-1}. In turn, the maximum copper content was determined as 16.38 mg·kg^{-1}, whereas the average was 7.12 mg·kg^{-1}. Another heavy metal which concentration was high in propolis was lead. Its mean concentration level was 6.91 mg·kg^{-1} (maximum 19.86 mg·kg^{-1}). The present study demonstrated that arsenic content in propolis amounted to 0.745 mg·kg^{-1} on average, and in individual samples ranged from 0.009 to 1.921 mg·kg^{-1}. Whereas, concentration of cadmium in samples was an average of 0.218 mg·kg^{-1}. However, the dispersion of results was very wide from 0.008 to 0.889 mg·kg^{-1}.

DISCUSSION

The concentration of some toxic elements in the propolis samples presents high variability. Numerous authors draw an attention to the fact that there is a high correlation between the concentration level of minerals in bee products and their content in an external environment [1, 6, 13, 14, 19]. Therefore, these authors indicate the possibility of environment pollution degree determination through the analysis of the contamination of bee products. Present study demonstrated that propolis was contaminated with toxic elements. This is confirmed by numerous research of other authors, which showed high concentrations of heavy metals in propolis [6, 7, 12] and also claimed that propolis is much more contaminated with heavy metals than any other bee products. In the present research the highest concentration was demonstrated in the case of Zn with average of 56.28 mg·kg^{-1}. The content of this element observed by Dogan et al. [8] in propolis samples from different regions of Turkey was 176-676 mg·kg^{-1}. Whereas, Cvek et al. [7] demonstrated even the value of 9326 mg·kg^{-1}. Next was copper where average content was 7.12 mg·kg^{-1}. Roman [15] observed several times higher concentration of that metal in the propolis from the region of Głogów which ranged from 23.51 to 34.15 mg·kg^{-1}, while Dogan et al. [8] received higher value in the propolis from different regions of Turkey - an average of i.e. 45-96 mgCu·kg^{-1}. Lead is one of the most burdensome heavy metals in the environment. Its concentration was also high in propolis and reached an average level of 6.91 mg·kg^{-1} (maximum 19.86 mg·kg^{-1}). In other studies, Roman [16] showed the mean level of Pb concentration amounted to 18.39 mgPb·kg^{-1} in the propolis from Głogów and from Rudna area from 6.73-17.83 mg·kg^{-1} on average [16]. Similar results were obtained when examining the mean concentration of this element in propolis from the region of Opole (6.62-13.63 mg·kg^{-1}). It can be thus concluded that lead concentration in propolis was high and relatively stable. The present study demonstrated that arsenic content amounted to 0.745 mg·kg^{-1} on average. Very similar results were shown by Roman [15] in propolis originating from the Opole and LGOM area, i.e. 0.561 and 0.670 mg·kg^{-1}, respectively. This is most likely the result of a significant contamination of soils in the areas of metallurgical and chemical industries and extensive urban areas, where the accumulation of arsenic in the soil reaches up to 2500 mg·kg^{-1} [12]. Generally, the cadmium concentration in the examined bee product was the smallest and reached an average of 0.218 mg·kg^{-1}. In earlier studies conducted by Roman [15] lower levels of Cd, ranged from 0.043 to 0.116 mg·kg^{-1} were observed in propolis originating from the region of the copper industry, but significantly higher in the product coming from the region of the cement industry - an average of 0.513 to 0.795 mg·kg^{-1}. Moreover, Roman [16] noted higher Cd content in the propolis from the Wałbrzych region (0.260 mg·kg^{-1}).

The results of the present study and review of other authors results allow to conclude unequivocally that the level of toxic trace elements concentration in propolis depends on the state of environmental pollution in the area of the material sampling. Therefore, many authors [4, 7, 10, 13, 15, 20, 21] believe that propolis can be used as a bioindicator of environmental pollution, e.g. with heavy metals.

CONCLUSIONS

In present study propolis has shown high contamination level by toxic elements. The results suggested that this bee product is very good bioindicator and can be used to evaluate environmental pollutions degree trough determination the level of toxic elements accumulated in his samples.

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Table 1: Concentration of chosen elements in bee propolis (N=30)

No. of sample	Chemical elements (in $mg \cdot kg^{-1}$)				
	As	Cd	Cu	Pb	Zn
Minimum	0.009	0.008	1.09	0.39	11.49
Maximum	1.921	0.889	16.38	19.86	118.32
Average	**0.745** ^{A}	**0.218** ^{B}	**7.12** ^{C}	**6.91** ^{C}	**56.28** ^{D}
SD	0.607	0.200	4.56	5.69	32.10
Variation coefficient	81.5	91.6	64.0	82.3	57.0
LOQ	$\leq$ 0.001	$\leq$ 0.01	$\leq$ 0.01	$\leq$ 0.100	$\leq$ 0.001

A, B, C, D - differences between the elements assessed highly significant on a level of $p \leq 0.01$

OXIME REACTIVATION OF ACETYLCHOLINESTERASE INHIBITED BY ORGANOPHOSPHORUS COMPOUNDS (Abstract)

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The effectiveness of currently used (pralidoxime, obidoxime, and HI-6) and newly synthesized, K-serius oximes to reactivate dichlorvos (DDVP)-inhibited acetylcholinesterase from sheep, cattle, and pigs. The dissociation constant (K_{dis}) for oximes were obtained a highest affinity for the intact acetylcholinesterase (AChE) to HI-6 for sheep, cattle, and pigs. AChE activity was determined by the Ellman method, adapted for a plate reader. K_R affinity of new oximes to dichlorvos-inhibited AChE indicates that the high affinity to K048 for sheep and obidoxime for cattle, while K005 was highest for pigs. First-order rate constant for reactivation (k_r) was obtained highest for pralidoxime for sheep and HI-6 for cattle and pigs. Oxime K048 has the highest bimolecular constant of reactivation (k_{r2}) for sheep and pig, while HI-6 was highest in cattle. The purpose of this study was to find suitable reactivator of AChE and to recommend the most efficacious oxime compounds for the next evaluation as antidotes for intoxication by DDVP. Furthermore, the results of this study confirm that the reactivation effect depends on: (i) number of pyridinium rings, (ii) number of oxime groups and their position, and (iii) length and the shape of the linkage bridge between pyridinium rings. In conclusion, our result confirms that there is no single, broad-spectrum oxime suitable for the antidote treatment of poisonings with OP compounds for food animals. These results also indicated that the developed bisquaterenary symmetric (K027) and asymmetric bispyridinium (K048) oximes seem to be promising reactivator of DDVP-inhibited AChE for sheep and pigs while HI-6 was for cattle. However, obidoxime, ineffective against DDVP, is the most efficacious oxime for the reactivation of liver food animals inhibited AChE. Furthermore, little change in the structure of the AChE reactivator can greatly affects its affinity to the intact or inhibited enzyme.

ROLE OF ANTIOXIDANTS IN CONTROLLING REPRODUCTIVE TOXICITY IN RATS

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ABSTRACTS

50 rats at the age of weaning were used for these studies. Rats were divided into three groups. The first group fed on diet containing 80mg lead acetate / kg.bw. The second group fed on diet containing 80mg lead acetate / kg.bw and antox in a dose of 20 mg/animal/day, group three used as a control . It has been conclude that lead toxicity has a deleterious effects on both the female and male genital organs. These effects were manifested by qualitative and quantitative histopathological and serobiochemical changes in both ovaries and testes. Moreover a lead genotoxicity of the female reproductive tract was reported for the first time including metaplastic changes in the epithelium of the fallopian tube, uterine glands , cervix and vagina.

KEY WORDS

reproductive toxicity, lead, rats .

INTRODUCTION

The harmful effects of lead exposure are numerous. Lead has been reported as a cause of infertility in both male and femal.On the other hand, it was speculated that the antioxidant could prevent these harmful effects.

MATERIALS AND METHODS

50 rats were divided into three groups, as following:

Group one (Gr1): (Lead treated group) consisted of 19 rats which were subdivided into 9 males and 10 females. They were fed on diet containing 80 mg lead acetate / kg.Bw.

Group two (Gr2): (Lead and Antox treated group) consisted of 15 rats which were subdivided into 7 males and 8 females. They were fed on diet containing 80 mg lead acetate / kg .Bw. and Antox in a dose of 20 mg / animal / day.

Group three (Gr3): (Control group) consisted of 16 rats which were subdivided into 6 males and 10 females.

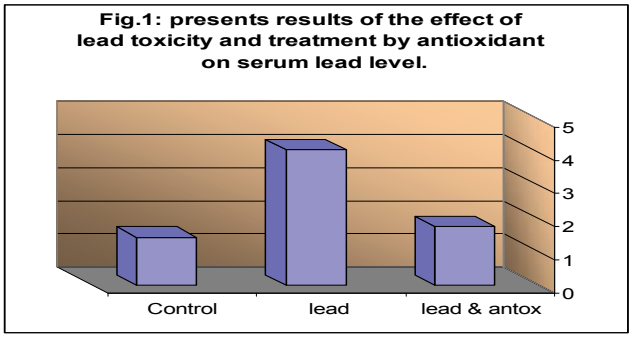
- This treatment continued for two months with observation of rats. After the end of the experiment, all rats were slaughtered .Testes, ovaries, fallopian tubes, uterus, cervix and vagina were collected and examined grossly. Samples for histopathological examination were obtained.

Serum was collected for determination of lead levels and estimation of testosterone hormone.

Counting technique: In females, serial sections of ovaries were taken. All types of follicles and corpora lutea were sorted and counted per ovary, both right and left for each case. In males, all types of spermatogenic and sertoli cells were counted in 10 cross sections for each right and left testicle for each case. The mean number of ovarian structures and spermatogenic cells were counted and statistically analyzed according to the following scheme.

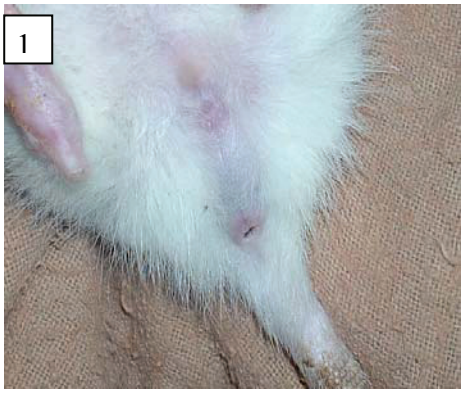
RESULTS

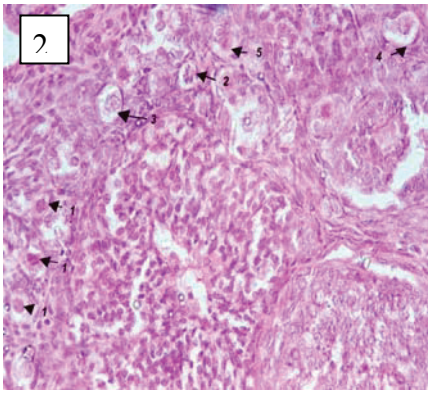
- Effect of lead toxicity on the Lead serum level:

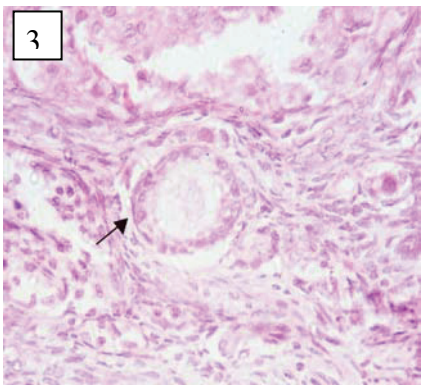


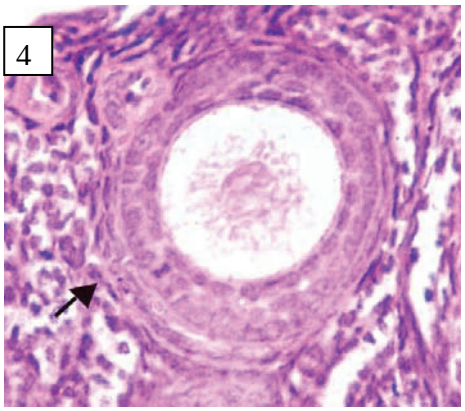
-Total number of examined follicles in different groups:

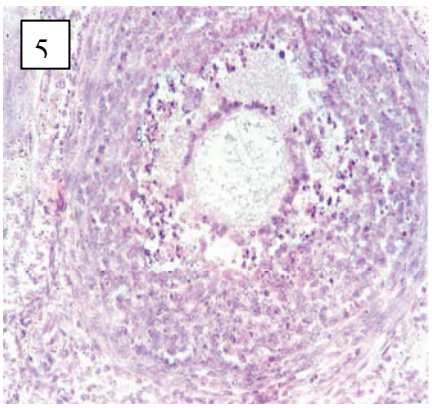
	Control group	Lead group	Antioxidant group	Total
Primordial follicles	226	195	188	609
Primary follicles	67	74	65	206
Secondary follicles	69	44	60	173
Vesicular follicles	137	100	102	339
Mature follicles	10	6	10	26
Corpora lutea	90	25	24	139

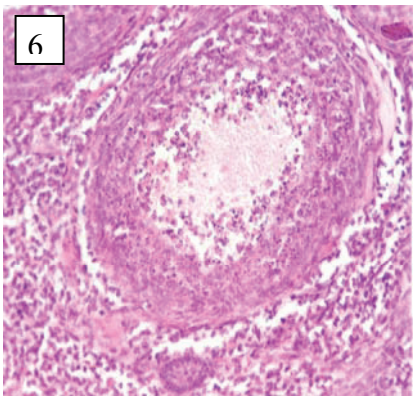


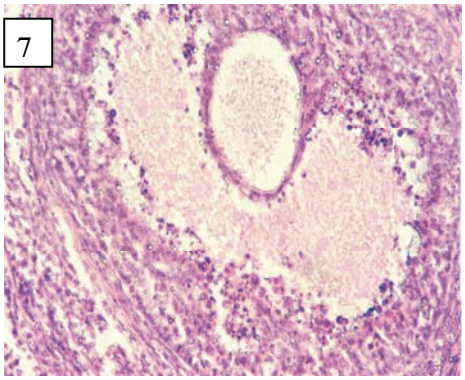


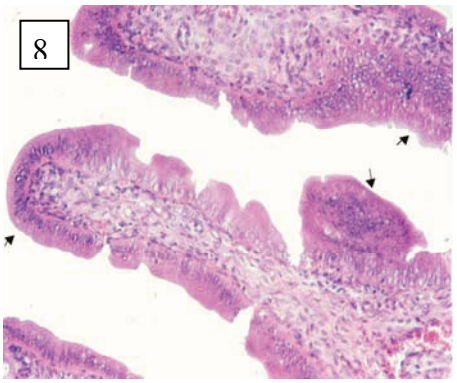


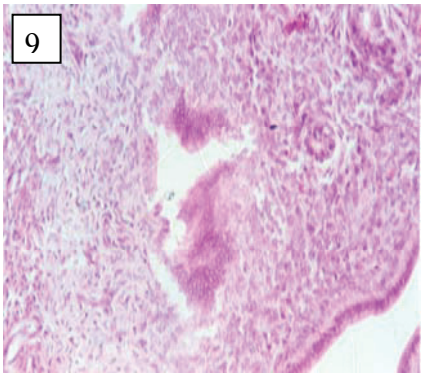




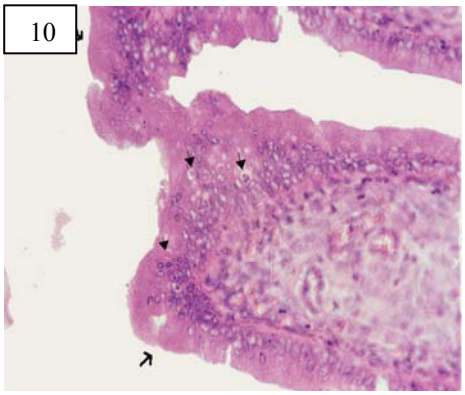


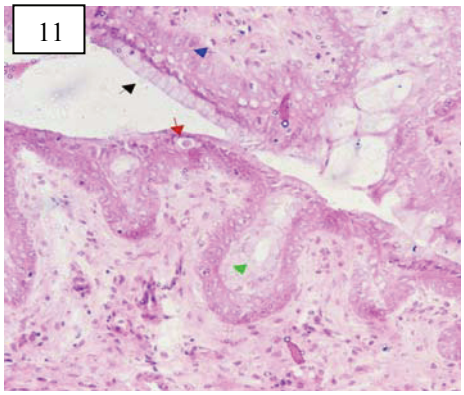


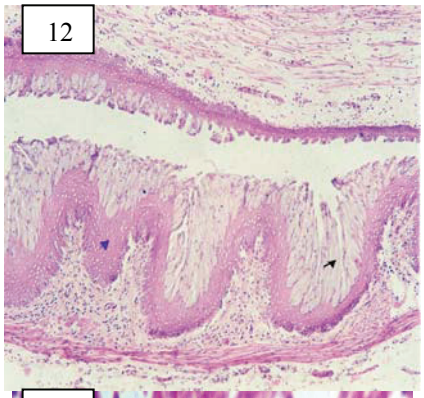


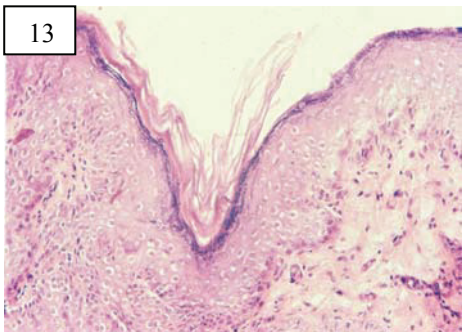


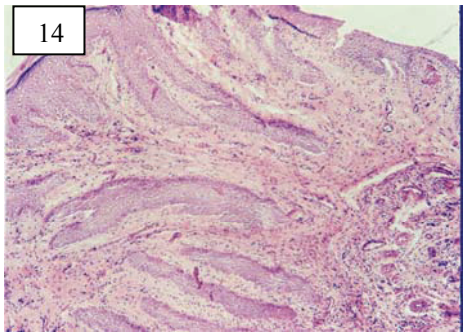
1. The lead toxicity delayed the day of vaginal opening.
2. Lead degeneration of primordial follicles → 1Karyopyknosis → 2 Karyorhxis → 3 karyolysis → 4 Cytoplasmic vaculation of oocyte. → 5 desquamation of the follicular cells. H&E X 40.
3. Lead degeneration of the primary follicle. Karyolysis and cytoplasmic vaculation of oocyte. Karyopiknosis of follicular cells. H&E X 40.
4. Lead Atresia of secondary follicles. Karyo and cytolysis of oocyte and some follicular cells. X 40.
5. Lead atresia of vesicular follicle. Karyo and cytolysis of oocyte.Pyknosis of granulosa cells. Desquamation of the pyknotic granulosa cells into the vesicular cavities. H&E X 40.
6. Lead atresia of the vesicular follicle. Complete lysis of the oocyte.Desquamation of pyknotic granulosa cells into the vesicular fluid.Pyknosis and The Thecal cells are suffering. H&E X 40.
7. Lead atresia of mature follicle. Lysis of oocyte with its vetelline membrane. Karyopiknosis and coagulation of the granulose cells and thecal cell. H&E X 40.
8. Lead toxicity, Focal metaplasia of the epithelium of the oviduct villi. The columnar epithelium has transferred into several layers with the following character: atypical aggregation of nuclei with slight atypism in size. H&E X 25.
9. Lead toxicity. Uterine gland metaplasia. H&E X 40.

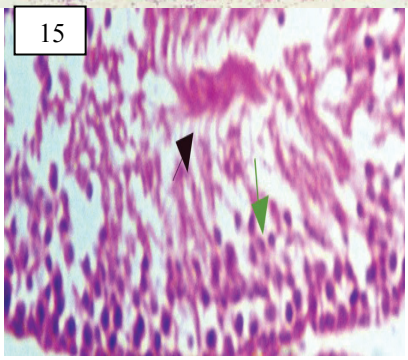


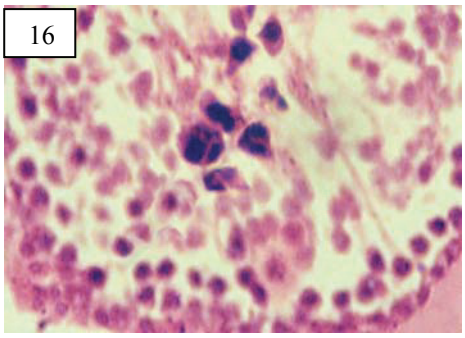


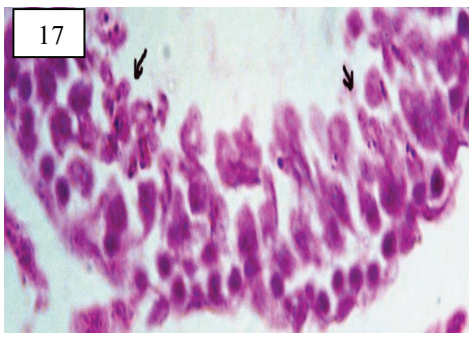


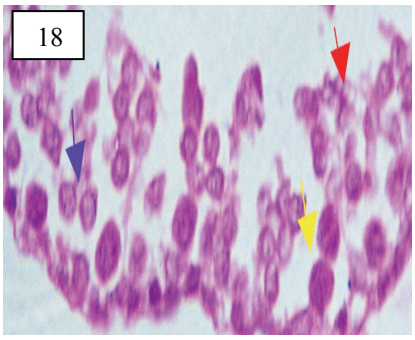




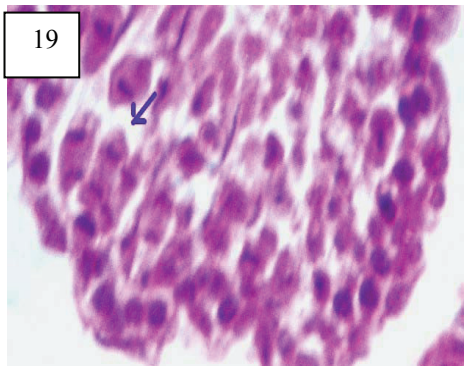


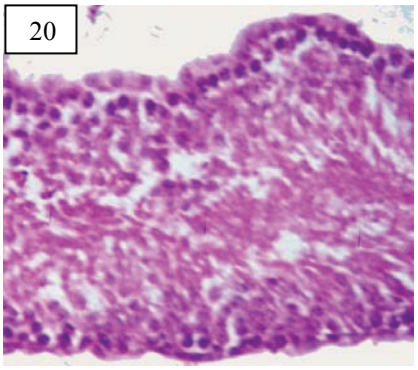


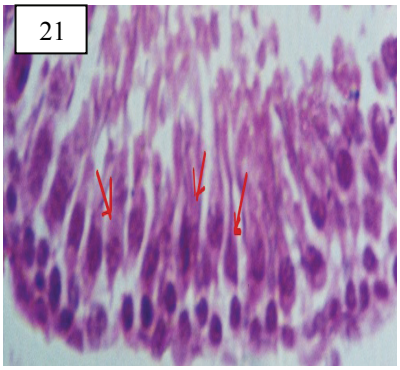


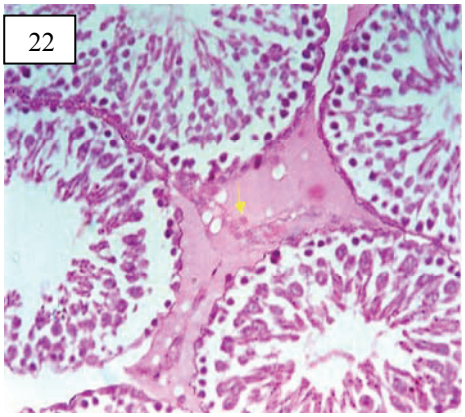


10. Lead toxicity, Focal metaplasia with dysplasia of oviduct villi. Large arrows point to metaplastic area where the columner epithelium has transformed into several layers Small arrows point to acidophilic intranuclear inclusion body. H&E x 40.
11. Lead toxicity uterine cervix. Normal cervical lining (⟶).Metaplasia of transitional cells (→). Metaplasia with vaculation of cervical gland (→). Acidophilic intranuclear inclusion body (→). H&E x 25.
12. Lead toxicity uterine cervix. The Superficial columner foamy cells (progesterone effect) underwent hyperplasia and metaplasia (lead effect) (⟶). Sever metaplasia of transitional cells (lead effect) (→).Compare to more or less upper lining wall in the photo. H&E x 10.
13. Lead toxicity vagina. Hyperkeratosis, the non keratinized stratified squamus of the vagina become keratinized. Note the presence of the stratum granulosum. H&E x 25.
14. Lead toxicity vagina. Hyperplasia of stratum germinativum with excessive formation of reta pegae invading deeply the subepidermal lamina. H&E x 10.
15. Lead toxicity, seminiferous tubule showing necrosis of spermatozoa and pyknosis of rounded spermatid. H&E x 25.
16. Lead toxicity, seminiferous tubule showing necrobiotic rounded spermatids with spermatid giant cells. H&E x 40.
17. Lead toxicity, seminiferous tubule showing karyorhexis in elongated spermatid. H&E x 40.
18. Lead toxicity , seminiferous tubule showing degenerated spermatogonia, primary spermatocyte (→) , secondary spermatocyte (→) , rounded spermatids (→) and aspermiogenesis. H&E x 40.









19. Lead toxicity, seminiferous tubule showing high meiotic index of the secondary spermatocyte. H&E x 40.
20. Lead toxicity, seminiferous tubule showing only surviving spermatogonia and necrosis of the all layers of spermatocytes, spermatids and spermatozoa. H&E x 25.
21. Lead toxicity, seminiferous tubule showing bared sertoli cells suffering necrobiosis. H&E x 40.
22. Lead toxicity, edema of the interstitial testicular space with vacuolated degeneration and desquamation of the interstitial cells. H&E x 40.

CONCLUSION

1. It had been concluded that lead toxicity has deleterious effects on both female and male genital organs. These effects were manifested by qualitative and quantitative histopathological changes in both ovaries and testes. Moreover, a lead genetotoxicity of the female reproductive tract was reported for the first time including metaplastic changes in the epithelium of the fallopian tube, uterine glands, cervix and vagina. Intranuclear inclusion bodies in the metaplastic epithelium of oviduct and cervix were reported for the first time.
2. It has been also assumed that lead reproductive toxicity in both male and female experimental rats might result in a secondary toxic infertility or at least lowering the fertility with percentage varies from (47 - 87 %) .
3. It has been also concluded that antioxidant represented by Antox might play an important role in antagonizing the reproductive toxicity of lead for both male and female genital organs.
4. Practically, our results demonstrated that it is advisable to protect all animals used for breeding from the deleterious effect of lead genetotoxicity as it might lead to secondary toxic infertility especially in the area suspected with presence of sources of lead toxicity.
5. Our results also demonstrated that antioxidant vitamins must be used as food additives for breeding animals in areas with sources of lead.

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TERATOGENIC AND GENOTOXIC EFFECTS OF PERFLUOROALKYL ACIDS ON EMBRYONIC AND NEONATE MICE

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SUMMARY

160 pregnant dams were used in this study and divided into two equal groups, PFOS and PFOA. Each of them was subdivided into two groups, treated of 60 dams and control group of 20 dams. Treated group was re-subdivided into three equal groups. Dams in PFOS group were treated with concentrations of 1, 10 and 20 mg/kg b.w., while dams in PFOA group were treated with concentrations of 1, 5 and 10 mg/kg b.w. Ten dams of each group were treated from gestation day 0 (GD0) till gestation day 17 (GD17). At GD18 dams were euthanized under anesthesia. The gravid uterus were removed and examined for prenatal evaluation of fetuses. The liver of the fetuses were dissected and used immediately for comet assay as an indicator for genotoxicity. Individual live fetuses were prepared for teratological evaluation. While the other ten dams were treated from GD0 till GD18 and then allowed to give birth. The neonates of 5 dams were monitored for 4 days for postnatal survival. Neonates of the remaining 5 dams were kept in the fixative till histopathological examination. Control group were received an equivalent volume of deionized water. PFOS caused DNA damage in fetal liver at 10 and 20 mg/kg. It reduces the number of live fetuses and increased fetal resorption. PFOS reduces fetal body weight in a dose dependent manner, while PFOA reduces the fetal body weight at dose of 5 and 10 mg/kg. Gross examination of the fetuses at GD18 showed presence of abnormal swelling in the back of the neck in fetuses of 20 mg/kg PFOS group. Teratological evaluation revealed the presence of several skeletal abnormalities in PFOS and few abnormalities in PFOA groups. Neonates borne with reduction in body weight and showed the presence of the bilateral swelling and accompanied by neonatal death. The study concluded that both PFOS and PFOA were toxic to neonates with different degrees. PFOS found to be more toxic than PFOA. Embryos may die from the lesion formed over the brain.

INTRODUCTION

Perfluorinated compounds (PFCs) have emerged as a new class of global environmental pollutants. Perfluoroctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) comprises a class of environmentally persistent chemicals have industrial applications. Genotoxicity of PFOS and PFOA was assessed by estimating tail moment of comet in single cell gel electrophoresis (SCGE) assay. Damaged cells have an appearance similar to astronomical comets, with long tails of DNA migrating from the center of the exposed nuclei. The reproductive toxicity of PFOS has been examined in several species as rabbits [1], rats and mice [8]. Teratological studies have been conducted in rat, rabbit and mouse with potassium and lithium salts of PFOS [3]. Observed developmental effects include reduction of fetal weight, cleft palate, edema, delayed ossification of sternum and phalanges, and cardiac abnormalities. These structural abnormalities were found in the highest PFOS dose groups, and significant reductions of weight gain and food consumption were also observed in the pregnant dams. PFOS also produced dose-dependent effects on neonatal survival and retarded the growth and development of neonates in rats exposed from gestational day 2–21 to doses ranging from 1–10 mg/kg/day and mice exposed on GD1– 18 to 1–20 mg PFOS/kg/day [3]. These effects were also reported in a two-generation study in rats exposed to doses ranging from 0.1 to 3.2 mg PFOS/kg/day [5]. Developmental toxicity from PFOA in rodents, including pregnancy loss, reduced fetal weight, reduced postnatal survival, and delays in postnatal growth and development in offspring were reported [4]. In the rat, PFOA and PFOS have been detected in placenta, fetus, amniotic fluid, and milk, and these chemicals have also been found in human breast milk. The aim of this work was to study the genetic and teratogenic changes in fetuses after maternal exposure to PFOS and PFOA.

MATERIAL AND METHODS

Perfluorooctane sulfonate, PFOS, (potassium salt 98% pure) was purchased from Fluka Chemie GmbH, Switzerland. Solutions were prepared at 0.1, 1 and 2 mg/ml of 0.5% Tween-20 vehicle and administered to the pregnant mice by gavage at a volume of 10 ml/kg/day. Perfluoroocatnoic acid, PFOA, (90% pure) was purchased from Fluka Chemie GmbH, Switzerland and prepared at 0.1, 0.5 and 1 mg/ml of deionized water and administered to the pregnant mice by gavage once daily from GD0 till GD17 at a volume of 10 ml/kg/day. The gravid uterus was

removed and examined for prenatal evaluation of fetuses. The liver of the fetuses were dissected and used immediately for comet assay. DNA damage in fetal liver was detected using comet assay (Single Cell Gel Electrophoresis) [7, 9]. Individual live fetuses were prepared for teratological evaluation. All the neonates were kept in Bouin's fixative (300 ml saturated picric acid, 100 ml formaldehyde and 20 ml glacial acetic acid) for three days then kept in 70% ethanol till histopathological examination. Fetuses were prepared for skeletal evaluation [6].

RESULTS

Treatment of dams with PFOS caused DNA damage in fetal liver at 10 and 20 mg/kg in the form of increased DNA migration represented by tail length and tail moment as shown in fig.1. Gross examination of fetuses at GD18 in PFOS group showed, presence of abnormal swelling in the back of the neck region in all fetuses of the 20 mg/kg group. After peeling of the skin, a bilateral firm swelling was observed and presented in fig. 2. In addition, some fetuses of the 10 mg/kg PFOA group showed mild swelling in the neck region. Teratological evaluation of the fetuses in PFOS group revealed, presence of several skeletal abnormalities such as cleft palate, delayed eruption of incisors, spina bifida occulta (delayed closure of the vertebral spine), delayed ossification of phalanges and sternum, wavy ribs, curved fetus (curved vertebral column) and abnormal tail as shown in fig. 3, mostly at 10 and 20 mg/kg PFOS groups, while there were few skeletal abnormalities in fetuses of PFOA group as delayed ossification of the sternum and phalanges accompanied by delayed eruption of incisors in the 10 mg/kg PFOA group.

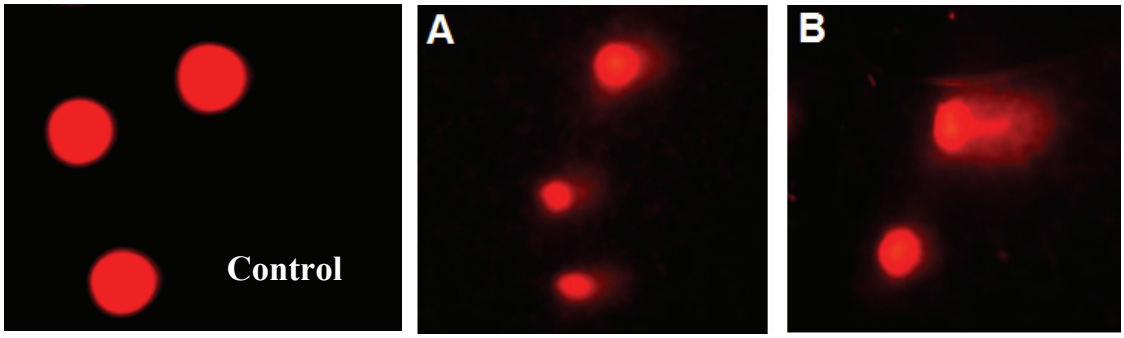

Figure 1: Image of an alkaline comet (hepatocytes) stained with ethidium bromide showing undamaged nucleus (control) and damaged nucleus, A) Partially damaged, B) Severely damaged.

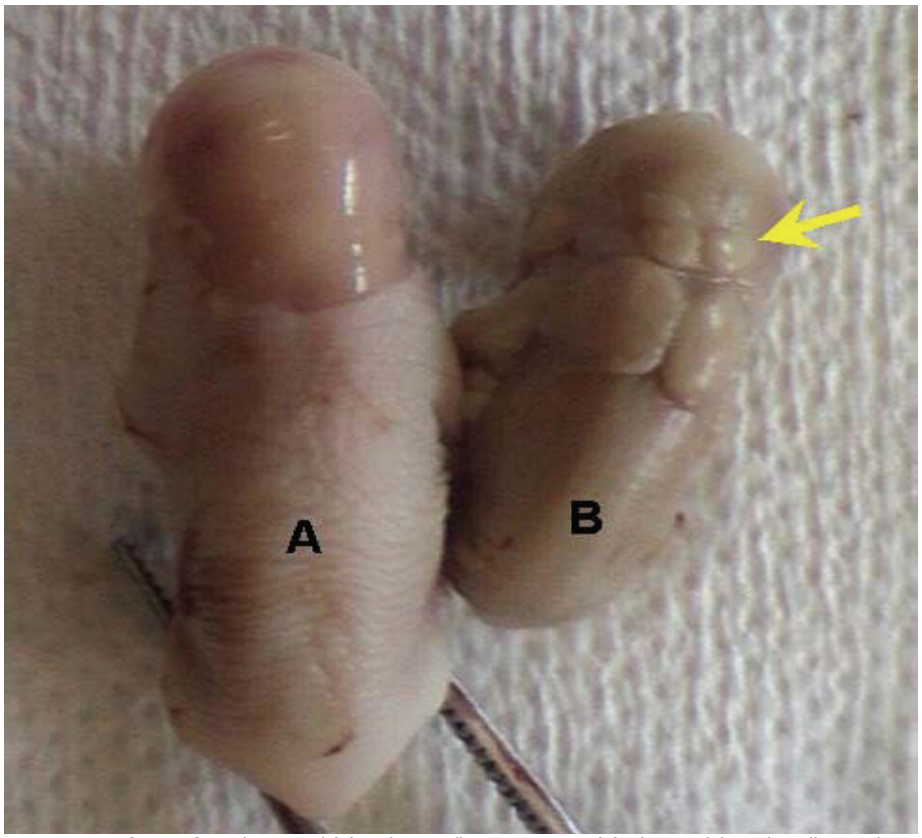

Figure 2: Mice fetuses from the control (A) and 20 mg/kg PFOS groups (B), showing bilateral swelling at the neck (arrow).

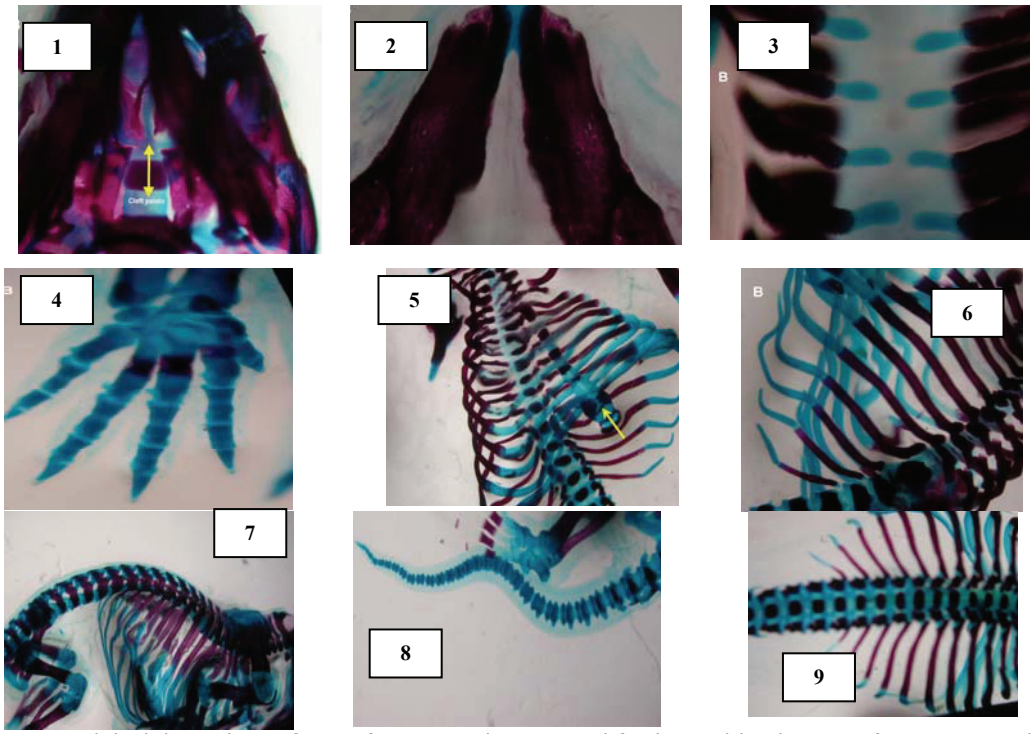

Figure 3: Skeletal abnormalities in fetuses of mice exposed to PFOS. 1. cleft palate, 2. delayed eruption of incisors, 3. spina bifida occulta, 4. delayed ossification of phalanges, 5. delayed ossification of sternum, 6. wavy ribs, 7. curved fetus, 8. abnormal tail. 9. extra ribs.

DISCUSSION

Results of this study indicated that PFOS and PFOA have genotoxic effects on hepatic cells because these compounds induced remarkable DNA damage in hepatic cells. Significant increase in tail length (DNA migration) was recorded in fetal hepatic cells exposed to PFOS (Fig.1). The increased tail length in fetal liver was 25.27 ± 1.60 and 25.93 ± 1.46. Tail moment was also recorded as 3.02 ± 0.38 and 3.45 ± 0.41 in the 10 and 20 mg/kg PFOS groups respectively. Meanwhile maternal exposure to PFOA has no significant indication in fetal tail length or tail moment. Maternal exposure to PFOS during pregnancy reduced the number of live fetuses and increased fetal resorption only at 20 mg/kg group, while reduced the fetal body weight in a dose dependent manner as the following 1.44 ± 0.01, 1.39 ± 0.01 and 1.07 ± 0.01 at 1, 10 and 20 mg/kg groups respectively. While the fetal body weight only was significantly reduced after exposure to PFOA in the following manner 1.36 ± 0.01 and 1.06 ± 0.01 at 5 and 10 mg/kg groups. The decrease in fetal body weight after exposure to PFOS and PFOA is similar to findings of previous studies. The effects of PFOS in rabbits were examined and noted reductions of fetal weight [1]. It was found that; exposure of pregnant rats to PFOS resulted in reduction of fetal body weight [8]. Maternal exposure of mice to PFOA was reported and recorded that, the fetal body weight was reduced [4].

The presence of an abnormal swelling in the back of the neck region in all fetuses of the 20 mg/kg of PFOS group is considered the first record in this respect and in some fetuses of the 10 mg/kg group as shown in Fig. 2. Other skeletal abnormalities recorded for the first time such as delayed eruption of incisors, spina bifida occulta, wavy ribs, curved vertebral column, and abnormal tail were found mostly in the 20 mg/kg dosage group and which represented in Fig. 3. The present study recorded that maternal exposure to PFOS caused skeletal abnormalities as cleft palate and delayed ossification of the sternum and phalanges which are similar to previous studies in rabbit [1], rats [2], and mice [8]. These results are reminiscent of the findings [10, 11], which reported severe toxic effects of both PFOS and PFOA in ICR mice. Observed developmental effects included reduction of fetal weight, cleft palate, delayed ossification of bones (sternebrae and phalanges), anasarca (edema) and cardiac abnormalities (ventricular septal defects and enlargement of the right atrium). Nonetheless, it should be noted that a preponderance of these structural abnormalities was found in the highest PFOS dose groups (10 mg/kg for the rat and 20 mg/kg in the mouse).

CONCLUSION

In spite of the fact that PFOS and PFOA are similar in causing neonatal death but the cause of death may be different. Further studies are required to explore the cause of neonatal death after maternal exposure to Perfluorinated compounds, PFOS and PFOA.

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APPLICATION OF HALLOYSITE AND BENTONITE AS FILTRATION BED TO AMMONIA REDUCTION

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SUMMARY

The paper describes the new method of reducing ammonia concentration in livestock buildings. The filtrating device ODOR1 was in the middle of each container. Filter packs in ODOR1 were filled with activated halloysite (container 1), roasted bentonite (container 2) or were empty (container 3 - control). The samples of air were collected on the beginning and every single day of the experiment, which lasted 10 days, with the use of AMZ-1 aspirator (Rotametr, Poland). The concentrations of ammonia in sampled air were determined with the use of spectrophotometer UV-VIS according to polish standards. It was found that the concentration of ammonia in air samples collected from experimental containers (ODOR1 with activated halloysite or roasted bentonite) was always lower than in the control container and the differences were statistically confirmed. The average reduction level for 10 days filtration period was 47 and 35 % for activated halloysite and roasted bentonite, respectively. Therefore, the investigated method could be useful to optimize microclimate in livestock buildings.

INTRODUCTION

European intensive animal production is connected with odorous compounds emission i.e. ammonia, having strong impact on our environment. Ammonia volatilizing in livestock buildings is producing detrimental effects on housing animals, also contributes to the eutrophication of terrestrial ecosystems and surface waters leading to potential adverse effects on biodiversity [1, 3, 14]. Therefore, abatement technologies for ammonia emission are of major importance when we want to obtain the sustainable intensive livestock production [8].

Strategies for reducing ammonia volatilization including dietary manipulation, different bedding materials, litter additives or uricase and urease inhibitors were examined last years frequently [4, 6, 7, 9, 13, 15]. Nevertheless, investigated methods were not decreasing NH_3 emission with satisfactory efficiency and moreover were often not cost effective and impractical. However, methods involving acid scrubbers, biotrickling filters, bubble column reactors or biofiltration are developing very fast in the last decade [2, 5].

The aim of the investigation was to estimate the application of activated halloysite and roasted bentonite as filtration bed to ammonia reduction during animal housing.

MATERIAL AND METHODS

The experiment was conducted in 3 identical containers (about 23 m^3). Fresh poultry droppings were placed in each of the containers (the surface of emission about 8 m^2). The filtrating device ODOR1 (patent application no. P-388329) was in the middle of each container. Filter packs in ODOR1 were filled with activated halloysite (container 1), roasted bentonite (container 2) or were empty (container 3 - control). The filtration efficiency of ODOR1 device was about 1000 m^3/h. The samples of air were collected on the beginning and every single day of the experiment, which lasted 10 days, with the use of AMZ-1 aspirator (Rotametr, Poland). The concentrations of ammonia in sampled air were determined with the use of spectrophotometer UV-VIS according to polish standards.

There were three series for each of the investigated sorbents and the results given are the averages. Effects were considered significant at a probability of $P < 0.01$. Differences among treatment means were tested for significance using Tuckey's multiple range test (Statistica ver. 8.1, StatSoft, Inc., Tulsa, OK, USA).

RESULTS

It was found that the concentration of ammonia in air samples collected from experimental containers (ODOR1 with activated halloysite or roasted bentonite) was always lower than in the control container (fig.1) and the differences were statistically confirmed. The average ammonia reduction level after 1 day of filtration was 71.88 and 58.33 % for activated halloysite (HA) and roasted bentonite (BR), respectively, when the mean concentration of NH_3 in the control container was on the level of 53.83 ± 10.74 ppm. While the average concentration of ammonia after 5 days of filtration was 55.13 ± 10.03 ppm, the reduction level was about 54.60 and 41.54 % for HA and BR, respectively. On the end of the experiment, the mean concentrations of ammonia

were 42.70 ± 7.39 and 46.47 ± 8.07 ppm in the containers where respectively HA and BR were used for the filtration, which was about 14.88 and 7.38 % lower than in the control container.

The mean reduction level for 10 days of filtration period was 47.37 and 35.28 % for HA and BR, respectively (fig. 2). The highest average reduction level was obtained for activated halloysite (49.78%) during the first series, nevertheless the difference between HA and BP was not statistically significant in each of the series.

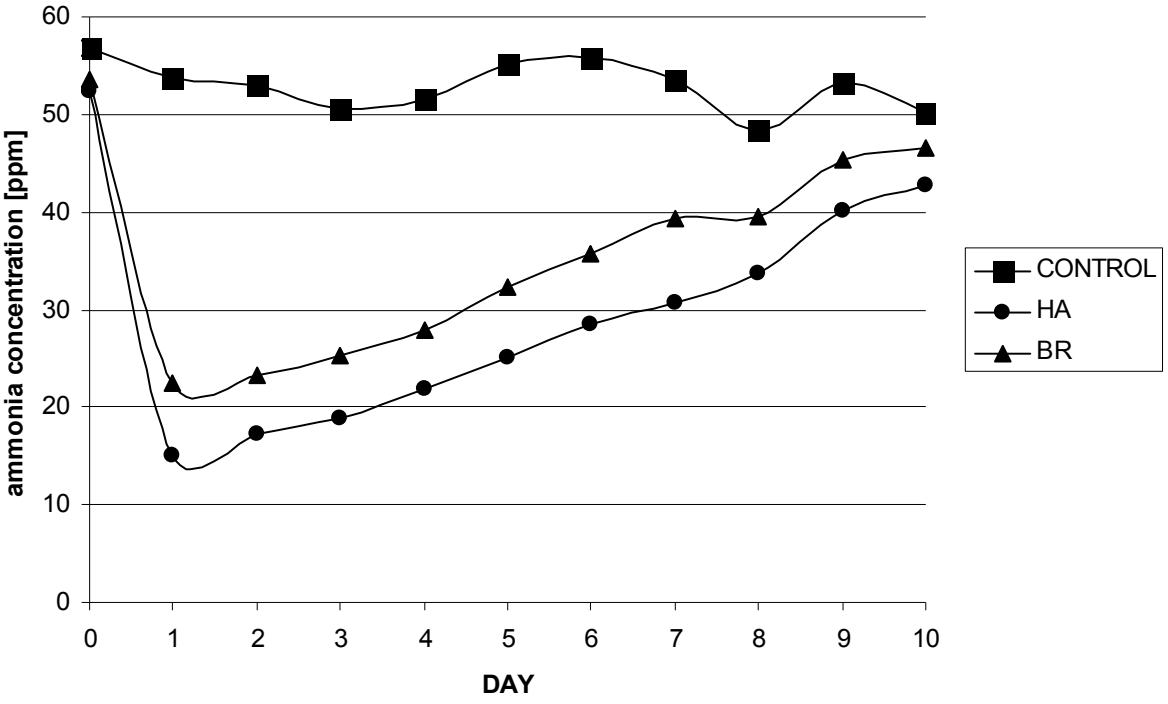

Figure 1: Effect of air filtration on ammonia concentration in the containers during 10 days (n=3)

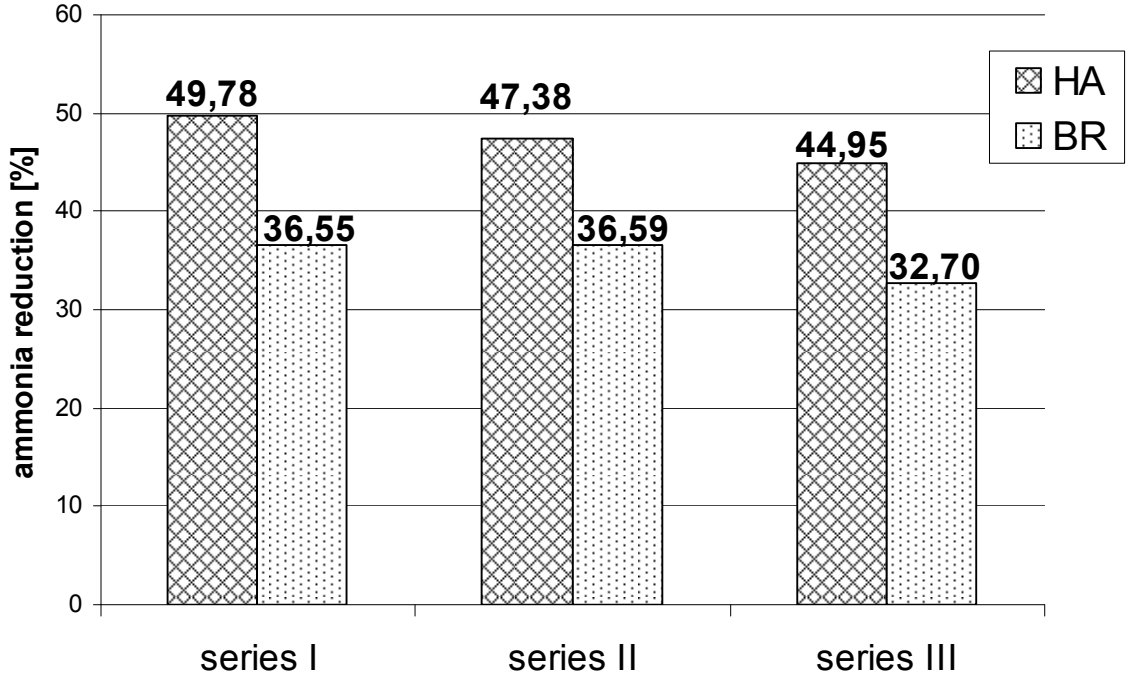

Figure 2: The ammonia reduction level for 10 days of filtration during each of the series

DISCUSSION

Taking into consideration that ammonia concentration in the control container was on the mean level of 52.55 ± 8.06 ppm, the results of our study showed that both activated halloysite and roasted bentonite are aluminosilites with high ammonia binding capacity.

The potential for removal of ammonia by aluminosilicate sorbents was confirmed in laboratory-scale examination. The reduction level of NH_3 was 93.6, 75.4 and 86.2 for activated, roasted and raw halloysite, respectively [10]. Moreover, the investigations with the use of ODOR1 device have been already conducted but the air was filtrated only 24h. It was found that all investigated sorbents reduced the concentration of ammonia and tentatively identified volatile odorous compounds in the air of experimental containers. The highest ammonia reduction level after one-day of filtration was determined for roasted bentonite and activated halloysite, 68 and 77 %, respectively [12].

It is worth to add, that the disposal method of aluminoslicate sorbents applied to air filtration was elaborated [11]. The activated halloysite and roasted bentonite used for aerofiltration were added to granulated Fe concentrates for clinker production in cement kilns. The iron concentration during the clinkerization process has to be on the appropriate level. It was found that investigated aluminosilicates can replace sodium bentonite, usually used during Fe concentrate production.

CONCLUSIONS

It was found that, both activated halloysite and roasted bentonite reduced effectively the concentration of ammonia in the air of experimental containers. Therefore, the investigated method could be useful to optimize microclimate in livestock buildings.

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TITANATE NANOTUBES AS ANTIBACTERIAL COATINGS FOR CONTROL OF *LISTERIA* IN FOOD PLANTS

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SUMMARY

The experiment of the synthesis and the coating stability of titanate nanotubes and the outcome experiment of their effects on *Listeria* reduction in food plants are the results of an interdisciplinary project within the Centre of Excellence NAMASTE. Our experiments in laboratory conditions showed that at least 35% of tested close related nonpathogenic bacteria (*L.innocua*) were reduced on titanate nanotubes coated glass under the conditions of UV photo influenced excitation (350 nm). The experiment in real conditions that is in poultry slaughterhouse showed more than 2 $\log_{10}$ (99.22%) (in average 40-70%) of test bacteria (*L. innocua*) reduction on the PE test slides coated by titanate nanotubes induced only by fluorescent light and existent air conditions.

INTRODUCTION

The human listeriosis caused by pathogen *Listeria monocytogenes* recently resulted in several fatal outcomes in humans in the years 2009 and 2010 in different EU-countries. The risk of the food-borne bacteria *Listeria* has brought to attention on the different pathways of *Listeria* contamination in the meat processing industry [1]. *Listeria* is ubiquitous, psychotropic and resistant at a wide range of temperatures, with the ability of growth under conditions which are unpleasant to *Listeria* competitive microorganisms [4]. Eighty *L. monocytogenes* isolates were screened for biofilm formation which are resistant to disinfection and from which cells can become detached and contaminate food products [2]. In addition it is difficult to achieve suitable sanitation measures to destroy *Listeria* in food processing plants. The ability of *Listeria* resistance to the ground sanitation refers to the study of the new biocidal materials in nano dimensions. This is an important step in the reducing of bacteria incidence, particularly pathogenic species such as *Listeria monocytogenes* in food plants. Biocidal coatings such as titanate submicron materials have shown to be bioactive materials, e.g. they photocatalyze the process of generating short-lived free radicals in wet conditions under the UV irradiation [3]. The mechanism of titanate nanotubes activity is the photo induced generation of free oxygen radicals penetrating the cell wall which fatally act on the physiological processes of the cell. Due to their physical properties, it is expecting to strongly adhere to surfaces, which may be the basis for ensuring the stability and safe use in the maintenance of hygienic surfaces in the food industry.

MATERIAL AND METHODS

The synthesis and deposition of titanate nanotubes coatings

We have determined the optimal synthesis conditions for the growth of titanate nanostructures: final titanate nanomaterial was made from anatase (325) form of TiO_2 and 10 M NaOH at T = 135 ºC, 3 days under hydrothermal conditions. The sample had uniform morphology, and the efficiency of reaction was high (visual estimate). Rinsing of the sample in 0.1 M HCl resulted in exchange of sodium ions with protons. The protonated form of titanate nanotubes (400 mg, HTiNC) was dispersed in 0.5 mM solution of Cu^{2+} (100 ml, the source of the Cu^{2+} was $CuSO_4 \times 5H_2O$) using an ultrasonic bath. Isolated material was finally heated in air at 400 ºC for 10 hours. The photocatalytic activtiy of synthesized titanate nanomaterials was determined using electron paramagnetic resonance spectroscopy (EPR) with spin trapping, which was optimized for measurement of primary radicals generated in the vicinity of the nanomaterial surface. This was achieved by measuring primary hidroxyl radicals in the presence of 30% ethanol with 5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide spin trap (DEPMPO). Electron paramagnetic resonance (EPR) spin trapping was applied to measure the generation of reactive oxygen species (ROS) production responsible for bacterial cell destroying. Titanate nanomaterials doped with a low content of copper had the highest photocatalitical activity when exposed to UV light or the wavelength range below 400 nanometers, which indicates that the antimicrobial action is possible even under ordinary fluorescent lamps. These materials were therefore chosen for experiments in food plants. The basic principle of titanate nanotubes suspension coating is thermal dehydration (120^0C) and adhesion on the surface owing to Van der Vaals force.

Experimental design

For the experiment the suspension of titanate nanotubes were coated on surfaces of glass- and polyethylene (PE) test slides, respectively. This two materials were used because the polyethylene is mostly used in the food industry, meanwhile the glass is most suitable material for the laboratory experiments. For security reasons antimicrobial properties were tested on non-pathogenic bacterium *Listeria innocua*, which is closely related to pathogenic *L. monocytogenes*. Initial bacterial suspension of *Listeria innocua* was prepared in liquid medium and incubated aerobically for 24h at 37°C. After incubation the culture contains approximately 10^9 colony forming unit (cfu) per mililitre. Small amount of suspension (0.1 ml, contained 10^7 cfu) was applied onto the tested surface coated with thin layer of titanate nanotubes. For negative control were used non-coated glass or PE slides. Samples were exposed to the different light sources for the limited time (6 min). The test polygon in the laboratory samples was the dark chamber illuminated by the constant intensity of UV radiation (350 nm) and same air conditions (60% R.H., 21,3^{0}C). The measurement point was fixed 2cm under the UV source. In the poultry slaughterhouse (real conditions) 4 measurement points were separated owing to the different intensity of UV irradiation of fluorescent light and the air microclimate conditions. Slides were exposed 180 min on the dry, wet, moderate and cold measurement point placed on different altitudes (0,5 m, 2,0 m) and positions (vertical, horizontal) regarding the influence of different air temperature, air moisture, aerosol and UV irradiation intensity of fluorescent lights in the evisceration and cold room place where the exposing and measurements were performed. The air aerosol saturation was estimated owing to vicinity of water sprayer, what was the case on wet measurement point. After exposure the samples were washed in saline (NaCl 0.9%) and examined bacteriologically to determine the number of bacteria. Survival rate of bacterial culture of *Listeria innocua* has been measured for samples with and without nano materials coat. Preliminary results of measurements of antimicrobial activity show a lowering of the survival rate in presence of nanotubes.

RESULTS

Laboratory test

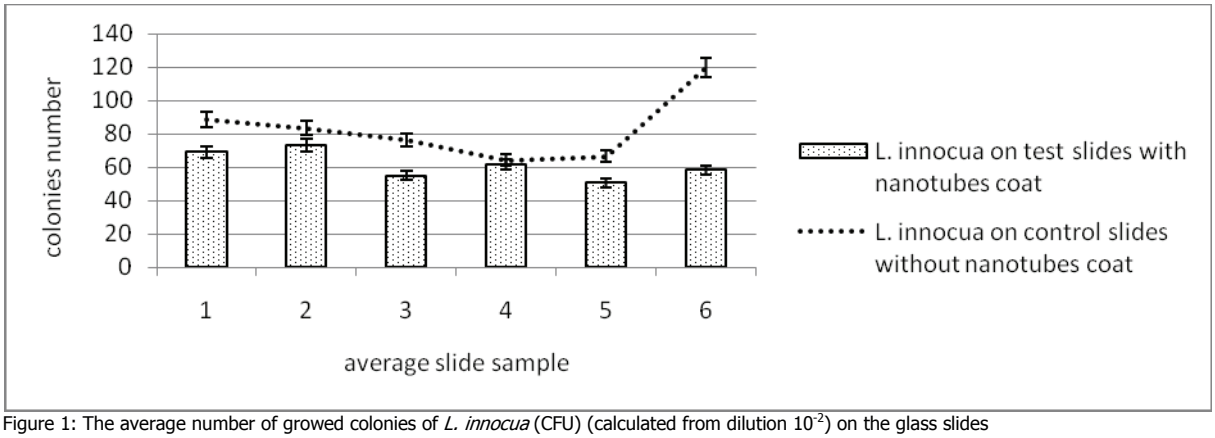

Figure 1: The average number of growed colonies of *L. innocua* (CFU) (calculated from dilution 10^{-2}) on the glass slides

The average number of *L. innocua* growed colonies on the test glass slides, coated by titanate nanotubes, varied between 2.700 to 13.200 what was significantly ($P<0.05$) less (35%) than the number of colonies (CFU) on control slides (without coating) in the range of 3.300 to 21.800 (Figure 1).

Test in poultry slaughterhouse

The average number of *L. innocua* growed colonies on the test PE slides exposed in evisceration and in cold room of poultry slaughterhouse, coated by titanate nanotubes, varied between 555 to 10.345 (CFU), what was significantly ($P<0.05$) less (40-70%) than the number of colonies (CFU) on control PE slides (without coating) in the range of 11.120 to 41.040 (Figure 2). On wet measurement point the highest effects of titanate nanotubes were achieved. On this point the average reduction of *L. innocua* was up to 99,2% where the air, owing to water sprayers vicinity, was saturated with aerosol (82% R..H.,16,8^{0}C). Lowest average reduction effects of *L. innocua* (53%) were determined on cold measurement point – cold room (80% R.H., 8,3^{0}C), where the air was not saturated with aerosol (no water sprayers). UV irradiation in the specter of existent fluorescent lightning of poultry slaughterhouse was in the range of 37,2 mV to 177,65 mV (average 90,2 mV). Average air humidity in evisceration and cold room was 80,5% R.H., meanwhile contact temperatures of test slides (16,9^{0}C) were the same as temperatures of the air (16,9^{0}C). Contact temperatures of test slides were lower (4,4^{0}C) as the air (8,9^{0}C) in cold room (Figure 2).

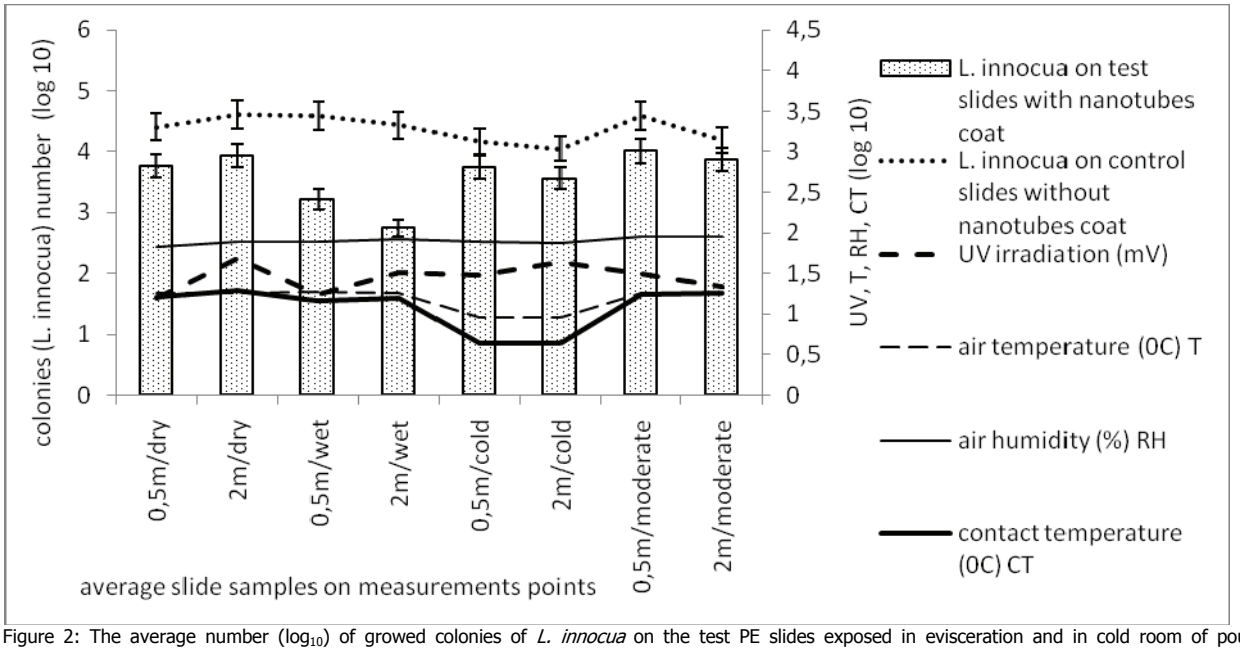

Figure 2: The average number (log_{10}) of growed colonies of *L. innocua* on the test PE slides exposed in evisceration and in cold room of poultry slaughterhouse

DISCUSSION

Results of existing laboratory tests on the influence of titanate nanotubes coating on reduction of *L. innocua* were rather moderate presumably to believable nanotubes aggregation and inhomogeneity, moreover on inconstant nanotubes coating thickness on the surfaces of tested glass. UV test lamp was radiated in the spectrum of UV-A (350nm). However the most efficient exposition time for radicals releasing should be explored in the future to separate the biocidal effects of the independent bactericidal rays in spectrum of UV-C (280-10nm). Results of tests in poultry slaughterhouse were rather different owing to changeable air conditions and fluorescent illumination with its UV-A (350 nm) specter irradiation. The UV irradiation from the specter of fluorescent light was obviously sufficient releasing free radicals, however best antibacterial effects were achieved where the air was aerosol saturated. Consequently test surfaces were wet, thus the moisture had significant influence on titanate nanotubes free radicals releasing. Presumably the contact temperatures of test slides have no significant effect on antibacterial effect of coatings.

CONCLUSIONS

This experiment indicates the possibility of the future use of titanate nanotubes coatings in food industry. The intensity of UV-A irradiation in light spectrum of fluorescent lights was enough for free radicals releasing, still the influence of different light sources and different exposing materials (stainless still, PET, PVC) have to be investigate in the future. Further research work will be focused to improve the bactericidal effectiveness by improving of surface coating homogeneity and stability against environment influences. The latter problem is an important starting point for studying local cellular reactions to this bioactive materials and possible health risks. Safe use of titanate nanotubes coatings in the food industry, exploiting their bactericidal activity, particularly against the *Listeria* should be an important contribution to improving food security.

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INFLUENCE OF ENDOTOXINS AND THERMOLYSIN IN AN *EX VIVO* MODEL OF EQUINE LAMINITIS

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SUMMARY

This paper describes an *ex vivo* model to evaluate the influence of endotoxins and thermolysin on the development of equine laminitis. Explants of equine hooves were cultured in medium for 48 hours with different concentrations of lipopolysaccharides from *Escherichia coli* 055:B5 and thermolysin from *Bacillus thermoproteolyticus rokko*. The separation of dermal-epidermal junctions of the explants was measured. Explants cultured with 100 – 10 µg/ml thermolysin and 200 – 10 µg/ml lipopolysaccharides could easily be separated. These results indicate that endotoxins and thermolysin play an important role in the development and progress of equine laminitis. Further experiments will focus on the effects of exotoxins and a combination of endo- and exotoxins, because lamintis is a multifactorial disease and cannot only be limited to one triggering factor.

INTRODUCTION

Endo- and exotoxins seem to play an important role in the pathogenesis of equine laminitis, although the mechanism is not fully understood. To evaluate influencing factors on the pathogenesis of lamintis an appropriate model is still needed. Alimentary carbohydrate overload laminitis has become one of the better-understood mechanisms of laminitis, and can be used for *in vivo* studies.

During a carbohydrate overload, the horse cannot digest all carbohydrate in the foregut, therefore the excess moves on to the hindgut and ferments in the cecum. This causes a rapid proliferation of bacteria (*Streptococcus bovis, Streptococcus equinus* and *Lactobacillus* spp.), which results in death of Gram-negative bacteria of the family Enterobacteriaceae, and leads to the release of endotoxins. Due to increased gut permeability, caused by irritation of the gut lining due to increased acidity, endo- and exotoxins can be absorbed into the bloodstream. This release can result in impaired circulation, particularly in the feet. Additionally, the increase of the matrix metalloproteinase (MMP) activity seems to play an important role, and causes an enzymatic separation at the hoof lamellar dermal-epidermal interface. Thermolysin is a bacterial metalloproteinase enzyme produced by the gram-positive bacteria *Bacillus thermoproteolyticus*. This enzyme can activate MMP activity [1].

Therefore, the aim of our study is to test the effects of endotoxins and thermolysin on lamellar separation in an *ex vivo* model.

MATERIAL AND METHODS

Hoof material and dissection

Hooves were obtained from healthy horses which were from a commercial abattoir. Hoof explants were prepared as described by Pollitt [2]. They consisted of 2 mm of the inner hoof wall, 6 intact epidermal lamellae and 2 mm of connective tissue (Figure 1.) Explants were washed 3 times in sterile sodium chloride solution and once in sterile PBS before cultivation.

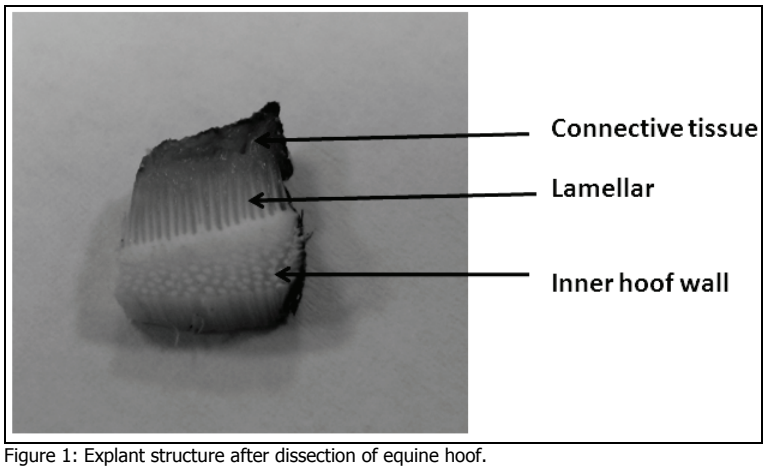

Figure 1: Explant structure after dissection of equine hoof.

Experiment 1: Explants were cultured in 24 well plates with 1 ml culture medium (D-MEM; 4.5 g glucose/ l; 0.1 mg/ml gentamicin) and 0.9% sodium chloride solution at 37 °C and 5% CO_2. After 1, 2, 4 and 5 days, explants were studied for lamellar separation.

Experiment 2: Explants were cultured in culture medium and in medium with different concentrations of thermolysin from *Bacillus thermoproteolyticus rokko* (100 – 2.5 µg/ml) and lipopolysaccharides (LPS) from *Escherichia coli* 055:B5 (200 – 1.25 µg/ml) for 48 hours. Explants cultured only in medium were used as negative control.

Structural integrity test

The test was performed as described by Pollitt [3]. Explants were fixed between two rat tooth forceps, one at the inner hoof wall and the other at the connective tissue. First the operator was pulling in a side to side direction and then up and down. Explants were counted as separated when it was possible to destroy lamellar connections, and if not, they were scored as intact.

RESULTS

Experiment 1: All explants cultured in medium for 1-5 days remained intact, and all cultured in 0.9% sodium chloride solution separated.

Experiment 2: All explants cultured in D-MEM without supplementation stayed intact. Explants incubated in 100 – 10 µg/ml thermolysin could easily be separated. At a concentration of 5 µg, 5 out of 12 explants separated and 7 stayed intact. At a concentration of 2.5 µg, 4 out of 11 explants separated, and 7 stayed intact. At a concentration of 1.25 µg/ml no explants separation could be observed (Figure 2).

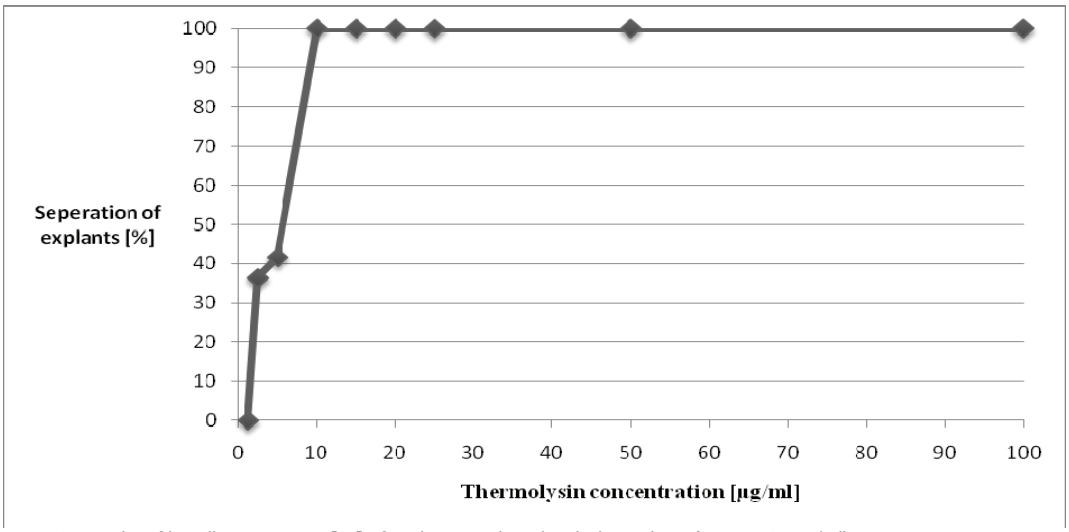

Figure 2: Results of lamellar separation [%] of explants incubated with thermolysin (100 – 1.25 µg/ml).

Explants cultured in 200- 20 µg/ml LPS could be easily separated. At a concentration of 10 µg/ml, only 3 explants out of 9 separated, and at 5 µg, only 1 explant out of 9 separated. At 2.5 µg/ml and 1.25 µg/ml no separation could be observed (Figure 3).

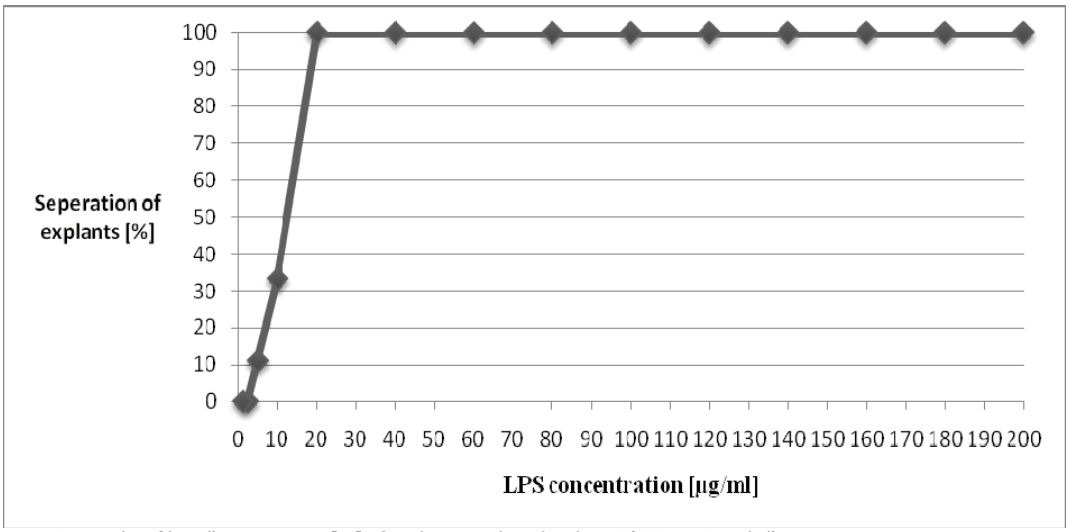

Figure 3: Results of lamellar separation [%] of explants incubated with LPS (200 – 1.25µg/ml).

DISCUSSION

The present study shows that LPS and thermolysin have an influence on lamellar separation.

Explants cultured in 200 – 20 µg/ml could easily be separated and also explants incubated in 100- 10 µg/ml thermolysin could easily be separated. The influence of thermolysin was already described by Mungall et al. [4, 5], who also described an influence on MMP-2 and MMP-9 activity. Thermolysin as a metalloproteinase is known to stimulate MMP activity. For further studies it is planned to analyze MMP-2 and MMP-9 activity in supernatants of explants.

Mungall et al. [5] also tested bacterial supernatant of *E. coli* for lamellar separation, and observed that it was possible to induce separation. In this paper LPS was also tested alone, but in contrast to our study no dose dependent response could be observed. Unfortunately, no details about type and concentration of LPS used were given.

Although laminitis has never been triggered *in vivo* with LPS given intravenously [6], the results of our study indicate that LPS plays an important role in the development and process of the disease.

CONCLUSIONS

Thermolysin and endotoxins seem to have an influence on the lamellar separation. Further trials with a combination of thermolysin and LPS will be studied to see if a synergistic effect can be observed. It is also important to evaluate the effect of exotoxins in this *ex vivo* model, and test it in combination with LPS, as exotoxins seem to be one of the potential trigger factors in the pathogenesis of lamintis. It is important to keep in mind that this disease is multifactorial, therefore the combination of several factors causes the pathological changes in the lamellar structures.

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EFFECTS OF DIFFERENT OIL SOURCES ON FEEDLOT PERFORMANCE AND FATTY ACID PROFILES OF LAMBS (Abstract)

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ABSTRACT

This study was investigating evaluation different oil sources such as Palm oil and Canola oil on feedlot performance and fatty acid profiles include daily weight gain, feed conversion ratio or feed efficiency and daily feed intake of native male lamb. Locally available breeds of livestock are important economic resources. Oil palm is one of the important oil and so it's by product is oil palm frond that they are feed resources in animal ration in South East Asia and cheaper than other feedstuff. Twenty-four male lamb (initial BW 14.23±1.46kg) were assigned to a completely randomized design were two kinds of oil includes Palm Oil and Canola oil. They were used for the first trial which lasted 16 weeks as fattening period and two weeks of adjustment period. Rations were isometabolizable energy (2.5 M Cal / kg DM intake) and isonitrogenous (14 percent crud protein on dry matter basis, (NRC 1985). Ration was consisted 65 percent concentrate and 35% oil palm frond. The rations were mixed and fed ad-libltum. The lambs bought of local folks and divided randomly in two treatments (12 lambs) of oil (Palm and Canola oils) and kept lambs individually in each box. At the end of feedlot period (16 weeks) all of lambs were slaughtered. The longissimus muscle has been taken as sample for detected fatty acids profiles. Total fatty acid from meat samples were extracted using a Chloroform-methanol solvent extraction system as described by Folch, Lees, and Sloan-Stanley (1957). Data were subjected to variance analysis by comparing the least square means by GLM procedure at a significant level of $p< 0.05$. The mean of initial, final weight, daily feed intake, feed conversion ratio and daily weight gain were not significant. In general, incorporation of Palm and Canola oils into the animal diet had significant effects on the muscle fatty acid composition of the important commercial muscle cut, such as longissimus muscle. The polyunsaturated fatty acid (PUFA) n-6/PUFA n-3 ratio was significantly increased due to the dietary supplement of Canola oil.

Key words: Palm oil, Canola oil, Feedlot performance, Fatty acid profile, Lamb

THE EFFECT OF PELLETED DIETS HAVING DIFFERENT FIBER LEVELS ON THE PERFORMANCE OF BROILERS (Abstract)

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INTRODUCTION

The possibility of feeding diets containing different levels of crude fiber (3.5, 5.0, 6.5, and 8.0%) in mash or pelleted form on the performance, carcass traits, meat chemical composition and blood biochemistry of broilers during a rearing period from 21-49 days of age was experimented on.

BIRDS, MATERIALS AND METHODS

A total number of 320 one day old of mixed Hubbard chicks were randomly allotted into four collections, each was subdivided to two groups of 40/each. A commercial iso-caloric iso-nitrogenous mash and pelleted diets of the same feed ingredients were fed for all 8 experimental groups from 0–3 weeks. In the first two groups, birds were fed ad-libitum on broiler grower-finisher experimental diets containing 3.5% crude fiber level in mash or pelleted form while the second, third and fourth collections (groups from 3 to 8) were fed diets with fiber levels of 5.0, 6.5 and 8.0% respectively. All single groups were fed on mash diets, while pelleted diets were fed to the paired groups.

RESULTS

The groups fed diet having 3.5 and 5.0% CF recorded nearly equal total feed conversion ratio (1.98 and 1.99, respectively). More food was consumed and less weight gained and by turn higher feed conversion values (2.27 and 2.52) were recorded by the groups fed diets having 6.5 and 8.0% CF respectively. The total weight gain of the groups fed the two diet forms was nearly equal, while pelleting the diet reduced the feed consumption and subsequently, improved the feed conversion during the whole experimental period. Along the whole experimental period, pelleting slightly improved BW, WG and FCR in groups fed diets having 3.5 and 5.0% CF. The groups fed mash and pelleted diets having 6.5 and 8.0% CF recorded higher values for FCR (2.34, 2.26, 2.68 and 2.45) than that recorded by the group fed mash diet having 3.5% CF. There were no significant differences in the dressed carcass, proventriculus percentages, serum biochemical parameters and meat chemical composition among all the treated groups. The group fed on mash diet having 8.0% CF recorded the highest gizzard weight, while the group fed on pelleted diet having 3.5% CF scorded the lowest gizzard weight. The groups fed the pelleted diets recorded significantly lower serum triglycerides levels than that recorded by the groups fed the mash diets.

CONCLUSION

The best performance was obtained for the groups fed on the pelleted diet than that fed on mash diet up to 6.5% CF level.

UTILISATION OF GLYCINATED AND INORGANICALLY-BOUND TRACE ELEMENT BY GROWING PIGS

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SUMMARY

Experiments were carried out with four groups of growing pigs with 4 pigs in each group (in the control A group 6) to estimate the utilisation of glycinated iron, copper, zinc and manganese supplements in comparison with their inorganic form. The pigs were kept individually in metabolic crates in chambers of the environmental laboratory. Group 1 (Ko-A) served as control and consumed feed with no supplementation. Group 2. (Ko-B) was fed with diet supplemented with inorganic microelement. Group 3 (Ki-A) had identical feeding regime with Group 2 but the pigs received glycinated microelement supplementation in quantities identical with the inorganic supplementation of Group 2. Group 4 (Ki-B) followed the feeding in Group 3 and the supplementation with glycinated microelements was 50% of Group 3. Faeces and urine was collected daily, blood samples were taken with 7 day intervals and their trace element concentration was determined. Weight gain of the pigs was also measured. At d 14 two pigs, than at conclusion of the experimental period all the pigs were sacrificed and samples were taken from the liver, kidneys, muscles and heart for determination of the trace element concentrations. Fe and Cu was utilised best by experimental pigs. Absorption of Zn was proven best in Ki-A pigs with remarking that absorption in Ki-B pigs was also favourable. Manganese absorption was found best in Ki-A pigs. Utilisation of Ki-B pigs was also good. Glycinated trace elements in feed ensure favourable trace element supplementation in a way that tissue concentrations of these elements are low; therefore they might be less toxic. Data of the present experiment proved that the best weight gain and the most optimum feed conversion efficiency were achieved by Ki-A pigs in the main period of the experiment.

INTRODUCTION

The chemical form of trace minerals can greatly influence the bio-availability as illustrated by the comparison of cupric oxide with cupric sulfate (Cromwell et al., 1989; Baker et al., 1991; and Xin et al., 1991), with the oxide form having very low bio-availability. A second major factor influencing bio-availability is the interference or interrelationship with other minerals. It is proved that organic forms of some trace elements provide a far superior biological effect than the inorganic form (Mertz, 1993). The essential Chromium for instance, meets the requirement at considerably smaller concentration than its inorganic variety (Mertz, 1993).
Good number of research have studied the absorption and metabolism of the organic and inorganic form of trace elements (Du et al. 1996., Mahan, 1997., Xin et al., 1991.). These investigations have proven that interactions among organically bound trace elements is decreased or vanished. The practical consequence of these findings is the need for more careful formulation of diets to avoid intoxications and/or disproportionate supplementation of trace element (Du et al., 1996).
The request for precision supplementation of trace elements has become, rightly, an important issue, because owing to the ever hardening rules of food and environmental safety, the applicable concentrations have been decreasing.

MATERIAL AND METHODS

The experiment was carried out with growing pigs to estimate the utilisation of organically (glycinate-) bound iron, copper, zinc and manganese supplements in comparison with their inorganic form.
The experiments were carried out with Hungahib (Large white x Landrace) hybrid pigs. Four groups were formed with 4 pigs in each group (in the control A group 6) pigs. The average weight of the pigs was 34.8±1.5 kg at the beginning of the experiment. The pigs were accommodated individually in metabolic crates in chambers of the environmental laboratory which provided optimal microclimatic conditions for each groups. All groups were housed in separate chambers. Illumination lasted for 12 hours per day.
Group 1 (Ko-A; Control-A) served as non treated control. Pigs of this group consumed pig starter feed with no trace element supplementation. The members of Group 2 (Ko-B; Control-B) were fed with the starter diet consumed also by Group 1 but in this case the diet was supplemented with an inorganic microelement supplement (220-650 Vitapig 4% premix; VITAFORT Co). Pigs in Group 3 (Ki-A; Experimental-A) had identical feeding regime with

members of Group 2. In this case, however, pigs received glycinated microelement supplementation in quantities identical with the supplementation of Group 2 pigs. The feeding in Group 4 (Ki-B; Experimental-B) followed the feeding in Group 3 with the difference, that in this case supplementation with glycinated microelements was 50% of that offered to pigs of Group 3 (Table 1).

The experiments consisted of a preliminary and a main period

- Preliminary period lasted for 14 days. In the first three days of this period the feed was gradually shifted from the home feed (viz. what piglets had consumed before they were transferred to the environmental laboratory) to the feed consumed during the experiment. This preliminary period gave good opportunity for the pigs to adapting themselves to the new surroundings and handling. From day 4 on feed and water consumption and faeces and urine production of the pigs was measured on daily basis. Due to initial technical problems, reliable data were collected only in the last few days of the preliminary period.

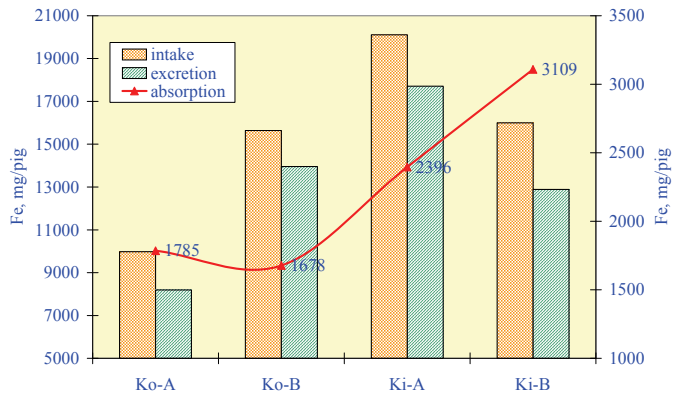
- Main period of the experiment took place for 24 days. From the very first day of this period the groups received the control and experimental diets. Daily feed ratios were distributed two times a day in equal quantities always at the same morning and afternoon hour. Drinking water was supplied ad lib through nipple drinkers. Climatic chambers were illuminated between 6.00 a.m. and 6.00 p.m. The environmental temperature was gradually decreased from the initial 25 oC to 21 oC towards the end of the experiment. Weights of the pigs were taken three times: at the beginning and at the end of the preliminary period and at conclusion of the main period. For studying the microelement concentration of the blood, samples were taken from the jugular vein with 7 day intervals. At the end of the preliminary period 2 pigs from the 6 of Ko-A group were sacrificed and muscle-, heart-, liver- and kidney-samples were taken (basal samples) for determination of the microelement concentration. On the very last day of the experiment all pigs were sacrificed and organ samples were collected. The microelement content of the organ samples were calculated per unit dry matter content. Urine and faeces was quantitatively collected on each day and three times a week (on Monday, Wednesday and Friday) samples were taken for further analysis of microelements (Fe, Zn, Cu and Mn).

RESULTS AND DISCUSSION

Microelement content of feed, faeces and urine samples; calculation of the digestibility coefficient

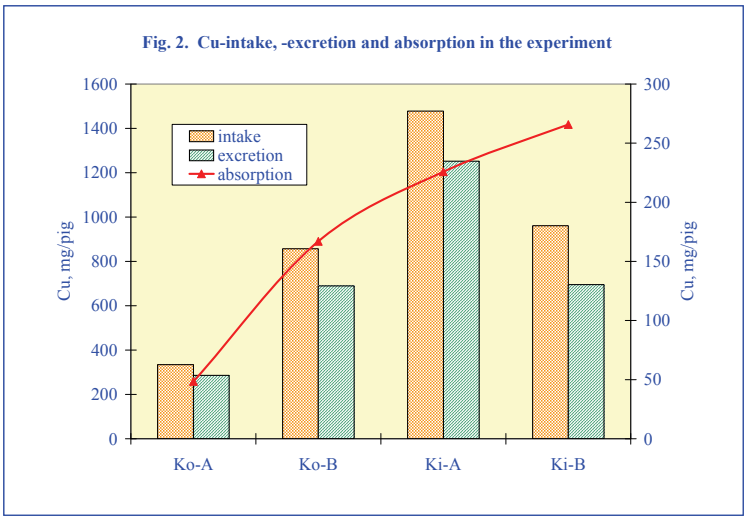
All feeds were sampled three times (at the beginning and ends of the periods) for determination of the concentration of micro elements. This made possible to calculate the microelement intake.
In order to be able to follow up the absorption of microelements throughout the main experimental period coefficients of digestibility was calculated. Daily faecal production of the pigs was registered in the main experimental period. Microelement intake was calculated by the actual microelement consumption. The trace element intake met the demand of the pigs (NRC, 1998). On basis of intake and excretion the absorbed amount of microelements was calculated and on this basis their coefficient of digestibility was estimated.

Fig. 1. Fe-intake, -excretion and absorption in the experiment



Absorption/utilisation of the micro¬¬elements examined

Most favourable absorption of iron was found in case of consumption of Fe-glycinate supplemented diets, however inverse relationship was found between intake and absorption. Therefore digestibility found in the Ki-B group was by 185% superior to that found in group Ko-B. The most favourable rate copper of absorption was found with Ki-B pigs. It was higher by 117% than that of the Ki-A group. The highest zinc absorption was produced by the Ki-A group that outnumbered that of the Ko-B group by 130%. Zn absorption of KI-B pigs was also superior by 7% to that of Ko-B pigs. Absorption of manganese was the best in the Ki-A group. This rate of absorption was by 317 and 123% higher in comparison with that of Ko-A and Ko-B pigs, respectively. Manganese absorption in Ki-B pigs was by 56% less than that in Ki-A pigs (Figure 1-2.).

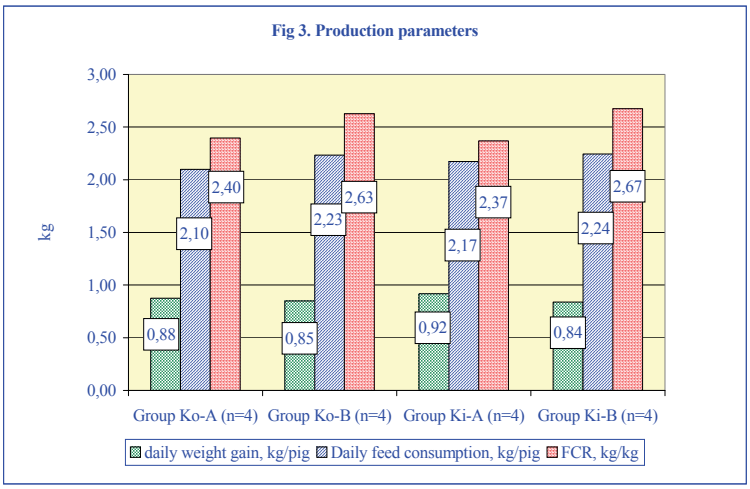


Trace element concentration of organ samples

The Fe and Zn content of the liver showed their smallest concentration at the end of the preliminary period. In contrast with the foregoing results manganese and copper content of the liver tissues showed its peak in the two pigs sacrificed at the end of the preliminary period. Iron and zinc concentration of the cortical tissue samples of kidneys proved smallest at the end of the preliminary period and the biggest at the end of the main experimental period in Ko-A pigs. Manganese concentration of the cortical tissue samples of kidneys was found the smallest and biggest in the Ki-B and Ko-A group, respectively. Copper concentrated at the least efficiency in the kidneys of Ki-B pigs while it was at its greatest concentration in Ko-B and Ki-A pigs. The iron and zinc concentration of the heart samples reached the highest levels in Ko-A pigs. Mn and Cu concentration of the heart samples was found at its highest peak at the conclusion of the main experimental period. Iron content of the samples taken from skeletal muscles was also found at its highest peak at the conclusion of the main experimental period with the smallest concentration in pigs of the Ki-A group. Zn concentration in the Ki-B group was considerably smaller than in the other groups. Mn and Cu concentrations of the skeletal muscles have followed similar trends.

Main production results

In the period of the experiment main production parameters of the experimental and control pigs were registered, in spite of the fact that only 4 pigs formed one group. In this light, production parameters can only be evaluated with certain reservations and can be regarded as informative results. Data has indicated that the best daily weight gain and feed conversion efficiency was produced by the Ki-A pigs in the main period of the experiment (Figure 3.).



CONCLUSIONS

Glycinated trace elements in feed ensure favourable trace element supplementation in a way that tissue concentrations of these elements are low; therefore they might be less toxic. Data of the present experiment proved that the best weight gain and the most optimum feed conversion efficiency were achieved by Ki-A pigs in the main period of the experiment.

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PRODUCTION OF CRUDE PROTEIN IN RYE GRASS AND ITS UTILIZATION IN RUMINANTS

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SUMMARY

Nonlinear parameters were estimated for crude protein rumen in cattle fed ryegrass harvested with different accumulation of days and dates of cuts: T1 (30 days and 15.03% CP), T2 (60 days and 19.80% CP), T3 (90 days and 16.02% CP) and T4 (120 days and 18.0% CP) at different incubation periods (36, 24, 12, 8, 4 and 2 h). The variables estimated were: crude protein degradability (CPD) with the exponential equation: $p = a + b (e^{-ct})$, where: p = rate of disappearance of nutrients in a period of time, a = intercept of the solubilized portion at the beginning of incubation (hour 0), b = potentially degradable fraction in the rumen, c h^{-1} = velocity or rate of degradability of fraction b, and t = incubation time. Potential degradability was obtained (pd) = a + b and effective degradability (ed)=a+b*c/(c+0.003) assuming a passage rate of 3%. There was no difference in the effective degradability (P>0.05) obtaining 23.09^{a} (T1), 24.27^{a} (T2), 22.28^{a} for T3, and T4. The treatments had a speed degradation rate ranging from 3.6 to 3.2%. There was no effect of cutting and number of days accumulated (T1 = 30, T2 = 60, T3 = 90, T4 = 120) in the estimated variables. We conclude that increasing the number of cuts and days accrued in the harvest of rye grass, which was used as feed for ruminants, did not affect the degradability and ruminal passage rate of crude protein (CP)

INTRODUCTION

Northwestern Mexico is known for its importance in ryegrass grazing (Rodriguez *et al.,* 2001). The efficiency of feed use is connected to the knowledge of its nutritional characteristics, being the main evaluation parameters, nutrient content, intake and digestibility (Guerra *et al.,* 2001). *In situ* digestibility is affected by diet type, animal species and physiological state, level and pattern of intake (Schneider and Flatt, 1975): this technique requires a process where you need to prepare surgically ruminants to be used as experimental units (Guerra *et al.,* 1998). The feeding value of forage is defined as the ability to promote livestock production as a result of the availability of nutrients, as well as consumption (Beever *et al.,* 2000). The aim of this study was to estimate nonlinear parameters rumen in cattle fed ryegrass harvested with different accumulation of days and dates of cuts for the content of crude protein (CP).

METHODOLOGY

This work was done at the zootechnical post belonging to the School of Agronomy of the Autonomous University of Sinaloa, located in the municipality of Culiacan, Sinaloa, Mexico. We evaluated four cut dates T1 = 60 d, T2 = 90 d, T3 = 120 d and T4 = 150 d (Table 1). The samples were dried at 60 □C for 48 h, for later analysis of chemical components such as: Crude Protein (CP), hemicellulose (HEMI), Cellular Content (CC), acid detergent fiber (ADF), neutral detergent fiber (NDF) (Goering and Van Soest, 1970; AOAC, 1975). Dry matter degradability (DMD) was estimated for different periods of incubation 48, 24, 12, 8, 6, 4 and 2 h. Subsequently, the bags were washed with running water for 5 min, until they were clean. For *in situ* degradability of dry matter (DM) the formula of Schneider and Flatt was applied (1975). We used two Cebu crossed male animals cannulated in rumen of 300 kg. Each animal was fed for treatment 105 plastic bags of 10x20 cm (ANKOM) with 5 g of ground sample (1 mm), weighed and identified by bag, animal and period, with a pore size of 50 ± 15 mμ, and area exposure of 18 mg cm 2^{-1}. Nonlinear rumen parameters were estimated (PNLR) DMS and NDFD (Ørskov and McDonald, 1979) with the exponential equation: p = a + b (e-ct), where: p = rate of disappearance of nutrients in a period of time, a = intercept of the solubilized portion at the beginning of incubation (time 0), b = potentially degradable fraction in the rumen, c = velocity or rate of degradability of fraction b, and t = incubation time. Potential degradability was obtained (pd) = a + b and effective degradability (ed)=a+b*c/(c+0.003) assuming a passage rate of 3% (Ørskov and McDonald, 1979). The data were analyzed using PROC NLIN of SAS version 9.2 (2004).

RESULTS

The treatments were a crude protein content of 15.03%, 19.80, 16.02 and 18.0% (T1, T2 and T3). Nonlinear parameters of the treatments are shown in Table 1, which shows that there was no statistical differences for any variables (P<0.05). In effective degradability (ed) ruminal crude protein was obtained 23.09%, 24.27%, 22.28% and 22.28% (T1, T2 and T3). At the speed the rate of degradation (c h^{-1}) results were 42.17%, 43.31%, 40.72% and 41.24% respectively.

DISCUSSION

The efficiency of the forage depends on the knowledge of its nutritional characteristics, the main evaluation parameters, nutrient content, intake and digestibility (Guerra *et al.,* 2001). For this reason, the nutritional value of forages is estimated by analyzing their chemical content (Bogdan, 1997).

CONCLUSIONS

We conclude that increasing the number of cuts and days accrued during the harvest of rye grass did not affect those treatments which had the same rate in the degradation rate of 3.0% and concluding that the above does not affect the effective degradability and the rate of ruminal passage of crude protein CP.

Table 1: Nonlinear parameters of *in situ* ruminal degradability: CP

Rye grass	RP				
Treatment	a	b	c (h^{-1})	dp (a+b)	ed
T1 (60 days)	3.00	39.17	0.032	42.17	23.09[a]
T2 (90 days)	3.48	39.83	0.033	43.31	24.27[a]
T3 (120 days)	1.39	39.33	0.036	40.72	22.28[a]
T4 (150 days)	1.41	39.83	0.033	41.24	22.28[a]

Values in the same row with different literal differ statistically (P<0.01)

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GROUPING OF NUTRITIONAL FACTORS THAT EXPLAIN THE QUALITY OF CORN SPROUTS

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SUMMARY

Corn sprout is excellent in its production of green material and its high nutritional value, with the leaves and young stems of high palatability for ruminants (Guerra *et al.,* 2001a). The objective of this research was to obtain the group of nutritional factors that explain the quality of corn sprouts and their effect on ruminant feed. The variables of interest were height (H, cm), green forage (GF) kg box^{-1}, dry forage (DF) kg box^{-1}, dry matter (DM%), crude protein (CP%) and acid detergent fiber (ADF%). We used a technique called Principal Component Analysis (PCA), selecting factors with own values equal to or greater than one, rotating them with the orthogonal method quartimax (Hair *et al.,* 1999, Guerra *et al.,* 2003). We used the PROC FACTOR and PROC CORR of the SAS package (2003). It is concluded that there were two groups for diagnosing latent factors that explain the nutritional value of forage maize, factor 1 consists of: A, GF, DM, CP and ADF (called succulent part) explains 82.412% of its quality and Factor 2 composed only of DF (called dry part without moisture), which accounts for 17.587%, the latter indicating that their influence is high, negatively impacting the quality of forage produced (Tejada, 1992).

INTRODUCTION

In ruminants fed forage one should be consider the fill volume as an important factor in voluntary intake (Balch and Campling, 1962). The use of germinated corn at levels of 50% based on dry matter fed sheep, the productive behavior is impacted negatively, by the volume that implies a low dry matter content (Guerra *et al.,* 2001b) preventing them to meet their nutritional requirements. The nutrient content of germinated maize is affected by age, as advanced maturity increases the content of dry matter and crude fiber, while digestibility, crude protein and ash fall (Guerra *et al.,* 2001 meetings, Guerra *et al.,* 2003). The objective of this research was to obtain the group of nutritional factors that explain the quality of corn sprouts and their effect on ruminant feed.

MATERIALS AND METHODS

This research was conducted in the School of Agronomy of the Autonomous University of Sinaloa, located at km 17.5 of the Culiacan-El Dorado road, County of Culiacan, Sinaloa. We evaluated a variety of commercial and germinated corn. Boxes of 54X37cm were used and 32 cm high, with perforations of 1 cm^2 in diamond shapes on the floor and walls, allowing the entry and exit of air, a germination technique reported by Gastelum (1998). The planting density was 1 kg box^{-1}, yielding 72% germination. The variables of interest were height (H, cm), green forage (GF) kg box^{-1}, dry forage (DF) 1 kg box^{-1}, dry matter (DM%), crude protein (CP%) Acid Detergent Fiber (ADF%) (AOAC, 1975). We used the multivariate analysis technique, applying the factor extraction method, called Principal Component Analysis (PCA), selecting factors with own values equal to or greater than one, rotating them with the orthogonal method QUARTIMAX (Hair *et al.,* 1999; Guerra *et al.,* 2003). We used PROC FACTOR and PROC CORR of SAS (2003).

RESULTS

Table 1: mean values and standard deviations of the variables used in the study of factors that explain the nutritional value of corn sprouts

Variable	Average	Estimated Standard Error
Height (H)	23.09	2.11
Dry Forage (DF)	2.93	0.11
Green Forage (GF)	13.34	1.31
Crude Protein (CP)	15.24	0.59
Acid Detergent Fiber (ADF)	20.61	1.69
Dry Matter (DM)	24.3	2.26

For standardization of the data we calculated the correlation matrix (Table 2), it is remarkable that there are high correlations between variables, which is one of the requirements of factor analysis by PCA (Hair *et al.,* 1999, Guerra *et al.,* 2003).

Table 2: Correlation matrix of variables used in the study of factors that explain the nutritional value of corn sprouts

	H	**DF**	**GF**	**CP**	**ADF**	**DM**
H	**1.00**	-0.636*	0.779**	0.787**	0.815**	-0.871**
DF		**1.00**	-0.210 ns	-0491ns	0612*	0.434 ns
GF			**1.00**	0.636*	0.772**	-0.950**
CP				**1.00**	0.729**	-0.669*
ADF					**1.00**	-0.883**
DM						**1.00**

In analyzing the behavior of the variables under study, it shows that by applying the methods of selection factors only two are kept, reducing it to bidimensional space, and able to keep 99.99% of the total variability. Table 3 presents the results of the resulting factors obtained by PCA before and after the rotation quartimax. The purpose of conducting the rotation of the factors is to define more adequately the factor values, placing as close as possible to 1 in one factor and 0 in the other (Davydova, 2002, Hair *et al.,* 1999, Guerra *et al.,* 2003). Factor 1 has high coefficients for the variables H, GF, CP, ADF and DM. Therefore, this factor can be designated as an *ad libitum* or factor succulent "as offered". Factor 2 has high capacities for dry forage (DF), so we call it dry with no moisture factor. The first two factors explain 82,412% of total variation. The spatial concentration for factor 2 is taken only by DF, the part without moisture of forage maize (Tejada, 1992); explaining 17,587% of total variation. The cumulative total variance explained by the two factors is 99.99% (Table 3), showing the relationship value of 5.366604 out of a total of 6 to explain, such that the specified total unexplained variance is 0.3463.

DISCUSSION

FAD content had no practical impact on the nutritional value, the low presence of the indigestible portion of sprouts and succulent. But it is important to note that sprouts in the form of dry forage is affected in a negative nutritional quality, since by reducing the presence of moisture and increasing the dry forage, ADF content is increased. The use of corn germinated at high levels in the diet of ruminants evidences that its main limitation is their high water content and low dry matter, preventing them meet their nutritional requirements (Guerra *et al.,* 2001a); these authors agree with Laredo and Minson (1973) who concluded that the leaf-stalk proportion and the plant growth stage has an effect on voluntary intake as it affects forage quality (Fontenot and Blaser, 1965), impacting negatively on productive behavior (Guerra *et al.,* 2001b), reducing the protein content in forage, and significantly affecting animal behavior (Minson, 1990).

Table 3: Factor extraction by principal compnents

	Factors					
Var	**Non rotation**		**Rotation quartimax**		**Relationship**	**Specific variance**
	F1	**F2**	**F1**	**F2**	h_i^2	**1-y_i**
H	0.94755	-0.07357	**0.92766**	-0.20669	0.903267	0.096733
DF	-0.62305	0.75588	-0.51002	**0.83632**	0.959544	0.040456
GF	0.86414	0.47332	**0.92234**	0.34649	0.970766	0.009948
CP	0.83454	-0.08063	**0.81478**	-0.19772	0.702957	0.029234
ADF	0.93438	-0.04015	**0.91934**	-0.17175	0.874686	0.125314
DM	-0.94486	-0.25024	**-0.97074**	-0.11425	0.955385	0.044615
Proper Values	**4.495071**	**0.871533**	**4.422754**	**0.943850**	**∑ 5.366604**	**∑ 0.3463**
TPV	**5.366604**					
PTVE	**83.76**	**16.23**	**82.412**	**17.587**		
TVEC	83.76	**99.99**	82.412	**99.99**		

Var = Variables, TPV = Total Proper Values, PTVE = Proportion of Total Variance Explained; TVEC = Total Variance Explained Cumulative.

CONCLUSIONS

We conclude that there were two groups for diagnosing latent factors that explain the nutritional value of forage maize, factor 1 consists of: H, GF, DM, CP and ADF (called succulent part) explains 82,412% of its quality and factor 2 composed only of DF (called the dry part without moisture), which accounts for 17,587%, the latter indicating that their influence is high, negatively impacting the quality of forage produced (Tejada, 1992).

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THE QUALITY OF FEED GRAINS ACCORDING TO EVALUATION CRITERIA

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SUMMARY

Aim of the study: to estimate quality of feed grain of 2009 harvest by the Grain processing enterprises criteria, estimate the correlation between grain qualitative and sanitary parameters. In the study was established that protein content was 12.5±0.32% in feed wheat, in feed barely protein content – 11.9±0.26%. Gluten content was 25.2±0.81% in feed wheat. Sedimentation indicator of feed wheat was 38.7±1.95 ml. Specific weight in feed wheat was 82.8±1.5 kg/hl, specific weight in feed barley – 76.3±1.5 kg/hl. Content moisture in feed wheat was detected 14.68±0.2%, in feed barely – 15.18±0.16%. Total content of impurities in feed wheat was 3.6±0.6% and in feed barley – 3.0±0.6%.

In the study was established that in the feed wheat grain samples total number of micro-organisms was 195.03 ± 25.65 thous. CFU/g on the average and in barley grain samples total number of micro-organisms – 329.83±51.05 thous. CFU/g on the average. Number of variables spores of fungi was average – 23.52 ± 3.25 thous. CFU/g on the average in the feed wheat, in the feed barley – 12.27± 18.46 thous. CFU/g.

Different concentrations of mycotoxins were tested in the feed wheat and barley, largest deoxynivalenol and zearalenone concentrations were detected in the feed wheat and barley. *Alternaria*, *Fusarium*, *Cladosporium*, *Rhizopus, Mucor, Helminthosporium, Penicillium* genera dominated in the feed grain samples.

Correlation was low between grain qualitative and sanitary parameters.

INTRODUCTION

Now wheat, barley, and corn are the most important cereal products, from which the most part falls on food and one-fifth – for feed [7]. Wheat and barley are grown in more than 140 countries in the world. They are the most common cereals in the world. Their grain is a valuable concentrated feed and raw material compound feed industry [10]. Grain quality largely depends on the grain type and its end use.

Grain quality is the whole of biological, physico - chemical, technological and customer-reflecting properties and grain characteristics. It includes a range of properties that can be defined in terms of physical (moisture content, test weight, kernel size, total damaged kernels, heat damage, broken kernels, stress cracking, breakage susceptibility), sanitary (fungi and mycotoxin count, insects and insect fragments, rodent excrements, foreign material, toxic seeds, pesticide residue, odor, dust), and intrinsic (milling yield, oil content, protein content, hardness, density, starch content, feed value, viability, storability) quality characteristics. The quality properties of a grain are affected by its genetic traits, the growing period, timing of harvest, grain harvesting and handling equipment, drying system, storage management practices [1; 2].

In the Lithuania, wheat quality is determined according to parameters of national standard „Wheat. Requirements for purchase and supply" (LST 1524:2003) and barley quality - according to parameters of national standard "Barley - Requirements for purchase and supply" (LST 1797:2003/1K:2008).

Cereals can be grown on various soils, using different agrotechnical and agrochemical implements. This way various groups of microorganisms have different conditions to develop in their growing environment and get a chance to contaminate grains when they are growing, maturing and being harvested [6; 8]. A great part of infectious microorganisms of grain is various species of fungi. Various secondary metabolites of different chemical composition – mycotoxins – are synthesized and excreted into the environment by fungi [3;5]. Recently it has been determined that toxins produced by *Fusarium* genera of fungi are especially dangerous. It was estimated that deoxynivalenol, zearalenone, T-2 toxin, fumonisins and many other mycotoxins are particularly hazardous to animal and human health.

Aim of the study: to estimate quality of feed grain of 2009 harvest by the Grain processing enterprises criteria, estimate the correlation between grain qualitative and sanitary parameters.

MATERIAL AND METHODS

In 2009 at harvest grain samples of feed wheat (*Triticum aestivum* L.) (n=50) and feed barley (*Hordeum distichon* L.) (n=50) were collected from different agricultures companies.

Qualitative characteristics (moisture, gluten, starch, protein, sedimentation index) of wheat and barley were tested with analyzer *Infratec*, were determined mass per hectolitre and impurities content.

The general number of microorganisms and number viable spores of fungi (forming units per gram sample (CFU/g)) in grain were detected dilution method. Internal grain contamination with fungi was determined by plating the surface-sterilized grains (200 for each sample) on Petri dishes with standard agar Czapek-Dox (Oxoid) supplied with chloramphenicol (50 mg/l) (Sigma) incubated for 7-8 days at 26±2ºC. The fungal contamination level on grain was determined by direct plating. The infection level of grain was evaluated in percent.

The mycotoxins deoxynivalenol (DON), T-2 toxin, zearalenone (ZEN) concentrations were analyzed by the ELISA method. RIDASCREEN® FAST DON, RIDASCREEN® FAST T-2 TOXIN, RIDASCREEN® FAST Zearalenon (R-Biopharm AG, Germany) were used for mycotoxins concentration analysis.

Statistical analysis was processed using the "SPSS for Windows", version 12.0.

RESULTS AND DISCUSSION

Grain quality largely depends on the grain type and its end use. Each year, some low quality grain enters the market channel following a growing season that is characterized by drought, extreme heat during a sensitive stage in crop development, excess moisture, an early frost, plant disease, or other malady. Grain qualitative characteristics are summarized in Table 1.

Table 1. Feed grains qualitative characteristics

Parameters	Feed grain	
	Wheat	Barley
Moisture %	14.68 ± 0.20	15.18 ± 0.16
Protein %	12.5 ± 0.32	11.9 ± 0.26
Sedimentation ml	38.7 ± 1.95	-
Mass per hectolitre kg/hl	2.8 ± 1.50	76.3 ± 1.50
Wet gluten %	25.2 ± 0.81	-
Total impurities %	3.5 ± 0.64	3.0 ± 0.62
Miscellaneous impurities %	1.5 ± 0.25	1.6 ± 0.43
Impurities consisting grain %	2.1 ± 0.53	1.4 ± 0.25

Moisture content in grain determines the length of time grain can be stored. High moisture grain is more prone to experience a deterioration in quality due to mold [4]. Moisture content in feed wheat ranged from 13.3 to 17.0% (S=0.95; C_v=0.91), in feed barely – from 14.3 to 16.7% (Sv=0.62; Cv=0.39).

The general number of microorganisms in wheat was 95.03 ± 25.65 thous. CFU/g (Sv=128.25), barely - 329.83 ± 51.05 thous. CFU/g (Sv=197.72).

The results of mycological tests indicate that feed wheat was contaminated with fungi 23.52±3.25 thous. CFU/g (Sv=16.23), in barely – 12.25 ± 18.46 thous. CFU/g (Sv=20.18).

Alternaria (in wheat - 46.6%, in barley – 41.0%), *Fusarium* (in wheat – 35.8%, in barley – 22.9%), *Cladosporium* (in wheat – 13.6%, in barley – 6.6%), *Helminthosporium* (in barely – 49.7%) genera dominated in the feed grain samples. The grain samples were contaminated and with *Rhizopus, Mucor, Penicillium* and other genera.

Various secondary metabolites of different chemical composition – mycotoxins – are synthesized and excreted into the environment by fungi. Recently it has been determined that toxins produced by *Fusarium* genera of fungi are especially dangerous. It was estimated that deoxynivalenol, zearalenone, T-2 toxin, fumonisins and many other mycotoxins are particularly hazardous to animal and human health.

Fungi can grow almost anywhere under a wide array of environmental conditions. Fortunately, not all fungi produce measurable levels of mycotoxins. The synthesis of mycotoxins, more than fungal growth, is dependent on specific weather and environmental conditions [9].

Results of mycotoxins analysis are summarized in Table 2.

Table 2 Concentration of deoxynivalenol (DON), T-2 toxin, zearalenone (ZEN) (µg/kg) in feed grains

Grain	Mycotoxin concentration, µg/kg		
	DON	T-2 toxin	ZEN
Feed wheat	727.1 ± 211.8	2.6 ± 2.6	53.5 ± 11.7
Feed barley	120.6 ± 42.9	-	16.6 ± 7.6

In 41,7% feed wheat samples DON concentration (1350.0 to 1700 µg/kg) exceeded the permissible norms. The maximum ZEN content was 65 µg/kg in feed barley.

Was detected correlation between grain qualitative and sanitary parameters. The correlation was low between grain qualitative and sanitary parameters.

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MILK REPLACER FEEDING AND THE FUNCTIONAL CONDITION OF CALF AND GOAT ABOMASUM

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SUMMARY

The objective of the study was to investigate the possible changes in functional state of the calf's and goat's abomasum in the relationship of animals age and milk replacement feeding. We summarize the data about the dynamics of pH, obtained by examination of 10 calves and 10 kid's in the age between five and 14 weeks. The intra-abomasal pH was determined by the uninterrupted long-lasting potenciometrical intragastric pH measurement method using pH-probe with was inserted through the abomasal fistula. We compeered intraabomasal pH dynamic before and after feeding with whole milk and milk replacement. We established that 15-10 minutes before routine feeding the radical increase of abomasum acid level was obtained, in calves. In 5-14 weeks old calf after whole milk feeding we established intra-abomasal pH 4.0, the lowest acid level was obtained in the first hour - not during 1.5-2 hour as it was in two - four weeks old animals. In 5-14 weeks old calf intraabomasal pH changes were different between animals who received whole milk or milk replacement.

INTRODUCTION

For a long time it has been noted in the literature that the proper feeding of the ruminants during milking period is complicated and responsible process, because fast-growing body needs valuable and energy-rich food. During this period animal's organs of the digestive system also continues to develop both morphologically and functionally. In recent years studies of the functional status of the stomach in calves and goats is associated with the development of a new power scheme incorporating a variety of manufactured milk substitutes, prestarters and forage supplements. There is studied their effect on the body as a whole as well as on the functional status and development of the digestive system organs [1, 2, 3, 5, 7, 8, 11].

In literature, there is little data on the functional status of the abomasum in calves and kids in connection with milk or milk replacer feeding. There are a few studies primarily related to improve scheme of the feeding scheme. It is proved that at the beginning of the milking period gastric enzymatic activity is increasing in the abomasum of the calves which significantly accelerates the abomasal emptying rate. There is data in the literature that animals what have been fed with different milk replacers have abomasal average pH values that ranged from 3.2 to 3.2 during 24 hours long period, but feeding the animals with whole milk, they were significantly lower - an average pH level was 2.7 [2, 3]. We could not find data for the milk or milk replacer feeding affects on the functional state of the abomasum in kids. Therefore the aim of this work is to establish a functional state of the abomasum basic in 5-14 week-old calves and the same age kids related with the milk or milk replacer feeding. Strategy: 1.Explore and compare dynamics of the intraabomasal pH of the 5-14 week-old calves before and after animal feeding with whole milk and milk substitutes.
2. Explore and compare dynamics of the intraabomasal pH of the 5-14 weeks old kids before and after animal feeding with whole milk and milk substitutes.

MATERIAL AND METHODS

The study in general used ten calves and ten kids ranging from five to 14 weeks of age. The research took place during the winter, so the nutrition of the Latvian base on developed feeding schemes for the age of calves and kids winter [6, 9].
During studies we followed the general health status of the animals – temperature, heart rate, the digestive process, blood biochemical parameters, as well as weight gain, what were within normal limits in the research included animals. Depending on the fed compound we created following research groups:

The first group – 5-14 weeks old calves. All these calves were fed with the age-appropriate amount of hay – from 0.3 to 1.0 kg per day and the combined concentrated feed for calves – from 0.5 to 1.0 kg. Starting with the fifth week calves were fed a little differently. Five animals were fed with the whole milk – by 3.5-4.0 l three times a day, and these animals formed the first A sub-group (1A). The other five – a milk substitute – by 3.5-4.0 l three times a day, which, in turn, represented the first group B subgroup (1B).

The second – group 5-14 weeks old kids. The animals were fed with the age-appropriate amount of hay – from 0.5 to 0.8 kg per day and the combined silage – 0.8 kg. Kids also

were fed little different: five animals twice a day were fed with two liters of whole milk (sub-group 2A) and the other five – two liters of milk replacer (sub-group 2B).

All animals of the study got abomasal fistulas. To study the *in vivo* changes of the abomasal pH in calves and kids before and after feeding we used chronic intraabomasal fistulas and pH– metric method. These methods are currently one of the more accurate and effective especially in long-term study performance [1, 2, 3, 10].

Gastric functional investigation in all animals were carried out using a 7-hour continuous pH-intraabomasal metering including morning feeding time at 6:15 during the cycle of investigation. We administered pH-probe through chronic abomasal fistula so that electrode of the probe could fix pH level close to the fistula's inner ring – that is in the fundic gland region of the abomasums. For the first group of calves we performed 119 tests, while for the second group of kids in general we performed 98 tests. During the study the animals practically did not feel any discomfort – they were free to eat, ruminate and sleep.

RESULTS

Intraabomasal pH changes of calves 5-14 weeks of age during one hour before and six hours after milk feeding (group 1A) and after feeding with milk replacer (group 1B) is showed in the first a image.

Analyzing the change in pH we found that in the morning before feeding 5-14 week-old calves (both 1A and 1B group of animals) acid level in the abomasum at the electrode location was on average 2.8 pH level. Since we started investigation during the time period from 5:00 and continuing it until 6:00 o'clock intraabomasal environment gradually became more acidic, but before the feeding of the whole milk or milk replacer there was observed rapid increase of the acid levels. It should be noted that, compared with baseline the acid levels significantly increased in group 1A before the calves were fed with whole milk when the pH decreased to 2.1 ± 0.21 level (P = 95%). But in the group 1B increase of acid level was less pronounced before the calves were fed with milk replacer (see fig.1.a).

Comparing the total intraabomasal pH dynamics after the morning feeding with milk or milk replacer, it has to be pointed that they were generally similar. Average intraabomasal pH values in the both groups of animals immediately after feeding were increasing above the 5.0 level (P = 99%), but in the following hours abomasal pH evenly began to fall.

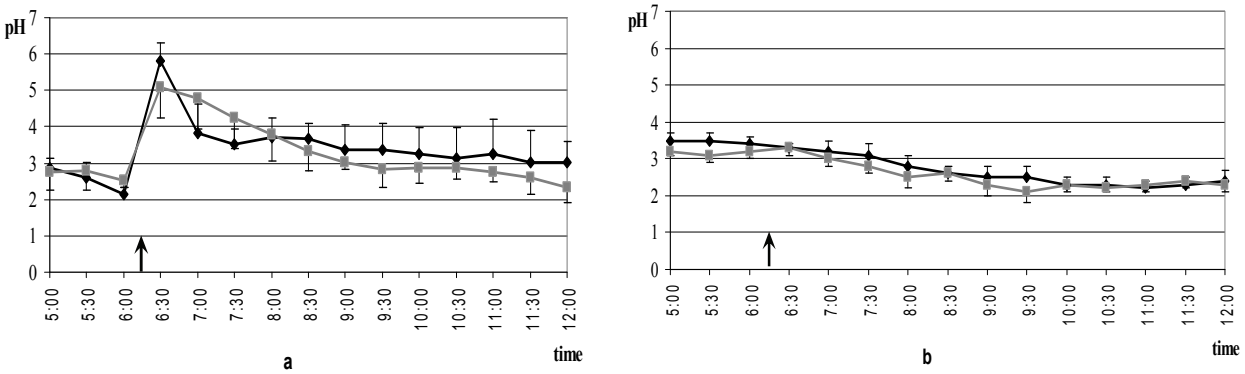

Figure 1: The intra-abomasal pH-dynamics in 5-14 weeks old calves (fig. a) and kid's (fig. b) before and after animal feeding with whole milk or milk replacer.
— - feeding with whole milk (pH average indices, T – standard deviation);
— - feeding with milk replacer (pH average indices, ⊥ – standard deviation);
↑ - animal feeding.

The main difference in the intraabomasal pH-dynamics of the animal subgroup 1A and 1B was found in 1.0-1.5 hours after the meal: in the first group of calves the acid levels evenly increased (decreased pH) to pH 3.8 ± 0.79 level already within one hour after receiving whole milk, but after feeding milk replacer for calves from group 1B hour abomasal pH level fell only to 4.8 ± 0.85, and only after two hours it reached average 3.9 - 4.0 level (see figure 1.a).

Intraabomasal pH changes in the kids before and after feeding the milk (group 2A) or milk replacer (group 2.B) is showed in figure 1.b.

It turned out that at 5:00 o'clock in the morning before the animal nutrition abomasal pH of the kid's ranged in the 3.2 - 3.4 ± 0.21 level. During the period from 6:30 after feeding the milk (2A groups of kid's) or milk replacer (group 2B kid's) pH in the abomasum gradually declined reaching 2.3 ± 0.2 pH level. It should be noted that intraabomasal pH levels pf the group of kids 2B were generally slightly lower than in group 2A (see figure 1.b).

DISCUSSION

Our research showed that in spite of fact that calves 5-14 weeks of age already receiving intensive fodder and silage there were rapid increase in acid level five minutes before the next feeding with whole milk or milk replacer in the abomasum (for some animals it was up to 1.8 pH level). Most notable it was observed in the animals what were fed with whole milk (P = 95%). A similar abomasal environment "before feeding acidification" in calves fed three times a day with whole milk has also been observed in studies by A.Ahmed with co-authors (2002). It should be noted that such "acidification" of the kid's abomasum before feeding the milk or milk replacer was not characteristic.

It should be noted that both calves and kids at this age have proventricular activity, because they show by regular periods of rumination and rumen movements. However before the morning feeding period with whole milk or milk replacer the sharp increase of the acid concentration had been seen only in abomasum of calves what is characteristic only in the mono gastric animals or milk feeding period of the ruminants [4]. One could therefore conclude that the calf in the second and possibly even the third postnatal month of life milk as a food product is still needed, and it is not ready yet to complete transition to only roughage fed at that age. While the two-three months old kids at this age can already completely switch to fodder as demonstrated in the literature [8, 9, 11].

CONCLUSIONS

1. A typical intraabomasal pH before feeding of the kids is 3.2 - 3.4 level, but of the calves - 2.8 pH level.

2. Calves unlike kids 14 weeks of age before the feeding with whole milk or milk replacer there is a marked increase of acid concentration in the abomasum reaching 2.1 ± 0.2 pH level.

3. Intraabomasal pH changes of the five-to 14-week-old calves was different in animals treated with whole milk, and animals receiving milk substitutes: the whole milk feeding intraabomasal pH 3.8 ± 0.79 level was reached within an hour, but after the milk replacer feeding pH level was reached only during 2-2.5 hours.

4. Intraabomasal pH dynamics suggest that milk feeding period for 5-14 weeks old calves has not yet expired, but the kids reaching this age is already completely ready to move on to forage feeding.

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GROWTH PERFORMANCE OF PIGS FED GREEN BERSEEM IN BASAL DIET OF KITCHEN WASTE

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SUMMARY

An experiment was conducted on 24 Large White Yorkshire finishing pigs aged 5 months to evaluate the effect of green berseem on growth performance and carcass traits in finishing pigs. The pigs were separated into four groups consisting of six piglets in each group in a completely randomized design and they were subjected to four different feding treatments *viz.,* group I: 10% green berseem + 50% kitchen waste + 40% concentrate, Gr. II: 15% green berseem + 50% kitchen waste + 35% concentrate, Gr. III: 20% green berseem + 50% kitchen waste + 30% concentrate and group IV: 25% green berseem + 50% kitchen waste + 25% concentrate. The pigs were reared up to 7 months of age and all animal were slaughtered at the end of experiment. Significantly ($p<0.01$) highest overall DM intake and overall daily weight gain was observed for Gr. I. Overall total body weight gain and FCR. Significantly ($p<0.01$) lowest value of blood glucose, total cholesterol and highest value of HDL cholesterol were observed for group IV followed by group III, II and I. Hot carcass weight, dressing percentage, back fat thickness, carcass length and loin eye area were non-significant among different treatment groups. However, significantly ($P<0.05$) highest 10^{th} rib fat thickness was observed for Gr. I. Significantly ($p<0.01$) lowest cost (Rs.) per kg live weight gain was observed for group IV (18.95) followed by group III (21.86), II (22.03) and I (25.65).

INTRODUCTION

In India, pig production systems are mainly based on low cost agro-industrial by-products unfit for human consumption or high-quality forages thus may avoid competition for human foods. Under the prevailing shortage of grains, attempts have to be made to use more of the garbage from hotels, kitchen wastes and green pasture, in place of grains to the extent feasible in pig ration without affecting the performance adversely. Legume forages can be used successfully in pork production but to a limited extent (9). The report on the use of berseem fodder in pig feeding is very scanty. Therefore, work had been planned to use more of the kitchen wastes and green pasture, in place of grains to the extent feasible in pig ration without affecting the performance adversely.

MATERIALS AND METHODS

An experiment was conducted on 24 Large White Yorkshire finishing pigs aged 5 months to evaluate the effect of green berseem on growth performance and carcass traits in finishing pigs. The pigs were separated into four groups consisting of six piglets in each group in a completely randomized design and they were subjected to four different feeding treatments *viz.,* group I: 10% green berseem + 50% kitchen waste + 40% concentrate, group II: 15% green berseem + 50% kitchen waste + 35% concentrate, group III: 20% green berseem + 50% kitchen waste + 30% concentrate and group IV: 25% green berseem + 50% kitchen waste + 25% concentrate. The pigs were reared up to 7 months of age and all animal were slaughtered at the end of experiment.

Table 1: Proximate composition (% on DM basis) of experimental diet

	T_1	T_2	T_3	T_4
Dry matter	52.32	48.52	44.73	40.93
Organic matter	92.69	92.41	92.13	91.85
Total ash	7.32	7.59	7.88	8.16
Crude protein	19.63	19.71	19.80	19.88
Crude fibre	4.34	5.12	5.89	6.67
Ether extract	8.06	8.02	7.98	7.94
Nitrogen free extract	60.58	59.48	58.38	57.28

Samples of feeds, residues and faeces were analyzed for proximate principles as per the methods described by (1). Estimation of glucose, Cholesterol and Protein were done by Span Diagnostic Kit. The data were analyzed using Analysis of Variance (ANOVA) and the Critical Difference (CD) to determine any significant difference among the treatment means (19).

RESULTS

During the entire experiment periods significantly ($P<0.01$) highest DM intake (Kg) was observed for group I (1.71±0.19) followed by group II (1.84±0.24), III (1.81±0.23) and IV (1.73±0.18). However no significant differences were observed among later three groups. Overall body weight gain (g) during finishing stage was 64.10 ± 7.27, 62.22 ± 5.84, 60.96 ± 5.61 and 64.69 ± 5.95 for group I, II, III and IV, respectively. However the difference among groups was non-significant. Significantly ($P<0.05$) highest daily body weight gain were observed for group I (505.21±69.44) followed by group IV (571.39±66.86), II (552.09±73.32) and III (538.69±56.91) however, no significant difference were observed among later three groups i.e. group II, III and IV. Replacement of concentrate for green berseem and kitchen waste decreased the Feed conversion Ratio (FCR) value. However, FCR did not differ significantly among treatment groups during entire experimental period. At the end of experiment, the glucose concentration (mmol/l) was significantly ($P<0.01$) highest in group I (4.90 ± 0.09) followed by group II (4.52 ± 0.08), III (4.37 ± 0.09) and IV (3.95 ± 0.05) however, no significant difference were observed between group II and III. Further, glucose concentration was decreased at the end of finishing stage as compared to growing stage in all the treatments. No significant difference were observed in plasma total protein (g/l) concentrations at the end of finishing stage and were ranged from 60.67 ± 0.33 to 59.17 ± 0.65 g/l. The total cholesterol (mmol/l) concentration was found significantly ($P<0.01$) highest in group I (3.68 ± 0.10) followed by group II (3.31 ± 0.06), III (3.01 ± 0.06) and IV (2.99 ± 0.08). However no significant differences were observed between group III and IV. High density lipoprotein (HDL) cholesterol (mmol/l) concentration in blood plasma increases at the end of finishing stage for all the treatment groups except for group I. Results clearly indicated that plasma HDL cholesterol concentration increases accordingly with the increase in percentage of green berseem in the diet. Significantly ($P<0.01$) lowest HDL cholesterol concentration were observed for group I (1.38 ± 0.04 mmol/l) followed by group II (1.52 ± 0.03), III (1.52 ± 0.03) and IV (1.57 ± 0.02). However, no significant differences were observed among later three groups. The dressing percentage (without head) in different treatment groups ranged from 65.37 ± 0.51 (group I) to 68.35 ± 2.98 (group IV). However, no significant difference was observed among different treatment groups. Maximum back fat thickness (cm) was observed for group I (3.41 ± 0.09) followed by group IV (3.21 ± 0.07), III (2.89 ± 0.36) and II (2.70±0.18) respectively, showing no significant difference. Fat thickness at 10th rib was also significantly (<0.05) highest for group I (3.57 ± 0.12 cm) followed by IV (2.73 ± 0.15 cm), II (2.50 ± 0.29 cm) and III (2.33 ± 0.27 cm) respectively. This finding might be due to the reason mentioned for back fat thickness. Loin eye area (cm^2) was observed to be higher for group IV (28.17±1.42) followed by group III (27.00±1.00), II (27.00±0.29) and I (24.50 ± 0.29). However no significant difference was observed among treatments. Cost involved for production of 1 kg live weight for group I, II, III and IV was Rs. 25.65, 22.03, 21.86 and 18.95 respectively, which differ Significantly ($P<0.01$) among groups but non-significant between group II and III.

DISCUSION

Decrease in DM intake in with increase in green berseem content in ration might be due to fact that bulky nature of fodder green berseem (5, 20, 21, 12). Reduced body weight gain and daily weight gain with increase in proportion of green berseem in diet might be due to low intake of protein and energy, ultimately resulting in lower growth rate. Higher rate of live weight gain were observed in pigs fed rations containing high level of energy (10, 12, 14, 15, 17, 21). Decreased FCR value with increase of green berseem in diet in different groups might be due to positive effect on digestive processes and nutrient metabolism (5, 7, 10). On the contrary, increased FCR value with increase in fodder radish in the diet was observed (21). Plasma glucose concentration decreases as the concentration of green berseem increases in diet. These trends might be due to increases in plant fibre content in diet (13). A definite decreasing trend of plasma cholesterol concentration was observed as the percentage of green berseem increased in diet. Our finding is in close agreement with the finding of scientists who used alfalfa meal in the treatment groups (4, 13). The average dressing percentage of 70.88 % in LWY crossbred pigs (2). Result clearly indicated that dressing percentage increases as the percentage of green berseem increases in diet (6, 8). The carcass length recorded in present investigation is almost nearer to the finding of scientists (2, 6). Longer carcass length in pigs maintained on hotel wastes than those maintained in farm management system was observed (6, 8, 17). Slightly lower back fat thickness (2.47 cm) in LWY cross bred pigs might be due to slaughtered at lower body weight (35-45 kg) (2). The higher back fat thickness in group I indicated the effect of high plane of nutrition which led to conversion of excess energy in to fat during the finishing period. Fat thickness at 10th rib was also significantly (<0.05) highest for group I (3.57 ± 0.12 cm) followed by IV (2.73 ± 0.15 cm), II (2.50 ± 0.29 cm) and III (2.33 ± 0.27 cm) respectively. This finding might be due to the reason mentioned for back fat thickness. Loin eye area (21.71 cm^2) in LWY crossbred pigs were reported (2). Pigs fed the experimental diets on alfalfa pasture were observed to be significantly less back fat thickness and significantly more

ham and loin-eye area than the pigs fed the diets in confinement (3). The result demonstrated a clear advantage of replacement of concentrate with green berseem and kitchen waste both in rate and economy of gain in respect of profit per kg of pork production over control (16). Green maize fodder can replace concentrate feed to a considerable extent and reduce the cost of pork production (20). Feed cost/kg gain decreased by 14% on inclusion of Lucerne forage (11).

CONCLUSION

On the basis of findings of the present study it could be concluded that Green berseem could be incorporated up to 25% of total dry matter intake without affecting the performance of pigs adversely. Substitution of concentrate for different levels of green berseem along with 50% Kitchen waste reduced the DM intake and growth but improves the FCR and significantly reduced the cost per kg pork production than that of the control diet.

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STUDIES REGARDING THE INFLUENCE OF SHEEP FEEDING UPON LAMBS DEVELOPMENT DURING MILKING PERIOD

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SUMMARY

In Romania sheep breeding represents a traditional occupation. Nowadays, Romania is situated on 4th place among E.U. countries, by a classifying depending on sheep and goat livestock.

The researches were carried out during the milking period upon 3 sheep batches in same breed. Every batch consisted in 50 animals. For all the 3 batches, the feeding norm was the same, but the structure was different. Batch 1 had a fibrous type fodder, 70-75% hay, 3-5% succulent fodder and 15-20% concentrates fodder. Batch 2 had a half succulent type fodder, 30-35% fibrous fodder 35-40% succulent fodder and 25-30% concentrates fodder. Batch 3 had a half concentrate type fodder, consisted in 30-35% fibrous fodder, 5-10% succulent fodder and 55% concentrate fodder of the dry substance.

The sheep feeding system during the milking period had correct feeding norms. The structure of the feeding norms influences the morph-productive parameters of mothers during the milking period. Even the half succulent rations were well fed by sheep, they assures weaker results then the fibrous or half concentrate type rations.

It is recommended that sheep be fed with fibrous or half concentrate type fodder because in these cases they had a good response and there are obtained the best technical results. The best economic results are obtained when sheep are fed with fibrous type rations; they are the cheapest, many of these fodders being secondary products of the agricultural vegetal harvests.

INTRODUCTION

During a calendar year, the nutritional requirements of adult sheep are hanging depending on their physiological status. So, during a year, the nutritional requirements can be grouped into: requirements for mating, requirements in the first months of pregnancy (gestation), andrequirements in the last month of gestation, requirements in the first and also in the second part of lactation.
The purpose of the research is to establish the influence of the type of the notion on the milk production in the first part of the lactation and also in what kind of influence the type of the ration in growing of the lambs has.

MATERIAL AND METHODS

All these groups of persons occupationally exposed to animals (treating live animals or handling carcasses) showed an MRSA colonisation.

The research was done in a company from Vaslui district, on a batch of Palas Merino sheep which were crossed with rams belonging Ile de France breed.

There were made three experimental groups of sheep which have given birth once but no more than twice, uniformly distributed in the three groups and with an average weight of 50 Kg.
These three groups of sheep were fed with the same norm forage, but with different structure of the rations.

Table 1: Experimental scheme

Group	Type of the ration	The proportion in dry substance		
		Fibrous	Succulents	Concentrated
I	Fibrous	70-75	3-5	15-20
II	Succulent	30-35	35-40	25-30
III	Concentrated	30-35	5-10	50-55

As seen from table 1, in feeding sheep, there were used three types of forage rations:
- group I was fed with a fibrous ration, consisting 70-75% hay, 3-5 % succulents and 15-20% mixture of grain.
- the second group, had a ration with more succulents, about 35-40% from dry substance(SU), fibrous in proportion of 30-35%, and mixture of grain in proportion of 25-30%.
- the third group was fed with a forage ration made from 30-35% hay, 5-10% succulents and 50-55% concentrated.

RESULTS

These three groups of sheep were fed with almost the same norm forage.

The contents of energy substances was expressed in UNC and had variations between 1, 26-1, 29 UNC, while the contents of protein substances varied between 113-120 PDIN.

Studying how consumed feed were, it can conclusion that all the groups of sheep ate very well the rations.

The concentrated forage was ate from all the groups of animals, the hay was ate in proportion of over 95% also from all the groups, while the succulents were ate in a bigger proportion by the first and the third group (93-96%).

The second group ate the succulents in a longer period of time and in a lower proportion (90-92%).

The forage rations from all three groups of sheep were completed (supplemented) with 20 g forage chalk, the salt was given all the day long (salt ball)

Sheep milk production was estimated in the first month lactation, when feeding lambs was made totally with maternal milk.

To estimate the sheep milk production, it was used the determining method of weight gain of lambs, which than was transformed in milk.

Special literature said that one kilo weight gain in the first month of the life of the lamb is obtained with consumption about 5 kg of milk.

This method of estimation used for the milk production is considered, in the first month of life, the most accurate because in the case of using the method of splitting the lambs and milking the sheep there retain a pretty big quantity for the lamb so that the results will be always smaller.

From the data presented in the table 2, it can be easily said that in the first month of lactation, the milk production exceeded at all groups the value of thirty five (35) kilos.

At group I, group that has been fed with a ration in which the fibrous were predominating, the average quantity of milk was 40,4 kilos.

At group II, group that was fed with a ration in which the juicy fibrous were predominating, the average quantity of milk was the smallest, 32,25 kilos.

At group III, group that was fed with a ration made of concentrated, the average quantity of milk was 41,05 kilos.

Table 2: Milk production in the first month of lactation

Specification	Group I	Group II	Group III
The body weight of the lambs/ seep			
n	50	50	50
X±sx	6,18±0,146	6,31±0,128	6,52±0,163
V%	13,86	12,24	14,36
X±sx	14,26±0,286	13,95±0,314	14,73±0,321
V%	15,19	15,12	17,32
The weight growth			
n	50	50	50
X±sx	8,08±0,328	7,65±0,286	8,21±0,319
V%	18,14	19,53	20,70
The quantity of the milk in the first month of lactation			
n	50	50	50
X±sx	40,4±1,53	38,25±1,87	41,05±1,49
V%	22,92	24,39	23,73

In the table number 3 is presented the body weight of the lambs at the birth and also after 30 days. Also the average body weight in the first month of life was established.
The body weight of the lambs from the third group had closes values of 4,02 kilos for the lambs group I, 4,16 kilos for the lambs from group II and 4,10 kilos for the lambs from group III.

Table 3: Body weight of lambs

Specification	Group I	Group II	Group III
The body weight of the lamb at the birth(kg)			
n	67	67	70
X±sx	4,52±0,062	4,66±0,036	4,73±0,045
V%	12,38	10,62	14,68
The body weight of the lamb at 30 days (kg)			
n	68	67	70
X±sx	10,38±0,263	10,28±0,187	10,64±0,207
V%	14,82	13,64	16,22
Average daily gain (g)			
n	68	67	69
X±sx	192±8,62	187±9,14	197±7,21
V%	16,4	12,8	15,4

At the age of 30 days, the weight gain was 10, 38 kilos for the lambs from group I, 9,96 kilos for the lambs from group II and 10,28 kilos for the lambs from group III.

Regarding the weight gain, this had an average values between 187 g and 197 g, the test results recording at lambs from group III, group that has been fed with concentrated rations, average weight gain of 197 g, lambs from group I . The group which has been fed with fibrous rations, realized a weight gain of 192 g, while the lambs from the group which was fed with juicy rations, realized the smallest weight gain of only 187 g.

DISCUSSION

The structure of the rations is influencing the sheep morph productive parameters in the suckling period of the lambs [3].

Milk production estimated in the first month of lactation is superior to sheep fed with mostly concentrate rations [2].

Fibrous food and concentrates are consumed with more pleasure than succulent food [1].

As the sheep milk production in the first month of lactation is higher, the body weight of lambs in the first month of life is higher [2].

The four concepts mentioned above are the same concluded in the reserches carried out by us.

CONCLUSIONS

From the research undertaken in the following conclusions are detached:

- the concentrated and fibrous rations assure the obtain of same superior parameters from the juicy rations;
- the milk production in the first month of lactation which is established on the weight gain from the lambs, was of 41,01 litres, for the groups fed with a concentrated and fibrous rations and of 38,25 litres, for the groups feed with a semi juicy ration.
- the daily average of body weight of the lambs in the first month of life has not recorded very big differences(197 and 192 g) at the group fed with concentrated and fibrous rations, and smaller at the group feed with a juicy ration, of 187 g.

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EPIDEMIOLOGICAL STUDIES ON ZOONOTIC DEEP MYCOSES BETWEEN ANIMALS AND MAN IN ASSIUT GOVERNORATE, EGYPT

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SUMMARY

This paper studies the epidemiology and effect of Zoonotic Deep Mycosis on the public health. A total of 100 cattle suffered from reproductive disorders were selected. Incidence of mycotic infection in examined vaginal swab was 68%. Incidence of *Aspergillus species* was 41%, while *Candida species* incidence was 17%. Incidence of mycotic infection out of the examined 100 subclinical mastitic bovine milk samples was 89 %. Incidence of *Aspergillus and Candida species* were 36% and 69%, respectively. A total 100 lung samples showing macroscopic lesions (abscess, anthracosis, congestion and area of hepatizition) was collected from slaughter houses. Incidence of *Aspergillus spp.* and *Candida spp.* in the examined bovine lung samples was 81% and 27%, respectively. A total of 44 mould species was isolated from soil samples. The most common isolated fungi were *Aspergillus spp, Pencillium spp, Fusarium.spp , Alternaria alternate, Trichoderma harzianum* , *Cladosporium spp*, *Sporothrix inflata* and *Stachybotrys chartum* . The most common fungal isolates obtained from 93 out of 100 examined bird dropping samples were *Aspergillus spp., Pencillium spp, Fusarium.spp*, *Alternaria alternate, Trichoderma harzianum, Cladosporium spp., Candida spp. and Trichosporon spp.* Incidence of pulmonary fungal infection in 100 examined sputum samples from patient admitted in Chest Hospital was 97%. Incidence of Aspergillosis in human patients was 88%. The higher incidence was recorded among male patients (89.4%) comparing with female ones (85.3%). Percentage of Aspergillus positive patients suffering from T.B. was 36.4 %,bronchial asthma 26.1%, bronchitis & chest pain 22.7 % and pneumonia 14.8%. Incidence of *Aspergillus fumigatus* by using culture method and ELISA IgG was 16 % and 14%, respectively. Concerning to Candidiasis, the incidence rate was 58% out of 100 examined patient sputum samples. Rate of infection among male and female was 59.1 %, and 55.9 %, respectively. Intensification of the infection (74.4%) among the age group 31- 50 years was discussed. This study clarified that 38% of Candida positive patients were suffering from bronchial asthma, 24.1% from T.B., 20.7 % from bronchitis & chest pain and 17.2 % from pneumonia. A total of 11 CSF samples was collected from patients admitted to Assiut University Hospitals, Psychic and Neurology Department. *Candida tropicalis* was isolated from two patients. In our study, we found that animal play an important role in the epidemiology of human mycoses either as a vector of pathogenic fungi or as a creator of environmental prerequisites for the development of fungi. Combination of culture method and ELISA can be ideal to confirm the diagnosis of mycoses especially in immunocompromised patients. Also, in cases of recurrent encephalitis, investigations should include fungal examination in CSF.

INTRODUCTION

Fungi as causative agents of human disease are widely distributed in nature. There are different sources which are responsible for spreading of fungal infections to human as soil and bird dropping. The epidemiology of systemic mycoses is quite complex and the soil is considered the natural habitat of these organisms. Animals also play a role in the environmental distribution of the systemic mycoses. These infections occur in debilitated or compromised patients after inhalation of the spores from their saprophytic habitats or direct exposure to animals or birds excreta or contaminated soil **[35]**. From the health point of view, Aspergillus and candida species have been incriminated as causative agents of pneumomycosis and meningeoenchephalitis in man **[34]**. In animals, respiratory disorders are the most common sign of systemic mycoses. Aspergilli have been recorded as causative agents of mycotic abortion in cattle **[41].** Moreover, mycotic mastitis in cattle has been recorded. This study attempts to isolate and identify pathogenic fungi from the collected samples, serodiagnosis of Aspergillosis by using ELISA, and study the epidemiology and the effect of Zoonotic Deep Mycosis on the public health.

MATERIALS & METHODS

The present study was carried out during the period from August, 2007 to October, 2008 in Mycology Center and Molecular Biology Research Unit, Assiut University. A total of 300 cattle (100 vaginal swabs, 100 milk samples and 100 lung samples) was selected from 2 governmental farms as well as slaughter houses in Assiut Governorate. An identification card for each animal to record age, sex, and a history of abortion, retained placenta, mastitis and pneumonia had been done. One hundred dropping samples (50 chicken and 50 pigeon) and 100 soil samples were collected. One hundred sputum and blood serum samples were collected from patients attending to Chest Hospital with a history of cough, bronchial asthma, and chest pain. A questionnaire was designed for each individual to determine the risk factors assessments regarding age, sex, residence and contact with livestock. Eleven CSF samples were collected from patients admitted in Assiut University Hospitals, Neurology Department, with a history suggestive of encephalitis and/or meningitis.

Collection and preparation of samples were done according to **[37, 28** & **27]**. Specimens were cultured on sabouraud's dextrose and Malt extract agar medium for isolation of fungi, Czapek yeast extract (CYA) and Potato sucrose agar media (PSA) used for identification of mould according to **[13, 4, 18** & **31]**. The identification was based on morphology of the colony, rate of growth and microscopic morphology of the isolates. Enzyme linked immunosorbant assay (ELISA) for IgG qualitative and quantitative ***Aspergillus fumigatus*** [IBL (RW56111) www.IBL-Humburg.com] has been done on 100 human serum samples collected from the same 100 patients in this study.

RESULTS

Table 1: Incidence of mycotic infection in cow's vaginal swabs, subclinical mastitis bovine milk & bovine lung samples

Samples	No. of samples	+ ve cases		-ve cases	
		No.	%	No.	%
Vaginal swabs	100	68	68	32	32
Milk samples	100	89	89	11	11
Lung samples	100	91	91	9	9

Table 2: Incidence of Aspergillus & Candidia spp. in cows vaginal swab's, subliclincal mastitic bovine milk & bovine lung samples

Samples	No. of samples	+ ve Aspergillus		+ ve Candidia	
		No.	%	No.	%
Vaginal swabs	100	41	41	17	17
Milk samples	100	36	36	69	69
Lung samples	100	81	81	27	9

Table 3: Incidence of mycotic infection in subclinical mastitic bovine milk samples according to residence

Farms	No.	Positive cases	
		No.	%
Farm (1)	64	55	86
Farm (2)	36	34	94.4
Total	100	89	89

Table 4: Percentage of fungi isolated from bird dropping samples

Kind of Samples	No. of samples	Positive samples	
		No.	%
Chicken	50	44	88
Pigeon	50	49	98
Total	100	93	93

Table (5): Incidence of mycotic infection in human sputum samples

No. of samples	+ve samples		-ve smaples	
	No.	%	No.	%
100	97	97	3	3

Table 6: Incidence of Aspergillus & Candidia spp. in human sputum samples according to sex

Sex	No. of samples	Positive cases			
		Aspergillus spp.		Candidia spp.	
		No.	%	No.	%
Male	66	59	89.4	39	59.1
Female	34	29	85.3	19	55.9
Total	100	88	88	58	58

Table 7: Percentage of patients have chronic disease history with relation to Aspergilloisis & Candidiasis

Patient complain	Aspergillosis (88)		Candidiasis (88)	
	No.	%	No.	%
Tuberculosis	32	36.4	14	24.1
Bronchial asthma	23	26.1	22	38
Bronchitis & chest pain	20	22.7	12	20.7
Pneumonia	13	14.8	10	17.2
Total	88	100	58	100

Table (8): Incidence of ***Aspergillus fumigatus*** in human samples

No. of tested samples	No. of +ve samples			
	Culture method		ELISA IgG	
100	No.	%	No.	%
	16	16	14	14

DISSCUSSION

The past three decades have witnessed dramatic change in man's environment and his immune defenses. Consequently, the fungi have assumed as a major role in respiratory and systemic infectious diseases. Mycotic infection is an important reproductive problem of cattle all over the world. Incidence of mycotic infection in examined 100 cows' vaginal swab (**Table 1**) was 68%. This finding are in accordance with the results that obtained by **[30]** (66.66%). While, it was considered higher than that previously recorded by **[19]** (21.42%). That difference can be accepted due to several factors as the density of animals, topographic variations and other ambient conditions (from hot humid to cold dry). Higher incidence in the study may be attributed to the site of sampling, as vagina is frequently exposed to the environment during oestrus and parturition [**43**]. Mycotic mastitis is considered one of the most common and economically important world wide diseases, leads to lowering in milk production and reduction of milk quality **[33**]. The incidence of mycotic infection among 100 subclinical mastitic bovine milk samples was 89 %. This high incidence may be attributed to the long course treatment with intramammary antibiotics without specific microbiological examination **[12]**. Moreover, large doses of antibiotics cause a reduction in the vitamin A content in cattle, leading to injury to the udder's epithelium, thus facilitating their invasion with fungi **[11**]. Incidence of mycotic infection in 100 bovine lung samples was 91%. Environmental exposure to any of fungi responsible for causing pneumomycosis.

Recorded results in (**Table 2**) showed that the incidence of *Aspergillus spp.* in 100 vaginal swabs of cows that suffered from reproductive disorders was 41 %. A variable incidence of *Aspergillus spp.* among cows suffering from reproductive disorders was obtained by many authors as **[29]** (14.86%); **[22]** (21.81%) and **[1]** (5.71 %). *Aspergillus spp.* was the most common fungal isolates in the study that carried by **[1]** on repeat breeders buffaloes and cows. The incidence of *Candida spp*. among 100 vaginal swabs was 17%. *Candida species* are opportunistic fungi, occurring as normal inhabitants of the digestive tract, oral cavity, and vagina of many domestic animals **[10]**. The shift from a harmless commensal to a virulent pathogen may be induced by many factors such as prolonged antibiotic therapy, malnutrition and vitamin deficiency **[10 & 17]**. Incidence of *Aspergillus* and *Candida spp*. in milk samples were 36% and 69% respectively. The high incidence of *Candida spp.* when compared to *Aspergillus spp.* may be accredited to their better perpetuation in the mammary gland and also to some genera of yeasts can utilize antibiotics like penicillin and tetracycline as a nitrogen source for growth **[11]**. Incidence of *Aspergillus spp.*was (81 %) out of 100 examined bovine lung samples. These results are quite similar to those obtained by **[21 & 45]** who stated that *Aspergillus spp.* are the most common factor of mycotic pneumonia. The incidence of *Candida spp.* in examined lung samples was 27%. Systemic candidiasis has been described in cattle as secondary to prolonged antibiotic or corticosteroid therapy **[25**].

This study was carried on two governmental farms. The incidence of mycotic infection was 94.4% and 86 % respectively (**Table 3**). The relatively high incidence in farm (B) may be ascribed to the improper use of milking machines which serve as a fomite for the transfer of microorganisms from animal to another. Moreover, it may reduce resistance of the streak canal by traumatizing the tissue and creating teat end lesions **[7]**.

In the present study, a total of 44 mould species were isolated from soil samples collected from different localities. The most isolated fungi were *Aspergillus spp, Pencillium spp, Fusarium.spp , Alternaria alternate; Trichoderma harzianum* ; *Cladosporium spp*; *Sporothrix inflata* and *Stachybotrys chartum* . These finding agree to Some extend with those noticed by many authors [**6; 42 & 44**]. The high prevalence of these fungi in soil,

suggested their role as potential pathogens in animals and human **[8]**.

The fungal isolates were obtained from 93 out of 100 dropping samples [pigeon (49 out of 50), Chicken (44 out of 50), as shown in (Table4). This result coincide partly with that reported by **[2]** who recovered fungal isolates from 100% of examined dropping samples. In recent year, the number of fungal infection in human has increased, which trigger an interest to examine the source and reservoir of such fungi **[3]**. The most common isolated mould from chicken and pigeon dropping were *Aspergillus spp.; Pencillium spp; Fusarium.spp*; *Alternaria alternate* ; *Trichoderma harzianum* ; *Cladosporium spp.; Candida spp.and Trichosporon spp*. Furthermore, the recoded yeast species in this study were *Candida albicans, Candida tropicalis and Trichosporon spp.*

The incidence of pulmonary fungal infection in 100 examined sputum samples from patient admitted in Chest Hospital, was 97% (Table 5). This percentage was relatively higher than that recoded by **[5]** and **[9]** (76%). This discrepancy may be ascribed to massive population growth and urban development which increased the prevalence of fungal infections in certain areas and putting more people at risk [**38**].

Incidence of Aspergillosis in human patients were 88% (Table 6). The higher incidence was recorded among male patients (89.4%) comparing with female (85.3%). Men had a greater chance of coming in contact with outdoor environment and exposure to dust particle through their work in agriculture, excavation and construction as well as animal rearing and livestock handlers. Incidence of Candidiasis in 100 examined sputum samples was 58% (male, 59.1 % & female, 55.9 %). The relative preponderance of males in this study can be explained by the fact that smoking and chewing tobacco, which leads to various obstructive lung diseases, is more common in males than in females **[24]**.

Out of 88 patient samples, 36.4 % of Aspergillus positive were suffered from T.B., 26.1% bronchial asthma, 22.7 % bronchitis & chest pain and 14.8% from pneumonia (Table 7). Tuberculosis as a predisposing factor in colonizing aspergillosis was found by many workers **[39& 36]**. Even today, tuberculosis remains the most important cause of subacute and chronic respiratory morbidity in many countries [**32**]. Bronchial asthma may occur in patient due to hypersensitivity reaction to *Aspergillus* antigen**].** Pulmonary aspergilloma (fungal ball) can occured in lung cavities as a sequel to T.B., lung abscess, lung cancer and cystic fibroses cases **[15].** Out of 88 patient samples, 38% of Candida positive patient were suffered from bronchial asthma, 24.1% T.B., 20.7 % bronchitis & chest pain and 17.2 % from pneumonia. These finding were quite similar to that discussed by **[40]** who observed that most people with bronchial asthma have Candida spores in their sputum and intestines. The presence of Candida in sputum of T.B. patients mainly depends on the duration of illness and chronicity of the disease **[23]**.

Incidence of *Aspergillus fumigatus* by using both culture method and ELISA IgG were 16 % and14% respectively (Table 8). Pulmonary Aspergillosis usually occurs in immunocompromised hosts whom immune response is less or may be absent. Therefore, such cases with negative serological finding may not be considered negative for infection **[26]**. Consequently, the combination of culture and serology can be ideal to confirm the diagnoses of pulmonary aspergillosis.

In our study, 11 CSF samples were collected from patients admitted to Assiut University Hospitals, Neurology Department. *Candida tropicalis* was isolated from two patients. A history of recurrent meningitis or/and encephalitis was recorded. *C. tropicalis* was previously detected in CSF cultures of a 49-year-old man by **[14]**.

CNS infections due to *Candida* species are frequently found in patients hospitalized for long periods in Intensive Care Units. However, infections due to *C. tropicalis* have increased dramatically on a global scale, thus, proclaiming this organism to be emerging pathogenic yeast **[25].**

In our study, we found that animal play an important role in the epidemiology of human mycoses either as a vector of pathogenic fungi or as a creator of environmental prerequisites for the development of fungi. The combination of culture method and ELISA can be ideal to confirm the diagnoses of mycoses especially in immunocompromised patients. Also, in cases of recurrent encephalitis, investigation should include examination for fungi in CSF.

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STUDY ON ZOONOTIC PATHOGENS IN RODENTS IN RECREATIONAL AREAS AROUND LEIPZIG, GERMANY

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SUMMARY

In this study, investigations on the occurrence of rodent-transmitted pathogens were conducted in three selected areas in and around the city of Leipzig. In altogether 47 investigated animals DNA of *Leptospira* spp. was found in 6 animals (13 %) and DNA of *Rickettsia* spp. was detected in 14 animals (30%).

INTRODUCTION

Rodents are known to host a variety of zoonotic pathogens [1]. Since knowledge is limited on prevalences of certain rodent-borne pathogens in regions such as the greater area of Leipzig, we collected rodents from urban and suburban areas in and around Leipzig and examined them for the presence of DNA of *Leptospira* spp. and *Rickettsia* spp..

ANIMALS, MATERIALS AND METHODS

Rodents were trapped at 3 different sites in and around Leipzig. In 2010, an overall number of 47 mice of the species bank vole (n=23), yellow-necked mouse (n=20), striped field mouse (n=2), and wood mouse (n=2) were trapped using Sherman-traps. Different tissue samples were taken from each animal. DNA was extracted from ears and kidneys with the QiaAmp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A real-time PCR was performed for the detection of DNA of *Rickettsia* spp. (ear) [2] and *Leptospira* spp. (kidney) [3]. Samples tested positive for *Leptospira spp.* in the real-time PCR were also run in a conventional duplex-PCR [4] in order to distinguish *L. kirschneri* from other pathogenic *Leptospira* species.

RESULTS

A total of 14 animals tested positive for *Rickettsia* spp. (30%) and 6 for *Leptospira* spp. (13%). The differentiation by the duplex-PCR resulted in the detection of two *L. kirschneri* and four *L. interrogans* or other pathogenic *Leptospira* species.

DISCUSSION

Although only a small number of samples was examined in this first study, the results already revealed a high prevalence of potential zoonotic agents in the collected rodents.

To further determine the species of the resident *Rickettsia* and *Leptospira*, sequencing of the *OmpB* gene [5] and the 16S rDNA gene [6] respectively will be performed.

CONCLUSIONS

The high prevalences of these potential pathogenic zoonoses found in urban and periurban rodents raise questions about their role as reservoir animals for these pathogens and their potential of transmitting them to humans. Since the captured rodents were infested to a great extent with ticks, fleas and mites, further examinations of these ectoparasites are currently outlined to evaluate their role as transmitting vectors. We will also screen the rodents for further pathogens like Hantavirus, Babesia spp., Anaplasma spp. and furthermore for endoparasites.

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EPIDEMIOLOGICAL FEATURES OF HUMAN BRUCELLOSIS IN GONBAD

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SUMMARY

Brucellosis is a Food-borne infection transmitted between humans and livestock and accounts as a major problem in human and animal heath in many developing countries including Iran. This survey was carried out to investigate the epidemiological features of human brucellosis in Gonbad, in a descriptive cross-sectional study during April 2010 to April 2011. All 21 brucellosis patients who were confirmed clinically and by serological methods at the Gonbad Health Center were entered to the survey. Prevalence of brucellosis was 7.25cases/100,000 inhabitants and was much lower compared to other areas in Iran. Brucellosis was more common in male than female. Consumption of unsafe dairy products could be the main route of infection and people who worked directly with livestock were at the maximum risk. High incidence of infection was seen in spring, summer and winter. Rural residents had the highest involvement.

INTRODUCTION

Brucellosis is caused by members of genus *Brucella*. These are small, non-motile, aerobic, facultative intracellular, Gram-negative coccobacilli. The ability of *Brucella* to replicate and persist in host cells is directly associated with its capacity to cause persistent disease and to circumvent innate and adaptive immunity [2]. Almost all domestic species can be affected by brucellosis except cats which are resistant to *Brucella* infection [9]. The species of *Brucella*, their major hosts and their geographic distribution are provided in *Table1*.

Table 1: Species of Brucella and their mojor livestock hosts

Organism	*Animal reservoir*	*Geographic distribution*
B. melitensis	Goats, sheep, camels	Mediterranean, Asia, Latin America, Africa and southern Europe
B. abortus	Cows, buffalo, camels, yaks	World wide
B. suis	Pigs (biotype 1-3)	South America, Southeast Asia, United States
B. canis	Canines	Cosmopolitan
B. ovis	Sheep	No known human cases

Human brucellosis remains the commonest zoonotic disease worldwide with more than 500,000 new cases annually [7]. Humans are infected either by direct contact with infected animals and their products or by the consumption of contaminated milk or dairy product [1, 13]. The disease is characterized by fever, arthralgia, sweating, back pain, malaise and anorexia [13].

Though it has been eradicated in many developed countries in Europe, Australia, Canada, Israel, Japan and New Zealand [3], yet it remains an uncontrolled problem in regions of high endemicity [10]. Middle-east has traditionally been considered as an endemic area [8].

This study was performed to investigate the epidemiological features of reported cases of human brucellosis to the Gonbad Health Center.

MATERIAL AND METHODS

Epidemiological, clinical and laboratory data relating to 21 consecutive cases of human brucellosis who attended the Gonbad Health Center in Golestan province during April 2010 to April 2011 were investigated in a descriptive cross-sectional study. All brucellosis patients who were confirmed clinically and by serological methods were entered to the survey. The department serves more than 289,647 people living in Gonbad city and the surrounding villages in the north of Iran in an area of about 5072 Km^2. There are also approximately 250,000 heads of goat and sheep, and 30,000 heads of cow and water buffalo in Gonbad.

In the present study a patient was considered to be suffering from brucellosis if, according to the World Health Organization (WHO) case definition, that person "showed intermittent or irregular fever of variable duration, profuse sweating, fatigue and other symptoms as well positive reaction to a serological test used for brucellosis diagnosis

[11]. Serological diagnosis of brucellosis was established by demonstrating a brucella titre of ≥1:80 in wright test for patients with clinical signs and symptoms compatible with brucellosis.

RESULTS

During the study period the infection was confirmed in 21 persons. Taking into account the number of confirmed cases of human brucellosis and the population in the prefecture, which was 289,647 inhabitants, the annual incidence of human brucellosis in Gonbad was calculated as 7.25 cases/100.000 inhabitants.

More males (n=14) than females (n=7) presented with brucellosis at Gonbad Health Center. The male to female ratio was 2:1. Sixteen patients (76.2%) had a history of unsafe dairy product consumption. Direct contact with infected animal or animal products was observed in the history of seventeen (80.9%) patients. More cases were from rural (90.5%) than from urban (9.5%) areas. Most cases were presented during spring (n=6) and summer (n=6) *(Table 2)*.

Table 2: Some epidemiological findings in 21 cases of brucellosis in Gonbad

Characteristic of patients		*No.*	*%*
Gender			
	Male	14	66.67
	Female	7	33.33
Consumption of unsafe dairy products			
	Yes	16	76.2
	No	5	23.8
Resident			
	Urban	2	9.5
	Rural	19	90.5
Season			
	Spring	6	28.6
	Summer	6	28.6
	Autumn	3	14.2
	Winter	6	28.6
Direct contact with infected animal or animal product			
	Yes	17	80.9
	No	4	19.1

Most cases (33.3%) were 10-20 years old. *Figure 1* demonstrates the age distribution of the brucellosis cases.

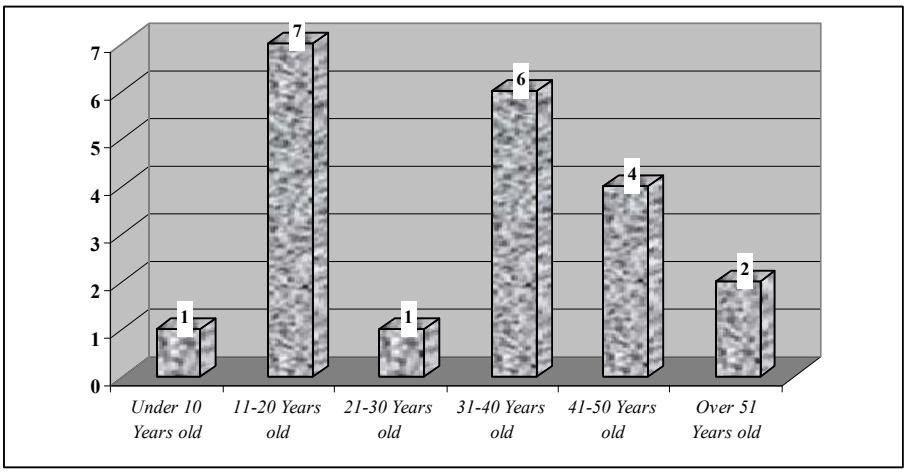

Figure 1: Distribution of cases of brucellosis according to age

DISCUSSION

There are so many factors that affect the prevalence of brucellosis. Prevalence of brucellosis can vary according to climatic conditions, geography, sex, age and diagnostic tests applied [5].

Brucellosis remains an important public health problem in Iran, causing serious complications and significant morbidity [6]. Indeed, five of the ten countries with the highest incidence for human brucellosis are in middle-east area. Syria has the highest annual incidence world wide, reaching an alarming 1603 cases per million per year according to data from OIE. The situation in Iran is improving, according to data from the National Commission on Communicable Diseases Control. In 2003, the annual incidence of human brucellosis in Iran had fallen to 238.6 cases per million [8].Based on the population of Gonbad, the incidence rate of brucellosis was 7.25 cases/100,000 inhabitants and was much lower compared to other areas in Iran.

In endemic regions, many cases occur in females. Although there are many reports on brucellosis in adulthood and children, only a few comprehensive studies have been carried out to investigate the different features of brucellosis between the sexes [4, 6]. Male: female infection ratio was 2:1 in this study, probably because in this region women are exposed to the unsafe dairy products. The finding that males are affected more than females is not consistent with the results of other studies in endemic regions, where consumption of dairy products and not occupational exposure is the most common route of infection.

Consumption of un-pasteurized dairy products could be the main route of infection. Much higher incidence of brucellosis in rural areas than urban can be due to the more direct contact with infected animals or consumption of unsafe dairy products in this area.

In Kuwait and some other countries most cases occurred during the spring and early summer [12], although in Gonbad most of the cases were observed in spring, summer and winter. The seasonal distribution of diseases in our region is similar to that normally seen in endemic areas.

From public health view point, brucellosis is considered to be an occupational disease that mainly affects slaughter-house workers, butchers, and veterinarians [5].

In some countries such as Iran, Jordan, Lebanon and Kuwait, the prevalence of brucellosis increases with age [12]. In this study the highest incidence rate was confined in patients with 10-20 years of old.

CONCLUSION

The results of this study show that endemic of brucellosis in Gonbad. The main route of transmission of brucellosis in our region is likely to be the consumption of unsafe dairy products. Avoiding consumption of un-pasteurized milk and dairy products, limiting exposure to infected domestic animals, education of higher risk groups (especially those in higher risk occupations) and eradication of disease (test and slaughter) may provide the best opportunities for human brucellosis prevention and control.

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RABIES IN THE CENTRAL REGION OF SÃO PAULO STATE, BRAZIL

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SUMMARY

Rabies is an infectious disease of global importance. In Brazil domestic and wild animals are important reservoirs, and the continuous activities pointed to diagnostic and surveillance of rabies are essential to prevent the increase of animal and human cases. The present study consists in a retrospective of the rabies diagnostic performed between August 2001 and December 2010 in the Zoonosis Diagnostic Service, at the Faculdade de Medicina Veterinária e Zootecnia, UNESP Univ Estadual Paulista, Campus Botucatu, Brazil. The laboratory is located at the central region of São Paulo State, and most of the animals submitted to diagnostic were from the surrounding cities. The techniques used were the immunofluorescent antibody test (IFAT) and the Bioassay in mice. In this period 4831 animals from 116 cities were tested. They included dogs (n=1545), bovines (n=339), cats (n=463), bats (n=2273), horses (n=87), small ruminants (n=56), pigs (n=9), and terrestrial wild mammals (n=90). Fifty seven (57) animals were positive (1.1%): 29 bovines, 22 bats, 5 horses and 1 pig. These results show that bovines, horses and bats are important reservoirs of rabies in the studied region. The absent of dogs and cats with rabies indicates the success of the preventive measures adopted for these species in the last years.

INTRODUCTION

Rabies is an infectious disease of global importance, characterized by its high lethality. The etiologic agent is a virus of *Lyssavirus* genera, and the main route of infection is through the bite of an infected animal (4). Every year thousands of people die of rabies, most of them in developing countries. In Brazil, although only a few cases of human rabies are reported per year, domestic and wild animals are important reservoirs (3,6,2). São Paulo is the most populous State of Brazil, with significant livestock production. The present study consists in a retrospective survey of the rabies diagnostic performed in the Zoonosis Diagnostic Service, at the de Faculdade de Medicina Veterinária e Zootecnia, UNESP Univ Estadual Paulista, Campus Botucatu, Brazil.

MATERIAL AND METHODS

The laboratory is located in Botucatu Municipality (22°53'S 48°26'W), in the central region of São Paulo State. Most of the animals submitted to diagnostic were from cities located in the same region. The retrospective study was performed using the files from the period of August 2001 to December 2010. The diagnostic was performed using the brain and cerebellum of the animals, whose were tested by the Immunofluorescent Antibody Test (IFAT) and the bioassay in mice, according to World Health Organization.

RESULTS

During the period 4831 animals from 161 cities were tested for rabies. They included dogs, cattle, cats, bats, horses, small ruminants, pigs, and terrestrial wild mammals. Fifty seven (57) animals were positive (1.1%). The results are shown in table 1.

DISCUSSION

Most of the animals were sent to diagnostic because they were sick, thus, these data cannot be interpreted as a prevalence of rabies in each species. These results show that bovines, horses and bats are important reservoirs of rabies in the studied region, as pointed by previous studies, not only in São Paulo State, but in other regions of Brazil (5,7,1). Although one pig had rabies, only a few animals were evaluated. A study including more animals could provide more reliable data, not only for pigs, but also for small ruminants and terrestrial wild mammal. A small percentage of positive bats were observed (0.9%) when compared to other species, such as horses (5.7%) and bovines (8.5%). The reason for this is that many bats were sent to diagnostic even without clinical signs of rabies, because they are considered one of the most important reservoirs (7), and also because the symptoms of rabies in these animals are more difficult to evaluate. The absent of dogs and cats with rabies indicates the success of the preventive measures adopted for these species in the last years, especially the municipal vaccination campaigns, whose focus are these animals.

CONCLUSIONS

Bats, horses and bovines are important reservoirs of rabies in the central region of São Paulo State Brazil. Activities pointed to these species are of great importance. The vaccination of dogs and cats remains an essential activity in the prevention of rabies.

Table 1: Animals tested for rabies in the central region of São Paulo State, Brazil, between August 2001 and December 2010. Botucatu, 2011

Year	Dog	Bovine	Bat	Horse	Cat	Small ruminant*	Pig	Terrestrial wild mammal**	Total	Positive
2001	25	32	68	6	8	1	3	1	144	1
2002	88	49	314	2	26	3	0	2	484	4
2003	121	31	172	5	43	4	0	3	379	2
2004	127	34	241	13	30	5	1	3	451	8
2005	225	56	425	6	58	1	3	18	774	12
2006	178	40	359	6	66	5	1	20	665	11
2007	240	23	229	13	56	8	0	6	575	3
2008	204	30	164	10	79	6	0	11	504	8
2009	206	24	158	11	63	18	0	12	492	3
2010	131	20	143	15	34	5	1	14	363	5
Total	1545	339	2273	87	463	73	9	90	4831	57
Positive	0	29	22	5	0	0	1	0	57	-
% [a]	0.0	8.5	0.9	5.7	0.0	0.0	11.1	0.0	1.1	-

*Sheep and goat
**The most common were opossums, foxes, monkeys, rodents, anteaters and deer
[a]Frequency of positive animals

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SEROLOGIC SURVEY FOR TOXOPLASMOSIS IN SYNANTHROPIC OPOSSUMS

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SUMMARY

Toxoplasma gondii is a protozoan distributed worldwide, that can infect several species of animals and men. Animals living in the urban environment can act as sources of infection, such as dogs, cats, rodents, pigeons, opossums, among others. The study aimed to investigate the presence of antibodies against *T. gondii* in synanthropic opossums to evaluate their possible role in the epidemiology of this parasite. Blood samples from 90 white-eared opossums (*Didelphis albiventris*) were submitted to the modified agglutination test (MAT). Four animals were positive (4.4%), with titers 25 (n=2), 50 (n=1) and 100 (n=1). The low frequency of antibodies against *T. gondii* suggests a low degree of infection in the studied area. Opossums have a diet extremely generalist, and they probably became infected by both oocysts and infected tissue ingestion. The white-eared opossum can act as a reservoir of *T. gondii* in the urban environment.

INTRODUCTION

Toxoplasmosis is a disease caused by the *Toxoplasma gondii*, a protozoan that affects several species of animals and men. The main routes of infection are the ingestion of raw or uncooked tissue from infected animals or food contaminated with oocysts of the parasite (7). The infection is usually asymptomatic, but in cases of immunosuppression can develop severe illness, such as neurologic/ocular disorders, abortion and death (11).

Many species of animals living in urban areas can act as sources of infection, like rodents, pigeons, opossums, stray dogs and cats, among others. Reservoirs of *T. gondii* are responsible for the infection of other animals and men, contributing to the life cycle of the parasite. The opossum (*Didelphis* spp.) occurs in most part of the American continent (2), and is frequently found in urban areas, having close contact with domestic animals and men. The present study aimed to investigate the presence of antibodies against *T. gondii* in synantropic opossums to evaluate their possible role in the epidemiology of this parasite.

MATERIAL AND METHODS

The animals were captured by the Department of Environmental Surveillance of Botucatu Municipality (22°53'S 48°26'W). The captures were performed by phisical restraint, and all the opossums were from the urban area. They were anesthetized with an intramuscular injection of ketamine associated with xylasine at the doses of 40.0 mg/kg and 4.0 mg/kg, respectively. The blood samples were colected by intracardiac puncture or from the caudal vein. After centrifugation, the sera were stored in -20°C untill the analysis. The serologic method employed was the the modified agglutination test (MAT) (3), with formalin-fixed tachyzoites, produced on the laboratory's own premises, in 30 day-old, female Swiss mice inoculated with RH strain and Sarcoma TG-180 cells (ATCC CCRFS-180 II). Animals with titers equal to or greater than 25 were considered positive.

RESULTS

A total of 90 opossums were captured. All the animals belonged to the species *Didelphis albiventris* (commonly named white-eared opossum). Four animals were positive in the MAT (4.4%), with titers 25 (n=2), 50 (n=1) and 100 (n=1).

DISCUSSION

The presence of antibodies against *T. gondii* indicates the previous exposure to the parasite. Opossums have a diet extremely generalist, and the infection probably occurred by ingestion of both infected tissue and oocysts.

Other studies involving opossums of genus *Didelphis* have found different prevalence of antibodies against *T. gondii* (6, 4, 7, 10, 9, 5). In most of them the studied species were other than *D. albiventris*, and the prevalence of antibodies varied from 13.0% to 29.0%. In the present study a low prevalence of antibodies were detected in a species that is poorly studied, although very common in urban areas of Brazil. In Botucatu municipality the white eared opossum is probably not such an important reservoir of *T. gondii* as reported in other surveys, despite the wide spread of the parasite in the same region, as reported previously in studies involving domestic dogs and cats (1,8).

CONCLUSIONS

The presence of antibodies against *T. gondii* suggests the participation of the white-eared opossum (*Didelphis albiventris*) in the epidemiological cycle of the parasite, and its possible role as a reservoir. Studies involving other infectious diseases in opossums should be performed, once that these animals are frequently present in the urban environment and have close contact with men and domestic animals.

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SEROLOGIC SURVEY FOR TOXOPLASMOSIS IN BATS: A PRELIMINARY STUDY IN BRAZIL

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SUMMARY

Toxoplasmosis is a zoonotic disease of global importance whose etiological agent is the protozoan *Toxoplasma gondii*. Several species of animals are considered reservoirs and potential transmitter of the parasite. The present study aimed to detect antibodies against *T. gondii* in blood samples of 62 bats. The sera of the animals were submitted to the Modified Agglutination Test (MAT). Two bats were positive (3.2%), a Seba's short-tailed bat (*Carolia perspicillata*) and a Palla's mastiff bat (*Molossus molossus*). Studies involving *T. gondii* in bats are scarce, and little is known about their role in the parasite cycle. In the present study a low prevalence of antibodies were observed, suggesting that these species rarely have contact with *T. gondii*.

INTRODUCTION

Bats are considered reservoirs of several infectious diseases, such as rabies, leptospirosis and histoplasmosis (1,2,4). The high number of species, wide distribution in the world, and the close contact with men and domestic animals in both rural and urban areas increase their importance as transmitters of zoonotic diseases.

Toxoplasma gondii is a protozoan able to infect men and a high number of animal species. The infection is usually asymptomatic, but can progress to disease when the immune system is affected (5). Synanthropic animals, once infected by *T. gondii*, can become reservoirs and infect other animals by ingestion of their tissues. This study consists in a preliminary investigation of the role of bats in the epidemiological cycle of *T. gondii* by the detection of antibodies in blood samples.

MATERIAL AND METHODS

The bats were from the urban area of Botucatu Municipality (22°53'S 48°26'W), São Paulo State, Brazil. They were collected by the Municipal Department of Environmental Surveillance and sent to the Zoonosis Diagnostic Service, at the de Faculdade de Medicina Veterinária e Zootecnia, UNESP Univ Estadual Paulista, Campus Botucatu, Brazil, to be submitted to rabies diagnosis. A total of 62 bats were studied, whose belonged to the following species: Palla's mastiff bat (*Molossus molossus,* n=16); black mastiff bat (*Molossus rufus,* n=7); greater bonneted bat (*Eumops perotis*, n=5); black bonneted bat (*Eumops auripendulus*, n=8); Wagner's bonneted bat (*Eumops glaucinus*, n=2); Seba's short-tailed bat (*Carolia perspicillata*, n=2); common vampire bat (*Desmodus rotundus*, n=2); Palla's long-tonged bat (*Glossophaga soricina*, n=2); great artibeus (*Artibeus lituratus*, n=5); and hoary bat (*Lasiurus cinereus*, n=3). In 10 animals was not possible to identify their species (6 *Molossus* spp.; 2 *Eumops* spp.; and 2 *Lasiurus* spp.). All the bats were suspitious of rabies because their were found in the ground and were unable to fly. Before euthanasia, the bats were anesthetized with sulfuric ether, and the blood was colected by intracardiac puncture. The sera was tested by the Modified Agglutination Test (MAT) (3), with formalin-fixed tachyzoites, produced on the laboratory's own premises, in 30 day-old, female Swiss mice inoculated with RH strain and Sarcoma TG-180 cells (ATCC CCRFS-180 II). Animals with titers equal to or greater than 25 were considered positive.

RESULTS

Two bats were positive (3.2%), a Seba's short-tailed bat and a Palla's mastiff bat, both with titer 25.

DISCUSSION

The detection of antibodies against *T. gondii* indicates previous contact with this parasite. The positive bats were insectivore, thus they probably became infected by the ingestion of oocysts.

Studies involving *T. gondii* in bats are extremely scarce. In Brazil there is only one study in common vampire bat (*Desmodus rotundus*), which used the same techniques and did not found positive animals (7).

The low prevalence observed can be justified mainly by two reasons: none of the studied species were carnivore, which reduces the risk of infection by ingestion of tissues from infected animals; and bats rarely have contact with the ground, which reduces the contact with oocysts. This fact has already been observed in species with different use of the vertical space (6).

Although there is no study related to the sensitivity and specificity of the MAT in bats, the possibility of cross reaction with other parasites cannot be excluded, because the only two positive animals presented low titers.

CONCLUSIONS

The studied species of bats appears to have a small importance in the life cycle of *T. gondii*. Studies, involving more individuals and more species should be performed to support this hypothesis.

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DETECTION OF COW RAW MILK CONTAMINATION BY *BRUCELLA ABORTUS* IN ARDABIL REGION OF IRAN BY ELISA METHOD

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SUMMARY

Brucellosis is a zoonotic disease caused by gram-negative bacteria *Brucella* that are pathogenic for a wide variety of animals and human beings .Brucellosis, in particular, is easily transmitted via raw milk. Despite its control in many countries, it remains endemic in Iran. The transmission of *brucella* from infected animals to humans occurs either by occupational contact or the consumption of contaminated animal products, especially milk, cream and fresh cheese. The purpose of this study was to evaluate the prevalence of cow raw milk contamination by *Brucella abortus* in Ardabil region, located in north west of Iran. A total of 100 cow raw milk samples were collected randomly from Ardabil suburbs dairy farms from September 2010 to December 2010. ELISA was performed using commercial ELISA brucellosis milk screening kit(Pourquier, France). An average prevalence rate of 4% was found in cow raw milk in Ardabil region. It is concluded from the current study that brucellosis is prevalent in cattle in Ardabil district, where animal breeding is common. Preventive and control measures should immediately and strictly be implemented to protect animals and humans from brucellosis.

INTRODUCTION

Brucella are Gram-negative, catalase-positive, oxidase-positive, short oval rods (0.3 mm 0.4 mm) which are non-motile and usually occur singly, in pairs, or, rarely, in short chains. It grows optimally around 37 °C and is killed by heating at 63 °C for 7–10 min. When shed in the milk of an infected animal it can survive for many days provided the acidity remains low [1]. Although brucellosis has sometimes been associated with the consumption of inadequately cooked meat from an infected animal, raw milk or cream are the principal food vehicles. Brucella is readily killed by normal milk pasteurization conditions so there is no risk from pasteurized milk or products made from it. Brucellosis is an infectious zoonotic disease that is associated with chronic debilitating infections in humans and reproductive failure in domestic animals. The transmission of brucellae from infected animals to humans occurs either by occupational contact or the consumption of contaminated animal products, especially milk, cream, butter and fresh cheese[10]. The disease characterized by fever, arthralgia, sweating, back pain, malaise and anorexia. It often results in complications and the musculoskeletal system in frequently affected [10]. Iran is an endemic area for brucellosis.

Among the serological techniques used, the ELISA method is the most recent application. This technique allows the analysis of milk samples, and it is particularly well suited to automation and it shows a very good sensitivity and often a better specificity than the Ring-Test.

The purpose of this study was to evaluate the prevalence of cow raw milk contamination by *Brucella abortus* in Ardabil region, located in north-west of Iran.

MATERIAL AND METHODS

At this study a total of 100 cow raw milk samples were collected randomly from Ardabil suburbs dairy farms from September 2010 to December 2010. ELISA was performed using commercial ELISA brucellosis milk screening kit (Pourquier, France). Milk samples were tested after removal of the fat layer following centrifugation of milk samples. The samples (controls and samples to be analysed) are diluted at 1/5 in Dilution Buffer". 50 µl per well of each milk sample were added and incubate for 1 hour and 30 min. (±5 min) at +21°C (± 5°C). Diluted a vial of "Concentrated Wash Solution" in 1900 ml of distilled water. This solution is hereafter called "Wash solution". The plate was washed three times, and Diluted the conjugate to 1/100 in "Dilution Buffer ". Dispensed 100 µl per well of this diluted conjugate then covered the plate (with a lid or aluminium foil) and incubated for 30 minutes (± 3mn) at +21°C (±5°C). The plate was washed three times. Dispensed 100 µl of "Revelation Solution 3" ready to use per well and incubated the plate at +21° (± 5°C), for 20 minutes (away from the light).The stop solution was added to each well. The optical density (OD) was measured at 450 nm. Sample /positive control ratio was calculated. Sample with a S/P % greater than or equal to 55% are considered positive for the contamination of *Brucella abortus.*

RESULTS

An average prevalence rate of 4% was found in cow raw milk in Ardabil region

DISCUSSION

At present study the prevalence of *Brucella abortus* was 4% in cow raw milk in Ardabil region. The prevalence of brucellosis varies in different regions of Iran. In the north west region of Iran, in Khoy city, in West Azerbayjan province, prevalence of cattle brucellosis has been reported 26.66% [5]. Maadi et al. reported 1.18% of cattle brucellosis in Urmia, West Azerbayjan, Iran [5]. In 2008, Bokaei et al. showed that the prevalence rate of brucellosis during 2002 to 2006 in human , sheep and cattle were 37/100000, 340/10000 and 56/10000 respectively [4].

Noorozias‌l et al. studied on the prevalence of brucellosis titers in buffalo in the Khoozestan province ,Iran. Of all 400 samples, 11% were positive for antibody against *brucella spp*. [6]. The countries of the Near East region with the highest incidence of human brucellosis are Saudi Arabia, Iran, Syria, Jordan and Oman [7].In Iran, Zowghi et al. isolated strains of *Brucella abortus* from cattle(612 cases) and sheep(6 cases) [11]. In Egypt ,the prevalence of brucellosis among buffaloes varied from 7 to 10% in 1939 and it was 0.3% in 1997 [7]. Dhand *et al.* (2005) reported that prevalence rate of brucellosis among buffalo in Punjab(India)was 13.4% [5].In Pakistan, the prevalence rate of brucellosis were reported 3% and 8.5% in cattle and buffaloes, by using MRT and i-ELISA methods, respectively [8].In India, Aulakh et al. showed that the overall apparent prevalence of brucellosis was 18.26% [3].

There has been a steady increase in the incidence of *Brucella* infection in the livestocks in Saudi Arabia over the past two decades. In 1977, the incidence of brucellosis in goats in Makkah, Saudi Arabia was found to be 0.8%, in sheep 0.5%, in camels 2.8% and in cows 3.6%. In 1987, the incidence of brucellosis had gone up to 18.2% in goats, 12.3% in sheep, 22.6% in camels and 15.5% in cows in the Asir region[2].

El Sherbini et al. stydied the prevalence of brucellosis among both human and livestock populations in an endemic area in Egypt [9].

CONCLUSIONS

It is concluded from the current study that brucellosis is prevalent in cattle in Ardabil district, where animal breeding is common. Preventive and control measures should immediately and strictly be implemented to protect animals and humans from brucellosis.

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DETECTION OF BRUCELLOSIS IN WILDLIFE, SWINE AND DOG IN AUSTRIA – CASE REPORT

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SUMMARY

We present four cases of brucellosis in wildlife (wild boar and European brown hare), livestock (domestic pig) and pet (dog). All animals were submitted for necropsy to our institute, and in the first three cases *Brucella (B) suis* biovar 2 and *B. canis* in the dog could be successfully detected by performing bacteriological, immunohistochemical and molecular analyses. The cases presented below indicate the existence of several different *Brucella* species in Austria.

INTRODUCTION

Brucellosis is an important zoonotic disease caused by several *Brucella* species. Among the numerous *Brucella (B)* species and biovars, a general host restriction pattern applies, meaning that they infect different preferred hosts. In Austria, brucellosis is a notifiable disease in cattle, sheep, goats and swine. Although our country is free from *B. abortus* and *B. melitensis*, occasionally *B. suis* biovar 2*, B. canis, B. ovis* and *B. microti* infection might be detected. In this case report, four cases of brucellosis in wild and domestic animals are presented.

MATERIAL AND METHODS

Material: (A) Farmed wild boar *(Sus scrofa)*, male, adult, originated from region Burgenland, euthanized because of *Brucella*-Ab seropositivity using complement fixation test; (B) European brown hare (*Lepus europaeus*), male, adult, originated from region Salzburg, found dead; (C) Domestic pig *(Sus scrofa domestica)*, male, fetus & placenta, originated from region Lower Austria, abortion in the 3rd month of pregnancy; (D) Dog *(Canis familiaris)*, standard poodle, female, fetus & placenta, originated from region Upper Austria, surgical specimen of gravid uterus containing 5 fetuses after sectio porro.

Methods: After necropsy, selected tissue samples were fixed in 7.5% neutral buffered formalin, processed into paraffin wax, and sections were cut at 3-4 μm and stained with haematoxylin and eosin (H&E), and examined using light microscopy. Smears and frozen sections of affected organs were stained using special staining methods (Stamp and Stableforth). Immunohistochemical staining of selected tissue samples for the detection of Brucella-antigen was performed using polyclonal rabbit antibodies derived from Brucella Positive Control Antiserum (AMS), (Becton, Dickinson and Company, Franklin Lakes, USA) [7]. Reaction was visualized by avidin-biotin complex (ABC) detection system (Vector Laboratories, Burlingame, CA, USA), according to the manufacturer's instructions. These antibodies detect *Brucella (suis)* in animals except for Leporids. A monoclonal antibody for detection of *Brucella* in the European brown hare was kindly provided by Prof. Moenning (Inst. für Virologie, Stiftung Tierärztliche Hochschule Hannover) [5].

Bacteriological investigation: Brucellae were isolated by direct culture using a *Brucella* specific selective medium. Columbia agar with addition of 10% defibrinated sheep blood and a selective supplement (Oxoid SR0209E) was incubated at 37°C in a 10% CO_2 atmosphere for up to seven days. The phenotypic identification and characterization of the isolates was performed using standard bacteriological methods including testing for CO_2 requirement, H_2S production, nitrate reduction, oxidase and catalase reaction, growth in the presence of dyes (thionin and basic fuchsin) and agglutination with monospecific antisera as described previously[1].

Molecular differentation of *Brucella* sp. was performed with the INgene Bruce-ladder V multiplex PCR (Ingenasa, Madrid, Spain) following the method of García-Yoldi et al.[2]. For typing of *Brucella suis* and B. *microti,* the INgene Bruce-ladder suis multiplex PCR kit (Ingenasa, García-Yoldi et al.[3]) and the specific PCR described by Scholz et al. [8] were used respectively.

RESULTS

Pathomorphological findings: (A) Farmed wild boar *(Sus scrofa)*: moderate multifocal granulomatous-necrotizing splenitis and hepatitis as well as severe seminal vesiculitis and prostatitis with giant cell formation;

(B) European brown hare (*Lepus europaeus*): severe granulomatous-necrotizing splenitis and orchitis in association with dystrophic calcification, amyloid deposits in spleen, liver and stomach;

(C) Domestic pig *(Sus scrofa domestica)*: moderate purulent-necrotizing placentitis (Fig. 1 & 2), in the fetal lung accumulation of detritus and bacteria.

(D) Dog *(Canis familiaris)*: beginning mummification of the fetus with slight purulent pneumonia, emboli of placental epithelial cells in the pulmonary vessels, moderate purulent placentitis.

Brucella antigen was demonstrated predominantly intracellularly but also extracellularly by immunohistochemistry, in association with the granulomatous-necrotizing and purulent lesions (Fig. 4) as well as in the fetal lung alveoles of the suid and canine fetus.

Further investigations: special stainings of smears and frozen sections of affected organs revealed numerous and mostly intracellular red coccoid bacilli (Fig. 3). Slow growing bacteria isolated from lymph nodes and glandula vesicularis of the wild boar, from fetus & placenta of the domestic pig and from lesions of the European brown hare were phenotypically identified and characterized as *B. suis* biovar 2. Slow growing bacteria isolated from fetus & placenta of the poodle were phenotypically identified as *B. canis*. All B.*suis* biovar 2 strains showed the expected DNA profile in both multiplex PCRs. However, the B. canis strains showed a DNA pattern in the first PCR most similar to the close genetically related *B. suis* strains, characterised by the absence of the 1.071 bp long fragment of *omp31* locus, which has been described before only for *B. abortus* strains. Final identification was performed with molecular methods of higher level of resolution.

DISCUSSION

Four cases of Brucellosis in wild and domestic animals were presented. The indentification of the infectious agent was carried out constructively and interdisciplinarily by different detection-methods. The morphological findings were consistent with the literature [4, 6, 7]. Wildlife constitutes a large reservoir for members of the genus *Brucella.* In Central Europe, the European brown hare and the wild boar act as reservoirs for *Brucella suis* biovar 2, as also ducumented by our cases. The presented canine case is the first report of *B. canis* infection in a breeding dog in Austria. Brucellosis has a major zoonotic potential. The source of human infection mostly resides in domestic or wild animal reservoirs [4]. *Brucella* species as facultative intracellular bacteria replicate without affecting cellular viability. The intracellular localization of these agents in specialized compartments affects both the natural history and the diagnostic and therapeutic principles of the disease. *Brucella* species can persist in the

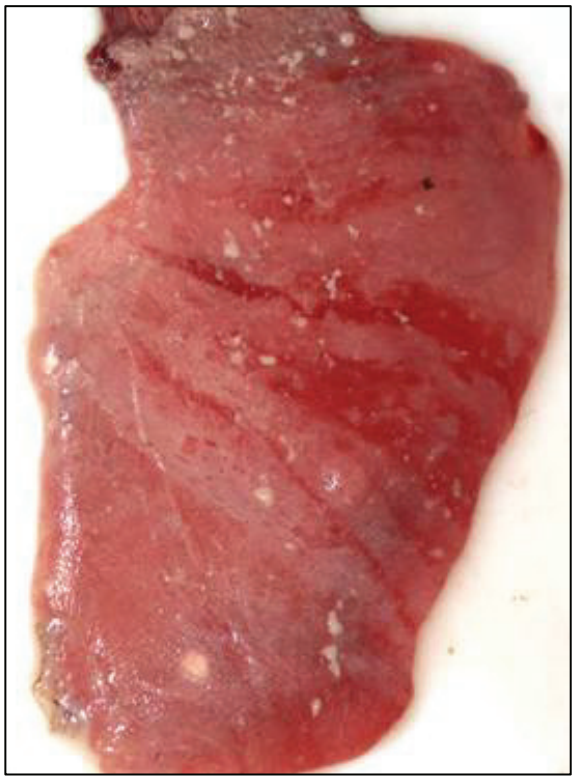

Figure 1: Brucellosis in domestic pig: purulent-necrotizing placentitis. Macrophoto, original size.

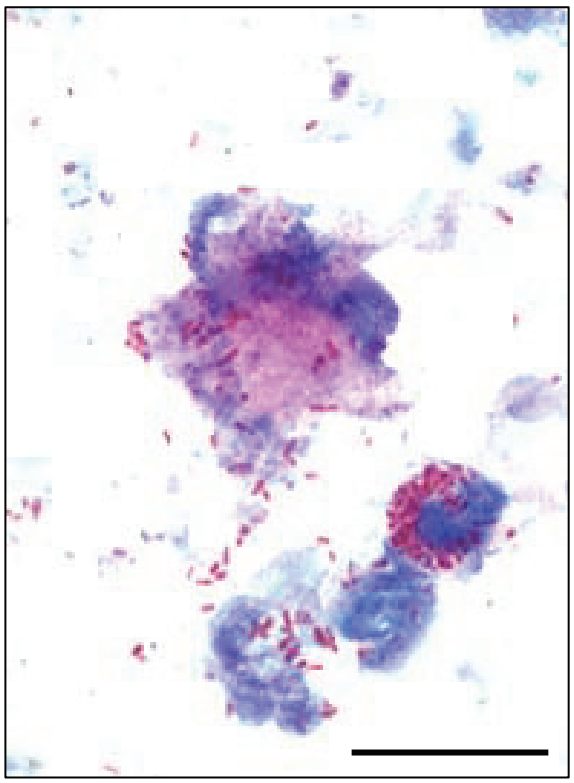

Figure 3: Brucellosis in domestic pig: intracellular demonstration of red, coccoid bacilli in placentar epithelial cells. Microphoto, placentar smear, Stableforth-stain, bar=20µm.

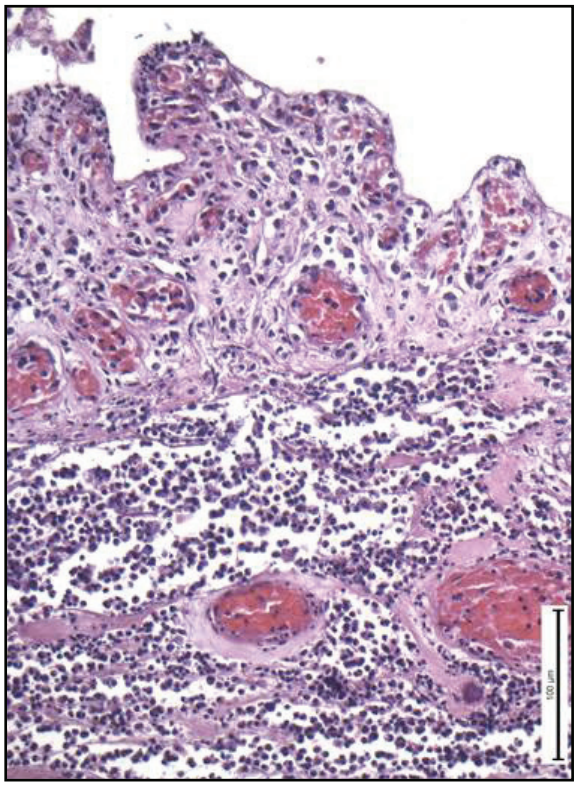

Figure 2: Brucellosis in domestic pig: severe diffuse neutrophilic infiltration of the placenta. Microphoto, H&E-stain, bar=100µm.

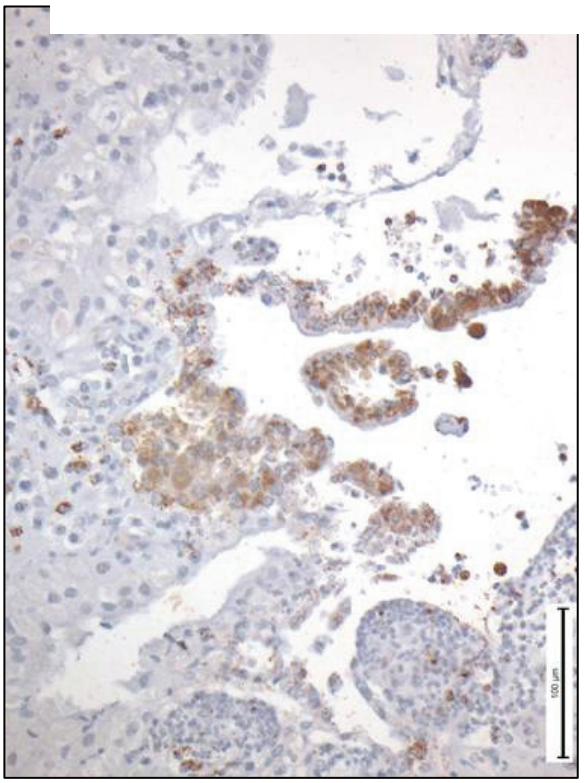

Figure 4: Brucellosis in domestic pig: predominantly intraepithelial demonstration of Brucella-antigen (red-brown signals) in the placenta. Microphoto, immuno-histochemistry (ABC-technique), bar=100µm.

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Periodical investigations of wildlife and free-range domestic pigs would be necessary to prevent the infection of livestock and complex bacteriological and serological investigations are advised in suspected kennels to protect the canine population. The prevention of human brucellosis depends predominantly on the control of the disease in animals.

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PREVALENCE OF *CRYPTOSPORIDIUM* SPP. IN CAMELS AND HUMANS RELATED TO CAMELS IN YAZD PROVINCE, IRAN

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SUMMARY

Although clinical infection with *cryptosporidium* in camels is rare, but regarding the zoonotic nature of this parasite, we investigated the prevalence of cryptosporidiosis in dromedary camels and humans related to them in Yazd province, a semi-arid region in center of Iran. During 4 seasons (winter 2008, summer 2009, winter 2009 and summer 2010), 200 fecal samples were collected from camels. Also, 100 abomasal mucosa and related fecal samples of the slaughtered camels were investigated. After staining by modified Ziehl-Neelsen method, the prepared specimens were studied microscopically. Results were analyzed using SPSS 16. The rate of infection in feces and abomasal mucosa of camels were 20.33 and 12 percent, respectively. Also, simultaneous fecal and mucosal infection was detected in 3 cases in winter. Statistical analyses showed no significant relation between infection and age of camels, their sex and season. Cryptosporidiosis in people who were in long-term touch with camels was also investigated microscopically by obtaining stool samples of 100 individuals (50 in summers, 50 in winters), 24 of them being infected with *Cryptosporidium* spp. The rate of infection was higher in winter than summer (16/50 compared with 8/50). The prevalence of *Cryptosporidium* spp. in camels and related humans in Yazd province is relatively considerable and of public health importance.

INTRODUCTION

Cryptosporidiosis is one of the important zoonotic diseases caused by *Cryptosporidium* spp. and its transmitting route is fecal-oral. Many mammals are affected by pathologic changes induced by this parasite, including human [1]. This pathogenic protozoan causes chronic diarrhea in those who have immune-suppression, but may induce only an acute self-limiting enteritis in those with intact immune system [2]. Infection with this parasite is a serious problem because of lack of widespread access to efficient therapy. Besides its medical importance, infection in animals may cause enormous economic impact because of high infection rate and decrease in production which is the result of emaciation and general malaise in diseased animals [1]. Gharagozleu reported the first case of cryptosporidiosis in Iran at early 1980s [3]. Iranian researchers indicated this infection in human and animals in different parts of the country. Nouri, Razavi et al., Borji et al. and Nazifi et al. studied the prevalence of the parasite in camels around Iran [4-7]. Nouri and colleagues reported the incidence of asymptomatic cryptosporidiosis in sheep and cattle and the people dealing with them to be 13% and 1.7%, respectively [8, 9]. The aim of this study was to determine the infection rate in asymptomatic camels and also individuals related to them in Yazd province, Iran.

MATERIAL AND METHODS

In four seasons (winter 2008, summer 2009, winter 2009, summer 2010), totally 200 fecal samples (100 in summers, 100 in winters) were collected from live camels. Also, 100 abomasal mucosa (50 in summers, 50 in winters) and 100 related fecal samples (50 in summers, 50 in winters) of slaughtered camels were investigated. Stool samples from 100 individuals (50 in summers, 50 in winters) who were in persistent touch with camels were also obtained, including farmers, slaughterhouse workers and veterinary students. The age and sex of camels were recorded and the camels were divided to three groups (<5, 5-10, and >10 years old). Microscopic exam of modified Ziehl-Neelsen stained smears was performed by 2 experienced parasitologists for the presence of oocysts. Statistical analysis was done by SPSS 16.

RESULTS

From 300 fecal specimens, 61 (20.33%) were positive for *cryptosporidium*. From 100 abomasal mucosa specimens, 12 (12%) were also positive, including 3 camels which showed concurrent fecal and abomasal infection in summer. There was no significant association between the infection and age, sex and season. Microscopic investigation of the stool of above-mentioned 100 individuals showed cryptosporidia in 24 of them, more in winter than summer (16 out of 50 compared with 8 out of 50).

DISCUSSION

Cryptosporidiosis in human was first reported in 1976 [10]. About 2% of examined stools were positive for this parasite in the US people [11]. Incidence of cryptosporidiosis in people ranges from 1 to 10%, and dependence on geography, standard of hygiene, season, age, proximity to farms and persistent contact with animals is well-known [12].

Results of the present study showed *cryptosporidium* infection in 24% of camel-related individuals. Since most of the sampled people in the present study were foreigner immigrants living in the study area, the infection rate was high likely due to their poor hygiene conditions. According to researchers' works, *C. muris*, *C. parvum* and *C. andersoni* are 3 species found in camelids [13-15]. Nouri reported that rate of fecal infection of camels to a *Cryptosporidium muris*-like parasite was 3.25% [4]. Razavi et al., Nazifi et al. and Borji et al. reported that the prevalence of *cryptosporidium* isolates from camels in different parts of Iran was 37.9%, 16.9%, and 1.9%, respectively [5-7]. The present study shows no significant difference in infection rate in two climatic situations; cold-dry and hot-dry. Many scientists have studied effects of season on occurrence and prevalence of the disease, and have reached different results. According to Garber et al. and Mohammed et al. the prevalence of cryptosporidiosis in calves and cattle was higher in winter [2, 16]. However, in the study by Becher et al., season had no effect on prevalence of the infection [17]. These contradictions may be due to climatic differences in the study areas or husbandry management systems. The Yazd province has two climates in the year; cold-dry and hot-dry, and cannot be regarded wet at all, therefore comparing effect of season on cryptosporidial infection rate with that of previous studies is very difficult.

In the present study, age of animals had no significant effect on prevalence of infection. Two things must be segregated, disease and asymptomatic infection. Most of the previous studies were done on animal neonates with clinical signs of infection, but asymptomatic carriers are especially important regarding infection spread. Our study indicates that camels of every age can affect public health. On the other hand, camels in the study area move freely in deserts almost all the year, and are only gathered for a short period. In this study there was no significant difference between male and female camel infection rates. Researches on the effect of age on prevalence of cryptosporidiosis in wild animal populations showed that infection rate did not depend on age of animals [18, 19]; this is similar to what happens to camels raised in Yazd province. Razavi et al. also could not find a relationship between sex of camels and infection rate. In their report, age of camels also had no effect on prevalence of cryptosporidiosis [5]. Our examined camels had no typical signs of the disease during sampling, so camels can be assumed as asymptomatic carriers for human and other animals in the Yazd province.

CONCLUSION

Since the treatment of cryptosporidiosis in human and animals is complicated and not yet universally used, and since camels can spread infection in human, domestic and wild animals populations, so its control and prevention strategies must be considered.

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FIRST REPORT OF SCRAPIE IN A SHEEP FLOCK IN NORTHERN CYPRUS

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SUMMARY

The aim of this study was to investigate the clinical cases and cause of deaths in a sheep flock suspected scrapie in Northern of Cyprus. Brains from 4 sheep showing clinical signs of classical scrapie were analysed for the presence of vacuolar degeneration by histopathologic exeminations and infective prion by ELISA. Clinically, hyperexcitability, aggressiveness, grinding of teeth, scratching, head tremor, ataxia, weakness of the hind limbs and paresis were observed. Infective prion was detected by ELISA in all sheep brains and also many well circumsized small vacuols in different size were seen in the neuropil of the grey matter of medulla oblongata, obex, caudal cerebral pedinculi, mid brain and proximal part of medulla spinalis. These findings describes clasical scrapie in sheep for the first time in Northern Cyprus.

INTRODUCTION

Scrapie is a fatal neurodegerenative disease affecting small ruminants [5-12]. Two forms of scrapie have been seen in sheep. The classical form is an infectious disease of sheep and goats known for 250 years. It has been reported in many countries in the world with excepition of Australia and New Zealand [5-7]. Classical scrapie affects certain genotype of sheep and those have to be exposed to infectious agent of scrapie. However, atypical form (first identified in Norway 1998 'scrapie Nor98') occurs in sheep resistant to classical scrapie agent and natural transmission from sheep to sheep has not been proven yet and therefore it occurs very low level in the farms across European Union [1-5] Reports indicate the presence of scrapie in Southern Cyprus since 1985 [9]. However, there is no report on the existence of scrapie in the Northern Cyprus. The aim of this study was to investigate the clinical cases and cause of deaths in a sheep flock suspected scrapie in Northern Cyprus.

MATERYAL AND METHODS

About 100 sheep showing clinical signs of scrapie died in 4 years time and the Veterinarian suspected from scrapie in the Northern Cyprus. To investigate the cause of disease in the flock, 4 sheep showing clinical signs of scrapie were euthanised and necropsie was performed. Brains were analysed for Scrapie histopathologically and by ELISA. For histopathology, conventional methods were used as described by others [12]. The prion extraction was performed from the obex of the medulla oblongata using the commercial test kit as described by the manufacturer (BioRad, Ref:355-1100). For the detection of infective prion protein the PLATELIA BSE test kit (BSE-scrapie detection kit=PLATELIA, Bio-Rad, Ref: 355-1103) was used and performed as described by the manufacturer.

RESULTS

Clinical findings

Affected sheep were 2-3 years of age. All sheep showed salivation in the beginning. By disease progression, abnormal behaviour like hyperexcitability, running away, fright from people, grinding of teeth and aggressiveness and motor changes like head tremor, ataxia, weakness of the hind limbs and paresis were seen by the Veterinarian. The sheep tend to scrap their head to walls and gates. Chronic emaciation was also observed although the appetite was normal. Most affected sheep in the flock died in 2-3 months after the clinical signs developed.

ELISA and Pathological Findings

Infective prion was detected by ELISA in all sheep brains analysed in this study. The OD values of 4 sheep were high titer on ELISA test.

No gross lesions were observed in the sheep brains. On histopathology, hyperemia and many well circumsized small vacuols in different size were seen in the neuropil of the grey matter of medulla oblongata, obex, caudal cerebral pedinculi and mid brain (Figure 1). Similar vacuols were also seen particularly in big neurons localised in the obex. Those vacuols in perikaryon of the neurons were either multiple or large-single. Increase in the number of astroglia cells was noted. In addition vacuols were also seen in the pyramid cells and neuropil in the stratum pyramidale of the cerebral cortex and in the neuropil and motor neurons in the proximal part of medulla spinalis.

DISCUSSION

Classical scrapie has been reported in 20 countries and 14 of them also reported atypical scrapie with lower prevalence [5]. Outbreaks of classical scrapie occured in Southern Cyprus after it was first diagnosed in 2 sheep flocks in 1985 [9-10]. By 1989, scrapie had been reported in 23 flocks and 356 out of 957 sheep showed histopathological lesions of classical scrapie [6-10]. However, there is no repoert on the presence of scrapie in sheep in Northern Cyprus. This is the first study reporting classical scrapie cases in the Northern Cyprus.

Amongst the 8 ELISA tests, the Bio-Rad Platelia/TeSeE rapid test has been recommended for detection of both type of scrapie in sheep by the European Food Safety Authority (EFSA) [3] and data from 13 countries has shown that 95% of the atypical scrapie cases were detected by the Bio-Rad Platelia/TeSeE rapid test [8]. Therefore, same test was used to detect infective prion in this study.

The criteria proposed by the EFSA and results of published papers indicate that atypical and classical scrapie cases are distinguished by the distribution of pathological changes, localisation of prion protein (PrPsc), age of animals, clinical signs, Western blot profile and genotype of sheep [1-3-4-5]. The clinical signs of atypical scrapie differs or less pronounced than the signs observed in classical scrapie [1]. In this study all sheep showed pruritis and other neurological signs which does not usually seen in atypical scrapie and fits the criteria of the signs of calssical scrapie published by EFSA and others [1-4-5]. In this study, the age of sheep analysed and cases of deaths occured in the flock were between 2-3 years old indicating the age of classical scrapie is mostly seen as reported by others [1-5]. Histopathological findings of this study (vacuolar changes, vacuols intensed in the obex) were also characteristic of TSE infections and similar to what seen in classical scrapie cases and also fits the criteria of classical scrapie [2-4-11-12].

CONCLUSION

Active surveillance of TSE's in small ruminants started in the EU countries in 2002 and non-EU countries were free to establish their own programme. In the sheep flock investigated in this study, control program has been applied and all suspected sheep were culled and burried. This study shows that scrapie is a serious health problem in sheep in the North of Cyprus. Further studies are necessary to determine the true prevalence of scrapie in the Northern Cyprus.

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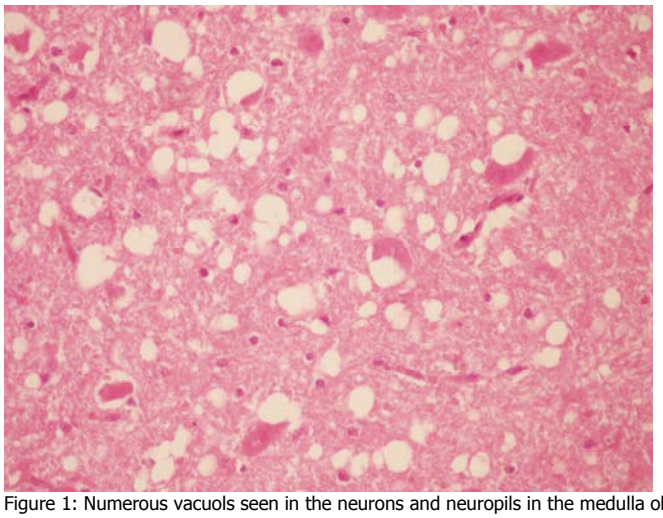

Figure 1: Numerous vacuols seen in the neurons and neuropils in the medulla oblongata.H.E. X200

RESEARCH OF TOXOPLASMA GONDII IN OSTRICHES (*STRUTHIO CAMELUS)* FROM BRAZILIAN SLAUGHTERHOUSE

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SUMMARY

Toxoplasmosis is one of the most widespread pathogen in the world, presenting high importance in production animals, like ostriches (*Struthio camelus),* and to human beings. This study was aimed to determine the prevalence of *Toxoplasma gondii* in ostriches from a Brazilian slaughterhouse. Serum samples of 344 ostriches slaughtered in a Brazilian slaughterhouse were researched for *T. gondii* antibodies by modified agglutination test (MAT), using 4 as cut-off titer. The brain of all seropositive and ten seronegative animals were bioassayed in outbreed mice for the research of the parasite. 3 8/344 (11.05%; CI95% 8.16-14.80%) animals presented *T. gondii* antibodies, with titers 4 (10, 26.31%), 8 (6, 15.79%), 16 (4, 10.53%), 32 (8, 21.05%), 64 (6, 15.79%) and 256 (4, 10.53%). No bioassay presented positive results for tachyzoites in peritoneal fluid or tissue cysts in brain tissue of the mice. The studied ostriches presented a homogeneous distribution of titers, and some of them with high titers, e.g. 64 and 256. *T. gondii* keep high titers for a long time in its hosts, characterizing a chronic infection. This fact is important when studying food animals, and slaughterhouse's process. Brain is a election site for *T. gondii,* but in this study this tissue did not present good results for this detection. The present study show the importance of ostriches in the epidemiological chain of toxoplasmosis in Brazil once these animals can be infected with this parasite, but the brain is not an important tissue for the multiplication of the parasite.
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INTRODUCTION

Toxoplasma gondii is a parasitic protozoan distributed all over the world. It infects warmblooded vertebrate species, including mammals and birds, presenting high importance in production animals, like ostriches (*Struthio camelus).* Cats are the definitive hosts, shedding oocysts in their feces. Transmission occurs through the ingestion of contaminated food with sporulated oocysts, and raw or undercooked meat with tissue cysts; transplacental transmission of tachyzoites also occurs. *T. gondii* leads to abortion in several production animals and has high public health importance since it causes an opportunistic zoonosis in immunocompromised and HIV-positive patients. In addition, it is a significant cause of abortion and congenital diseases during pregnancy [5,8,9,12,16].

The frequency of toxoplasmosis in chicken is high, as well as the risk for human beings [15], but in ostriches (*Struthio camelus)* few is know about the prevalence of this infection. In breeders, cats can transit being the main sources of infection for ostriches. They eliminate ooccysts in the place that the animals and their food are kept. Ostriches are breed in areas possibly contaminated with oocysts and can be infected by the ingestion of contaminated food and/or water [14].

This study was aimed to determine the prevalence of *T. gondii* antibodies and the isolation of the parasite in ostriches from a Brazilian slaughterhouse.

MATERIAL AND METHODS

The sample size was determined by using Epi Info 3.5.1 software [2]. A Brazilian expected prevalence of *7'. gondii* antibodies in ostriches of 14.36% [4], 1% significance level (α), 99% confidence level and 5% error limit were used to get at least 326 serum samples. Serum samples of 344 ostriches slaughtered in a Brazilian slaughterhouse were researched for *7'. gondii* antibodies by modified agglutination test (MAT), using 4 as cut-off titer [6]. Serum samples were serially 2-fold diluted from 1:4 in both tests in phosphate buffered solution (PBS), pH 7.2, 0.01M and endpoint titers were determined by means of serial dilution. A clear-cut button-shaped deposit of parasite suspension at the bottom of the well was interpreted as negative reaction and a complete carpet of agglutinated organisms was considered positive. The brain of all seropositive and ten seronegative animals were macerated, digested by pepsin-acid solution, and each brain samples was bioassayed in five outbreed mice, 30-days-old, for the research of the parasite [7]. The animals were kept in polypropylene boxes. Boxes were placed in an Alesco ventilated rack system (model ALE 99002-001, Brazil) during the experimental time (60 days p.i.).

RESULTS

In this study, 38/344 (11.05%; CI95% 8.16-14.80%) animals presented *7'. gondii* antibodies, with titers 4 (10, 26.31%), 8 (6, 15.79%), 16 (4, 10.53%), 32 (8, 21.05%), 64 (6, 15.79%) and 256 (4, 10.53%). No bioassay presented positive results for the identification of tachyzoites in peritoneal fluid or tissue cysts in brain tissue of the mice. Most of them survived for the evaluation period (60 days p.i.).

DISCUSSION

This study shows a similar prevalence to that found by Contente et al. [4] (28/196; 14.36%) in São Paulo State, Brazil, the unique study on ostriches in Brazil. In Rio Grande do Sul a similar study was carried out in rheas (*Rhea americana)* from commercial breeding facilities submitted to similar management. The authors reported prevalence equal to 8.10% as assessed by passive hemagglutination. Another study, in Canada, involving 973 ostriches observed only 2.9% seropositive animals, involving animals of commercial breeding facilities [10]. In wild ostriches from Zimbabwe the prevalence was 48%. As in the present study, these authors also used MAT, and kept high titers for a long time in its hosts, characterizing a chronic infection. This fact is important when studying food animals, and slaughterhouse's process. In one study on *7'. gondii* in ostriches, the authors studied *7'. gondii* in the serum of 50 wild ostriches in Zimbabwe and observed that 48% of the animals were reactors [13]. Brain is an election site for *7'. gondii,* but in this study the bioassay of this tissue did not present interesting results for this detection.

Sporulated *7'. gondii* oocysts are very resistant to environmental conditions, and remain infective in humid soil for more than 18 months. However, they do not survive long under cold or dry conditions. Oocysts of the parasite would have better conditions to survive in pastures of Brazil than in those of Canada, what would reinforce the hypothesis found in this study that the differences observed in the two studies are due to the climate, pasture contamination and sanitary and nutritional management to which the ostriches are submitted [10,11].

Ostriches do not depend only on grains for their nutrition, but also on other sources of fiber. The diet of birds such as ostriches is mainly based on insects, other small invertebrates and forage plants. A large part of the water they need comes from plants [1,3].

CONCLUSIONS

Thus, the present study shows the importance of ostriches in the epidemiological chain of toxoplasmosis in Brazil once these animals can be infected with this parasite, but the brain is not an important tissue for the multiplication of the parasite.

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CORRELATION BETWEEN COLIBACILLOSIS DIARRHEA IN CALF AND E. COLI ISOLATED FROM MILK AND SURFACE SKIN OF STAFF MEMBER (Abstract)

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INTRODUCTION

Colibacillosis diarrhea is the most important disease in the first week of calf life. *E. coli* has lots of serotypes and some of them are potentially pathogen.

Providing of sufficient amount of colostrums and health care are the most important aspects to disease control. Despite of all restrictions, some diseases become epidemic.

MATERIALS AND METHODS

For this study, 80 samples were collected from diarrheic faeces, Colostrum, milk and also skin surface of staff's hand.

At the first, the samples were transferred to specific media *to isolate E. coli.* Then, Isolated *E. coli* were characterized by using specific antisera (serotyping).

RESULT

The result of this study shown the positive correlation($P<0.05$) between serotype of *E. coli* that isolated from faeces and serotype of *E.coli* isolated from skin surface of staff's hand, and positive correlation between incidence of diarrhea and number of milk contaminated by *E.Coli* .

CONCLUSION

This study shown the importance of usage of gloves by staff specially in epidemic break out of diarrhea and the control of mastitis in the dry and post partum period.

THE AUSTRIAN POULTRY HEALTH DATA (PHD)

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SUMMARY

The Austrian Poultry Health Service describes himself as a partner for the public. The farmer and the veterinarian work together in the PHD and use its advantages. The data network was introduced for all kind of commercial poultry. So the data of parent flock, layers, broiler to turkeys are feed in by the farmers. Later on the veterinarians can use these data and work with it. So salmonella control has reached a unique level in Austria.

INTRODUCTION

The Austrian Poultry Health Service (QGV) was founded in 1999. In 2002 the national regulation for animal health services entered into force and since then, the QGV is approved as Poultry Health Service in Austria. All the national programs for Poultry were controlled by using the data originating from the PDH.

MATERIAL AND METHODS

In the PHD there is a web-module for each production stage. All Breeders, hatcheries, and farmers are connected via the GDV. The diagnostic laboratories, veterinary authorities and veterinarians also have the opportunity to communicate via the PHD.

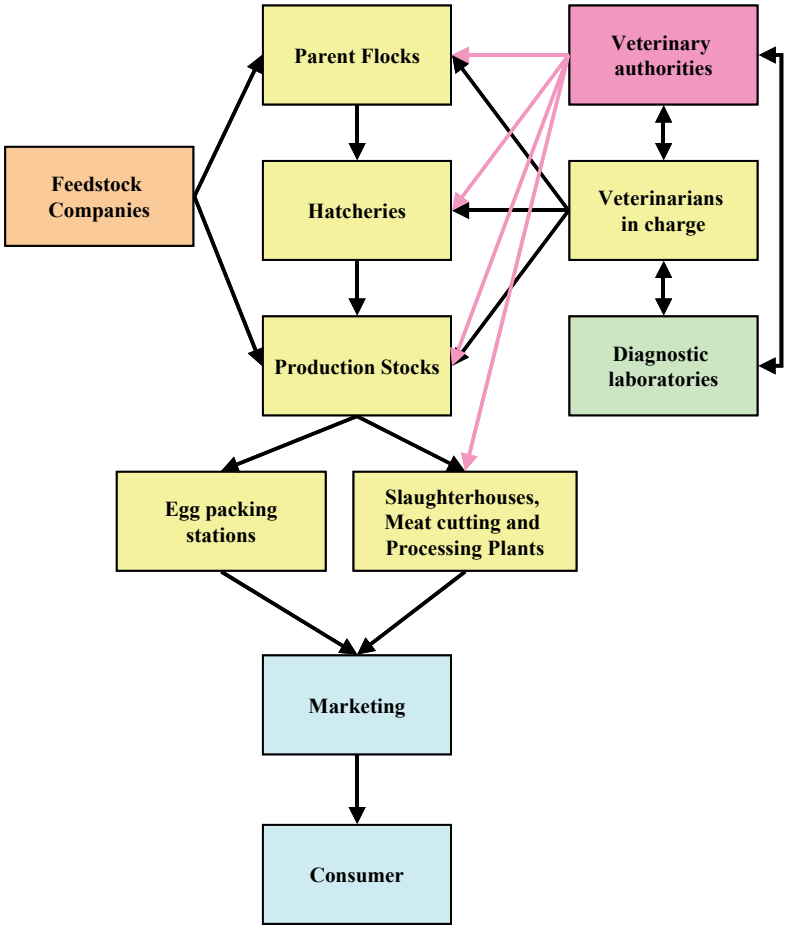
For example Data on the origin of the hatching eggs, the hatching success and the delivery of chickens to the broiler farms are fed into the system at the hatchery. The module for the veterinary in charge allows health relevant information(vaccination, diseases, treatment, waiting times) to be entered into the system in order to comply with statutory requirements. The veterinarian is also informed about the results of various tests (e.g. boot swabs) online by the laboratory and can thus react accordingly.

In Austria all boot swaps mentioned in the national programs are performed with the same product (Duo sterisox).

If positive findings occur in accordance to regulation 1273/ 2007 the flock is stopped for table egg production in the PHD.

Authorised persons, authorities and companies receive a pin code allowing them to generate information relevant to them from the data base in a quick and simple way, including e.g. optimum dates for ante mortem inspections, clinical findings, results of salmonella control, vaccinations, diseases, medications and records according to the register of laying hens.(EU Directive 2002/4/EC) and the EU Regulation No 2160/2003.

The figure illustrates the users working with the PHD.



RESULTS

The PDH fulfils following requests:

- Official Register for laying hens (EU-Directive Nr.4/2002)
- Official Salmonella Monitoring
- Official Monitoring of Residues at slaughtering and in poultry farms
- Official Monitoring of Zoonoses
- Official Monitoring of vaccination in rearing flocks for layers and parent flocks

DISCUSSION

In Austria using the PHD all statutory requirements can be fulfilled by several user groups.

In the future management data and new programs concerning animal welfare will be of interest.

CONCLUSIONS

The poultry industry needs to have valid data. So the PHD is an efficient instrument for a modern quality policy.
The advantages are widespread and can be listed incomplete:

- Valid data create a reliable situation for all users
- Healthy animals produce safe products
- Use of management data to reduce costs

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EFFECT OF BOVINE LACTOFERRIN FEEDING ON LIPID METABOLISM IN LIPOPOLYSACCHARIDE-INJECTED CALVES

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SUMMARY

The present study demonstrated that *in vivo* bovine lactoferrin (LF) administration can suppress hyperlipidemia in the lipopolysaccharide (LPS)-induced acute phase reaction. The study also demonstrated that LF, by reducing inflammatory cytokines, exerts an anti-inflammatory effect on lipid metabolism in LPS-treated calves. Supplementing calf diets with LF will probably improve their performance.

INTRODUCTION

Infection and inflammation frequently induce changes in lipid metabolism [1]. Hypertriglyceridemia, characterized by an increase in very-low-density lipoprotein (VLDL), accompanies bacterial infection and LPS administration [2]. The activation of the immune system in response to infection is now known to be modulated by cytokines, such as tumor necrosis factor-alpha (TNF-□) [3]. Previously, attention has focused on cytokines, in particular TNF-□, as the agents responsible for mediating the hyperlipidemia of infection [4]. Indeed, administration of TNF-□ to humans increases serum triglyceride (TG) levels [5].

LF, an iron-binding multifunctional glycoprotein, is abundant in the colostrum and milk of different species, including humans, bovines, and mice [6]. This protein is a key element in the host defense system [7]. A number of diverse biological roles have been proposed for LF, such as anti-inflammatory, immunomodulatory, and antimicrobial activities [8]. These effects greatly depend on the absorption and transportation of LF to specific tissues [8]. Recently, it was reported that LF provides a natural feedback mechanism to control the development of the metabolic imbalance of hyperlipidemia and protects against the deleterious effects of LPS [9, 10]. The aim of this study was to investigate the influence of LF feeding on lipid metabolism disturbances in calves injected with LPS.

MATERIAL AND METHODS

Twenty-one Holstein calves at 4 days of age were given one of 3 oral doses of LF (0, 1, 3 g/day) for 10 days (- 10 day to - 1 day). They were intravenously injected with LPS (50 ng/kg body weight) the day (day 0) after the end of LF treatment. Plasma samples were obtained on – 10 and 0 day (immediately before LPS injection), and at 2, 6, 12, 24, 48, 72, and 96 hours after LPS injection.

The procedures used to separate lipoprotein fractions were essentially those of Hatch [11]. All ultracentrifugations were performed with a Beckman-type MLA-130 rotor (Beckman Instruments, Fullerton, CA, USA) in a Beckman Optima Max ultracentrifuge. Three main lipoprotein classes were prepared by using the following density intervals recommended for ruminants [12]: VLDL, < 1.006 g/mL; low-density lipoprotein (LDL), 1.006 to 1.063 g/mL; high-density lipoprotein (HDL), 1.063 to 1.21 g/mL. The VLDL fraction was first removed from the plasma by ultracentrifugal flotation for 60 minutes at 1,000,000 g at 15°C. The LDL and HDL fractions were then separated from VLDL-free plasma by ultracentrifugation for 150 minutes at 1,000,000 g at 15°C, in a discontinuous density gradient. The concentrations of TG, non-esterified fatty acid (NEFA), total cholesterol (TC), free cholesterol (FC), and phospholipid (PL) in all plasma samples or lipoprotein fractions were determined with a commercial kit (Wako Pure Chemical Industries, Osaka, Japan) using a Hitachi 7070 automatic analyzer (Hitachi, Co., Ltd., Tokyo, Japan). The cholesterol ester (CE) content was calculated using the equation CE = (TC – FC) × 1.68 [13]. The VLDL, LDL, and HDL fractions were the sum of TG, CE, FC, and PL concentrations [12]. The intra-assay and interassay coefficients of variation for measurement of TG, NEFA, TC, FC, and PL were less than 2% and 3%, respectively. Plasma TNF-□ was measured by specific double polyclonal antibody ELISA (Endogen, Montgomery, IL, USA). The minimum detection limit was 80 pg/mL and the intra-assay coefficient of variation was 5%. The various characteristics (plasma lipid, lipoprotein, and TNF-□ concentrations) studied using the different treatments were analyzed by the repeated-measures ANOVA format outlined for the GLM procedure of SYSTAT 11 (SYSTAT Software, Inc. Richmond, CA, USA). The sources of variation in the model included treatments, calves, sampling times, and the interactions of treatments × sampling times. Using this model, responses to LF or LPS administration were compared with responses to the control at each time point. Differences in responses between treatments were considered significant at $P < 0.05$.

RESULTS

Plasma TNF-☐ concentrations at 2 hours after LPS treatment were lower (P < 0.05) in calves fed 1g/day LF compared with calves given 0g/day LF (control). Plasma TG concentrations were lower (P < 0.05) in the calves fed LF than in the control calves given 0 g/day LF at 12 and 24 hours after LPS injection. Plasma NEFA concentrations were elevated during the period between 6 and 24 hours after LPS treatment. At 12 hours, the concentration of plasma NEFA was lower (P < 0.05) in the calves given 3 g/day LF than in the control calves. On day 0, plasma TC and PL concentrations tended to be lower in the groups administered 1 and 3 g/day LF than in the control group, but did not differ significantly among the groups.

Plasma VLDL concentrations were lower (P < 0.05) at 12, 24, and 72 hours in the LF groups than in the control calves. At 0 hours, total lipid concentrations of the LDL class were lower in the groups fed LF than in the control group, but the differences were not significant. Thereafter, the concentrations remained lower (P < 0.05) in groups fed LF than in the control group at 12, 24, and 72 hours after LPS injection. Total lipid concentrations of the HDL class tended to be lower in the calves fed LF than in the control calves in the 96 hours after LPS treatment, though the changes were not significant.

DISCUSSION

Infection and inflammation of the host profoundly disturb intermediary metabolism [13]. Hyperlipidemia, resulting primarily from the accumulation of VLDL, is one such metabolic aberration [14]. Both an increase in hepatic lipid synthesis and a decrease in the metabolism of circulating lipoproteins can be seen in animal models of infection and LPS administration [2, 13]. Many of the responses to infections are mediated by cytokines [15, 16], and the cytokine TNF-☐ is thought to be primarily responsible for the hyperlipidemia that accompanies infectious diseases [17]. The administration of TNF-☐ rapidly increases serum TG levels in heifers [12, 18].

Several studies have revealed that LF, administered either i.p. or i.v. prior to i.p. or i.v. administration of lethal doses of *Escherichia coli*, protects mice against infection [9, 19]. These studies suggest that this prophylactic effect of LF involves the inhibition of the production of several cytokines, including TNF-☐ and IL-1☐, which are key mediators of the inflammatory response leading to death from toxic shock [20]. Indeed, the anti-inflammatory properties of LF have been demonstrated by its inhibition of LPS-induced TNF-☐, IL-1☐, and IL-6 activity *in vitro* and *in vivo* [21, 22]. We previously showed that LF given orally inhibited the elevation of LPS-stimulated plasma haptoglobin level in calves [23]. On the other hand, in the digestive tract, intact LF and partially digested LF peptide, which retain biological activity, may exert various physiological effects, including a bacteriostatic effect due to iron binding and anti-inflammatory effects [24]. Therefore, it seems likely that oral LF or its digestion products act initially on the intestinal immune system and then promote systemic protective immunity as a secondary effect [25].

CONCLUSIONS

Although there is no acute thread to human health due to MRSA ST398 it is advisable to closely watch the occurrence and epidemiology of the laMRSA clonal line ST398

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AN INFLUENCE OF VARIOUS FEED FATS ON OXIDATION POTENTIAL AND IMMUNOLOGICAL INDICES OF LAYING HENS BLOOD

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SUMMARY

The effects of different feed fats on oxidation potential and immunological parameters of laying hens blood were studied. Laying hens were fed experimental diets containing fish oil and linseed. Total antioxidant status (TAS), activity of glutathione reductase (GR), glutathione peroxidase (GPx) and the levels of IgA, IgG and IgM immunoglobulins were examined in Lohmann Brown laying hens after ingestion of different sources of fat (in group K – soybean oil, sunflower and soybean meal, in group A – addition of linseed oil (LS), in group B – addition of fish oil (FO), and in group C – addition of LS and FO) for 32 weeks of laying period. The mixtures were isoprotein (16.9-17.1%) and isoenergetic (ca. 17 MJ/kg).

The applied kinds of fats (LS, FO) did not influence significantly TAS level (1,33-1,42 mmol/l), however glutathione reductase (GR) exhibited some statistically important differentiation (27,92 – 33,49 U/l) and was the lowest in group C. Significant increase in glutathione peroxidase (GPx) activity was in turn observed in experimental groups A, B and C. Level of immunoglobulin M was significantly higher in groups A, B and C (fed with supplement of LS and FO) as compared with group K. IgG was characterized by decreasing tendency with respect to the control group, but the differences were not statistically significant between the experimental groups.

INTRODUCTION

It is known that the blood parameters of laying hens are variable and depend on many factors, especially on diet and phase of production [5, 6].Various feed fats are used in laying hens feeding in order to improve nutritional value of eggs, including an increase of polyunsaturated fatty acid (L-PUFA n-3) contribution [8]. There are not numerous studies concerning an influence of various fats on physiological parameters of laying hens in an intensive breeding

Fish oils are rich sources of n-3 fatty acids (FAs), especially eicosapentaenoic acid (EPA; C20:5) and docosahexaenoic acid (DHA; C22:6) [1]. Several trials have shown that dietary fish oil or n-3 fatty acids supplementation has immunomodulation effect and therapeutic benefits in animal disease models or humans with various disease conditions. Furukawa et al. [2] showed that oral or enteral supplementation of EPA with soybean oil emulsion reduces serum interleukin (IL)–6 levels.

The aim of the present work was to assess of two different dietary fats (linseed, fish oil) on oxidation potential and immunological parameters of laying hens blood.

MATERIAL AND METHODS

The research material were 120 16-week-old Lohmann Brown laying hens kept in battery cages (4 groups, 6 cages, 5 hens in each) for 32 weeks of laying period. Birds were divided into 4 experimental groups. The source of fat (and energy) in control group K was soybean oil, sunflower and soybean meal. In group A linseed (LS) was introduced, in group B fish oil (FO), and in group C – LS and FO. Laying hens were fed *ad libitum* with standard feed mixtures (Tasomix Company) according to the Polish Standards of Poultry Feeding [2005] The feed mixtures were isoprotein (16.9-17.1%) and isoenergetic (ca. 17 MJ/kg). The mixture was analysed using standards methods and calculated assays were presented in Table 1. The analyses of hens blood were conducted three times (every 10 weeks) and included the following items: total antioxidant status (TAS), activity of glutathione reductase (GR), glutathione peroxidase (GPx). The level of IgA, IgG and IgM immunoglobulins was determined.

Table 1. Ingredient and chemical composition of the basal diet

Ingredients		Group K	Group A	Group B	Group C
Corn ground	%	25,00	24,96	25,10	24,97
Triticale	%	18,00	18,00	18,00	18,00
Wheat ground	%	13,80	12,90	13,60	12,80
Sunflower crush	%	13,40	11,10	13,40	11,10
Linseed	%	-	4,50	-	4,50
Fish oil	%	-	-	1,20	1,20
Soybean oil	%	2,50	1,30	1,40	0,20
Humobentofet	%	1,40	1,40	1,40	1,40
Dicalcium phosphate	%	1,00	0,83	1,00	0,83
L-lysine 98	%	0,19	0,20	0,19	0,20
DL-methionine 99	%	0,09	0,09	0,09	0,09
Sodium bicarbonate (NaHCO3)	%	0,06	0,06	0,06	0,06
Vitamin E	mg/kg	20,00	45,00	45,00	45,00
Alkosel	%	0,0025	0,02	0,02	0,02
Calculated analysis*					
Metabolisable Energy	MJ/kg	15.04	16.78	17.47	15.88
Dry matter	%	88.46	89.50	89.68	88.85
Ash	%	8.56	9.44	8.73	8.07
Crude protein	%	17.16	16.76	17.03	16.51
Crude fiber	%	4.79	4.40	4.80	5.35
Crude fat	%	3.79	4.87	4.62	5.15
Calcium	g/kg	29.68	37.09	31.21	26.70
Phosphorus	g/kg	7.64	6.71	6.75	6.66
Sodium	mg/kg	1.02	0.69	1.79	1.11
Potassium	g/kg	5.03	5.32	5.08	4.79
Magnesium	g/kg	1.89	1.97	2.30	1.23
Copper	mg/kg	12.72	8.62	16.94	9.25
Zink	mg/kg	97.80	43.88	140.25	101.03
Manganese	mg/kg	100.42	43.55	147.98	93.18
Iron	mg/kg	384.72	409.31	371.97	367.43

*According to Polish Standards of Poultry Feeding

RESULTS

The applied fats (linseed, fish oil) did not influence significantly TAS level (1,33 – 1,42 mmol/l), however GR exhibited some significant differentiation (27,92 – 33,63 U/l) and was the lowest in group C (Table 2). Statistically significance of increasing glutathione GPx activity was in turn observed in experimental groups A, B and C (max 30961 U/L). Furthermore the immunological indices (IgA, IgM, IgG) were characterized with different tendency between groups. The level of immunoglobulin M was significantly higher in groups A, B and C) as compared with group K. Maximum value was 1,172 mg/ml. IgG was characterized in turn by decreasing tendency with respect to the control group, but the differences did not differ statistically between groups. Maximum value was 12,35 mg/ml.

Table 2. Oxidation and immunological blood parameters (mean ± SD)

Group (n=30)	Total antioxidant status (TAS)	Glutathione reductase (GR)	Glutathione peroxidase (GPx)	Ig A	Ig M	Ig G
	mmol/l	U/l	U/l	mg/ml	mg/ml	mg/ml
K	1,33±0,23	31,67ab±8,80	20392a±6884	0,551 ± 0,268	0,921a ±0,425	12,35 ± 4,71
A	1,40±0,24	30,82ab±8,13	25479b±5677	0,627 ± 0,271	1,152b ±0,410	11,42 ± 5,24
B	1,42±0,25	33,63b±10,92	30961c±10472	0,680 ± 0,363	1,172b ±0,554	11,04 ± 5,18
C	1,35±0,22	27,92a±6,49	25536b±5486	0,584 ± 0,220	0,920a ±0,321	10,67 ± 3,34

a,b,c $p<0.05$

DISCUSSION

Fish oil is rich in polyunsaturated fatty acids, with five or six double bonds, so lipid peroxidation may increase after administration of fish oil. In another study liver antioxidant enzyme activities were analyzed in rats infused with fish oil, safflower oil, or fed with chow. The result showed that the fish oil group had lower superoxide dismutase activity than did the chow-fed control group but did not differ from the safflower oil group. Liver glutathione peroxidase activity was the lowest in the fish oil infusion group [9]. Because superoxide dismutase and glutathione peroxidase are enzymes that protect tissues from the effect of free radicals and lipid peroxidation, and the enzyme activities are elevated after free radical–mediated injury and lipid peroxidation [3], the lower superoxide dismutase and glutathione peroxidase activities in the fish oil group may indicate less accumulation of lipid peroxidation products in the liver tissue of the fish oil group.

Husvéth et al. [4] investigated the influence of unsaturated fish oil with vitamin E supplementation on the antioxidant status of broiler chicken cockerels. Vitamin E supplementation reduced liver serum glutathione (GSH) and raised blood serum total antioxidant status TAS. Serum GSH was the same for vitamin E supplemented diets regardless of the fat supplement. The results suggest that feeding oils rich in n-3 PUFA increases tissue lipid peroxidation and reducing the antioxidative status of broiler chickens. Supplementing high levels of vitamin E with such oils may increase tissue oxidative stability. Serum TAS or GSH may be used as a measure of antioxidative status in chickens.

Rama Rao et al. [7] concluded that sources of oil for broiler diets doesn't have any effect on immune responses or the activity of anti-oxidizing enzymes. Their higher concentrations of dietary α-tocopherol (50 or 100 mg/kg) reduced lipid peroxidation activity and enhanced activities of anti-oxidative enzymes. Activities of glutathione peroxidase was reduced with concentration of α-T for each source of oil (sunflower, palm and safflower).

Own studies showed the greatest analyses (TAS, GR, GPx, Ig) in groups supplemented with linseed or fish oil separately.

CONCLUSIONS

The feed fat applied (linseed and fish oil) had a limited influence on an oxidation potential and immunological indices of laying hens blood.

The obtained results indicate the opportunity to apply fish oils and plant oils to poultry diet for further examinations, also including physicochemical parameters of eggs.

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EFFECTS OF PHYTOGENIC FEED ADDITIVES CONTAINING *QUILLAJA SAPONARIA* ON AMMONIA IN FATTENING PIGS

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SUMMARY

The objectives of the presented studies were to investigate the effects of two phytogenic feed additive (PFA) containing extracts of *Quillaja saponaria* and essential oils as active ingredients on aerial ammonia (NH_3) and odor concentrations in growing-finishing pig houses.

Both trials were conducted in climatic chambers where temperature and ventilation were at the same level. With both products, Aromex ME Plus (AME+) and Fresta® F Plus (FF+) the ammonia emissions were reduced by 38% and 32%, respectively. The odor units were reduced by 34% with AME+ and 29% with FF+.

INTRODUCTION

Structural changes in agriculture towards fewer farms with higher numbers of animals per farm are causing problems due to ammonia but especially odor emissions. Approval processes for new buildings are often delayed due to conflicts between farmers and neighbors. Most EU countries have admitted to reduce their ammonia emissions. Thus, extensive research is done to identify solutions for ammonia abatement. Feed additives including saponins have been reported to reduce ammonia emissions (Colina et al., 2001; Veit et al., 2010). This effect might be explained by the direct binding of ammonia to saponins (Killeen et al., 1998) and/or the inhibition of the bacterial enzyme urease (Nazeer et al., 2002; Yeo and Kim, 1997), which catalyzes the hydrolysis of urea into ammonia and carbon dioxide. In comparing techniques like air cleaners or slurry spreading, Phytogenic feed additives can reduce the ammonia production/concentration already at animal level, which is increasing animal health and welfare. Calculations on economics for ammonia reducing techniques reveal that feed additives which increase performance can result in profits instead of costs per unit of ammonia.

MATERIAL AND METHODS

In both trials a total of 32 three-way hybrids, (Large White x Landrace) x Pietrain, were distributed to two treatment groups by weight and sex. Pigs were housed in two identical barns with two pens each with fully slatted floors for 8 fattening pigs from 30 to 110 kg live weight. Both groups received a basal diet during a 10 day adaption phase. Water was available *ad libitum* during the whole trial period. Feed intake was recorded per group, while weights of animals were recorded individually.

During the experiments, the control groups were fed on basal diets while the treatment groups received the basal diet plus 100 ppm of the phytogenic feed additives AROMEX® ME Plus in trial 1, and 150 ppm FRESTA® *F Plus* in trial 2. The quantity of *Quillaja Saponaria* is the same for both trials. In trial 2 diets were provided as a grower (day 1 - 35) and finisher phase (day 36 - 78). Feed intake has been restricted in trial 1 while feed was available *ad libitum* in trial 2.

Table 1: Calculated feed values for trial 1 and 2.

	AME+	FF+ Grower phase	FF+ Finisher phase
Energy (MJ/kg)	12.8	13.20	13.10
Crude protein (%)	17	17.50	16.00

Ammonia was measured with two portable devices (Dräger, Germany) at an interval of 10 minutes. Temperature and humidity were measured in the barns at animal level, the attic and outside. The means of 10 minutes were recorded in a Mikromec-multisens-data-logger.

Samples for odor measurement were taken 4 times during the trial. Each sample was analyzed by 2 teams composed of 4 subjects each. For quality assurance purposes, the subjects were tested with n-Butanol prior to each measurement, pursuant to DIN EN 13725.

For the evaluation of the odor reducing effect of the additives an olfactometer (Mannebeck, Germany) was used. The odor substance concentration of the exhaust air sample to be measured is determined by thinning it out with synthetic air until the odor threshold is reached. In

addition, an increasing concentration of a constant, odorless stream of air is mixed with an odor-intensive gas flow that is led through a flow meter. This mixture is provided to the test subjects through nose masks for evaluation. In order to determine the personal odor threshold, each subject must make a yes/no decision (it smells/it doesn't smell). The results of the odor substance concentration measurements are provided in OU/m³ (odor units per cubic meter), with all corresponding statistical values.

RESULTS

In both trials no differences in temperature and humidity were observed between the two barns. There were also no significant changes in feed intake and performance detected.

Analysis on ammonia concentrations and odor units showed significant differences between control and treatment groups. In trial 1, results show that the addition of AME+ resulted in an aerial ammonia reduction of 38% for the whole fattening period and the reduction of OU/m³ was 34% (Figure 1). The statistical analyses of trial 2 (FF+) showed a reduction of the ammonia concentration of 32% for the whole period and an average reduction of 32% OU/m^3 (Figure 2). The results of the olfactometric investigations showed a correlation of odor and ammonia concentrations.

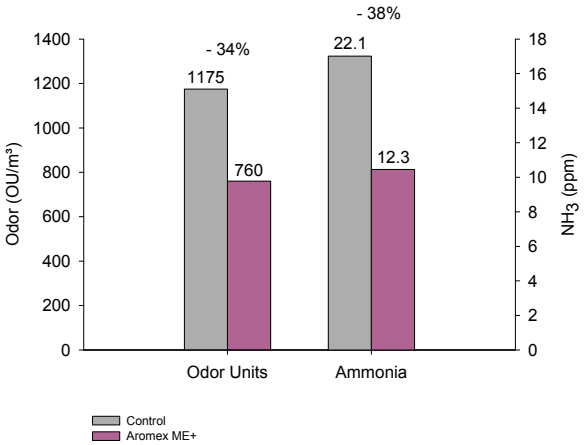

Figure 1: Odor and ammonia concentrations of trial 1 (AME+)

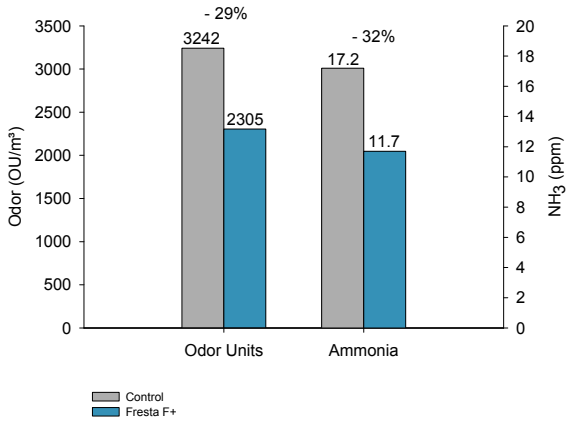

Figure 2: Odor and ammonia concentrations of trial 2 (FF+)

Figure 3 shows the ammonia concentrations for the grower and the finisher phase in trial 2, where lower reductions were shown in the grower phase (25%) than in the finisher phase (36%).

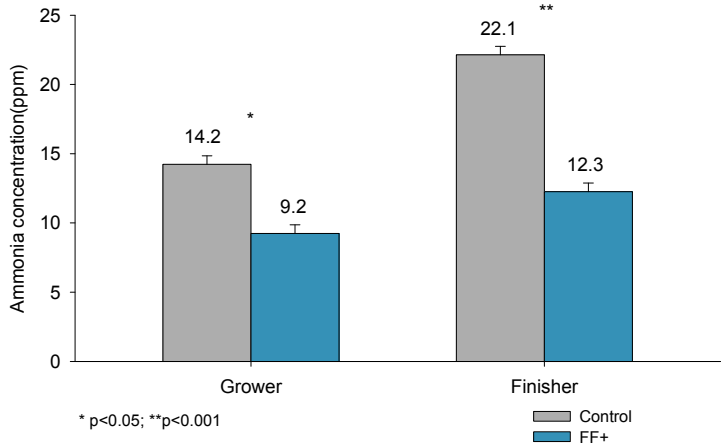

Figure 3: Ammonia concentration in grower and finisher phase of trial 2 (FF+)

DISCUSSION

The results of these trials indicate that the ammonia and odor reduction is due to the inclusion of *Quillaja Saponaria*, which was included at the same quantity per ton of feed in both trials. Same temperature and humidity values, as well as the equal feed intake and performance levels in the trial groups provide comparable conditions for control of feeding strategies on ammonia emissions.

The inhibition of urease is reducing the splitting of urea into ammonia and CO_2 which happens within hours after excretion and leads to an immediate reduction of the

ammonia and odor concentration. The remaining protein in the manure is also fermented by microbes to ammonia, but this is a process which takes days to weeks, which might explain the higher reductions in the finisher phase of trial 2 (Figure 3). The results on ammonia reduction are in line with field trials in piglets (Veit et al., 2010). Although the values for odor units where at different levels in the trials (Figure 1 and 2), a correlation between ammonia concentration and odor units is likely. Reasons for the differences in OU/m^3 might be different feed components or different test persons.

CONCLUSIONS

Both feed additives, AME+ and FF+, showed significant effects on ammonia and odor from growing finishing pigs under identical conditions between control and treatment groups. This indicates that *Quillaja Saponaria* is the active ingredient in these phytogenic feed additives reducing ammonia and odor levels. The reduction of ammonia concentrations at animal level helps to improve health and stress status and thus, contribute to animal welfare. Reduction of OU/m^3 achieved with the tested feed additives is of importance for production sites that have immediate neighbors, and improves working conditions.

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EFFECT OF PROBIOTIC ON GUT DEVELOPMENT OF DOMESTIC FOWLS

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This experiment was conducted to study the effects of effective microorganisms (EM) and Zinc bacitracin on gut development, digestibility coefficient of nutrients and intestinal histology of Inshas chicken (a local Egyptian chicken strain) . Five hundredand fourty chicks were randomly assigned to 1 to 6 dietary treatments for 41 wk. The dietary treatments were 1) control; 2) Basal diet + EM (2.5 ml/kg diet); T3) Basal diet + EM (5.0 ml/kg diet); T4) Basal diet + EM (7.5 ml/kg diet); T5) Basal diet + EM (10.0 ml/kg diet) and T6) Basal diet + Zinc bactracin (500 mg/kg). The obtained results showed that, villi height , villi thickness and villi surface area were significantly increased in birds fed EM with different levels and Zinc bactracin diets . The data on the digestibility coefficient of nutrients revealed that, all nutrients of EM diets were more efficiently digested than that of Zinc bactracin diet ($p \leq 0.01$) . While, digestibility coefficient of OM, DM, CP, EE, CF and NFE was significantly increased as compared with chicks kept on the control diet. Moreover, It was generally noticed that intestinal histology was almost following the same trend observed with gut development and digestibility coefficient .

INTRODUCTION

The impact of biotechnology in poultry nutrition is of significant importance. Biotechnology plays a vital role in the poultry feed industry. Nutritionists are continually putting their efforts into producing better and more economical feed. Good feed alone will not serve the purpose but its better utilization is also essential. Dietary changes as well as lack of a healthy diet can influence the balance of the microflora in the gut thus predisposing to digestion upsets. A well-balanced ration sufficient in energy and nutrients is also of great importance in maintaining a healthy gut. A great deal of attention has recently been received from nutritionists and veterinary experts for proper utilization of nutrients and the use of probiotics for growth promotion of poultry.

In poultry nutrition, probiotic species belonging to *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida*, and *Saccharomyces* have a beneficial effect on poultry performance [1], modulation of intestinal microflora and pathogen inhibition [2], intestinal histological changes [3], immunomodulation [4], certain haematobiochemical parameters [5], improving sensory characteristics of dressed broiler meat [6] and promoting microbiological meat quality of broilers[7].

Effective Micro-organisms (EM) is a microbial preparation developed by Professor T. Higa of University Of The Ryukyus in Japan. The EM is composed of different microbes that include bacteria, yeasts and/or fungi. Some of the benefits claimed to accrue from the use of EM include improved meat and manure quality, improved animal health, reduction of foul smells and absence of toxic effects on bird growth [8]. Use of EM in Africa is a new innovation and novel idea. There is no available literature regarding use of microbial preparations in broiler production.

Therefore, this experiments was designed to investigate the possibility of using probiotic namely, (EM) effective microorganism (instead of using antibiotics) to Inshas chickens (Egyptian local strain) , and to evaluate its effects on gut development .

MATERIAL AND METHODS

A total number of 540 unsexed vaccinated Inshas (local Egyptian chicken strain) one day-old-chicks were weighed , wing banded and randomly divided into six experimental groups (three replicates each group) . The birds were placed in a room (floor pens) maintained at a constant temperature of 28+/-3 oC and a relative humidity of 70+/-3% .Food and water were always available *ad libtum*. The basal diet was formulated to meet the nutrient needs suggested by the NRC, 1994. For enteric morphometric analysis, birds on the designated evaluation day were euthanized, and small intestines were collected. A 1-cm segment of the midpoint of the lower ileum were removed and fixed in 10% buffered formalin for 72 h. Each segment was then embedded in paraffin, and a 2µm section of each sample was placed on a glass slide and stained with hematoxylin and eosin for examination with a light microscope [9]. The parameters evaluated were villus height, villus and thickness, villus surface area. Morphological parameters were measured using the Image Pro Plus v 4.5 software package. Fourteen measurements were taken per bird per parameter. Villus height was measured from the top of the villus to the top of the lamina propria. Villus surface area was calculated using the formula $(2\pi)(VW/2)(VL)$, where VW = villus width, and VL = villus length [10], and we used anther way villus surface area index = perimeter length × mucosal thickness [9].The experimental design consisted of six dietary treatments as follows; (T1) Basal diet (control), (T2) Basal diet + EM (2.5 ml/kg diet), (T3) Basal diet + EM (5.0 ml/kg diet), (T4) Basal diet + EM

(7.5 ml/kg diet), (T5) Basal diet + EM (10.0 ml/kg diet) and (T6) Basal diet + Zinc bactracin (500 mg/kg).. The results obtained were statistically analyzed using Duncan's Multiple Range Test [17]. Statements of statistical significance are based on P<0.05.

RESULTS AND DISCUSSION

Morphometric Analysis of the Gut

Villi height

Results obtained showed that villi height was significantly (p≤0.05) increased in birds fed EM with different levels and Zinc bactracin diets in Fig (1). The villi length was longer (P < 0.05) by about 12.6 to 15.2% for birds fed EM and Zinc bacitracin diet as compared with the control. There are no significant differences among chicks fed diets with different levels of EM.

Villi thickness

Statistical analysis of the results obtained proved that EM and Zinc bacitracin diets had a significant effect on the Villi thickness Fig (1). Villi thickness was significantly increased in birds fed EM and Zinc bacitracin diets as compared with control diet (as average 3.8 vs 1.6 µm; P < 0.001).

Villi Surface Area

Villi surface area as influenced by dietary EM with different levels and Zinc bactracin during the experiment of period is presented in Fig (1). Villi surface area was increased (*P* < 0.001) from 17.11 to 71.3 µm as the level of EM increased from 0 to 10 ml/kg in bird diets. Also, increased (*P* < 0.001) by 101.3 % in chicks fed T6 diet as compared with those fed control diet . These results agree with **[**11]. Upon consumption, probiotics deliver many lactic acid bacteria into the gastrointestinal tract. These microorganisms have been reputed to modify the intestinal milieu and to deliver enzymes and other beneficial substances into the intestines [12].

It is well established that probiotics alter gastrointestinal pH and flora to favor an increased activity of intestinal enzymes and digestibility of nutrients [13]. Mechanisms by which probiotics improve feed conversion efficiency include alteration in intestinal flora, enhancement of growth of nonpathogenic facultative anaerobic and gram positive bacteria forming lactic acid and hydrogen peroxide, suppression of growth of intestinal pathogens, and enhancement of digestion and utilization of nutrients [14].

Illuminating work from Gordon's laboratory provides evidence that manipulating the microbiota with probiotics can influence the host. Germ-free mice were colonized with *Bifidobacteria thetaiotaomicron*, a prominent component of the adult human gut microbiota, and *Bifidobacterium longum*,a commonly used probiotic. *B. longum* repressed *B thetaiotaomicron* expression of antibacterial proteins that may promote its own survival in the gut, as well as influence the composition, structure, and function of its microbial community.

Digestibility Coefficients

Data on digestibility coefficient of nutrients as shown in Table (1) revealed that all nutrients of EM diets were digested more (p≤0.01) efficiently than of Zinc bactracin diet. While, digestibility coefficient of OM, DM, CP, EE, CF and NFE were significantly increased by 3.7, 3.8, 3.9, 9.6, 4.1 and 4.4 %, respectively, in chicks fed T6 diet. Also, increased (p≤0.01) by 7.3, 7.5, 5.4, 9.0, 53.9 and 7.0 %, respectively, in those fed EM diets (T2, T3,T4 and T5) as compared with chicks kept on control diet.

[15] reported that supplementing broiler diets with probiotics tended to increase the digestibility of CP in both fresh maize and 10% moldy maize diets**.** Also, [16] showed that, supplementing broiler chick diets with growth promoter significantly improved digestion coefficient of nutrients except CF compared to un supplemented diet. The increased number of beneficial microbes was confirmed and explained by [17] who found that the number of anaerobic bacteria and cellulytic bacteria was increased, when the diet was supplemented with yeast, due to enhancing Lactate utilization and moderating pH of the media, therefore, yeast improved the nutrients digestibility coefficients.

In this connection, [18] reported a positive effect of probiotics on apparent protein digestion and attributed this effect to the proteolytic activity of bacteria. It is worthy to note the absence of significant differences in the data as a result of the combination effect of dietary CP level and tested probiotics. Such observation confirmed the previously mentioned opinion that the tested probiotics had a sparing effect of nearly 2.0 % CP. Similarly, the better (P>0.05) digestibility obtained with probiotics supplementation suggests that such addition improved feed and nutrients utilization, which in turn explain the better growth and FCR values obtained with the probiotics supplemented diets. In general, the improvement (P>0.05) due to adding the probiotics may be attributed to improving intestinal microbial balance. In other words, probiotics help to keep the intestinal tract healthy and when the epithelial tissue is healthy, there is improved and better absorption of all nutrients [19].

Improvement of nutrient digestibility by supplementing chick diets with either microbial probiotics could be attributed to different stimulators such as change in the enteric flora and reduction of E. coli population, lowering gastric pH, synthesis of catabolic enzymes that help in

releasing cell compounds including amino acids, sugar and fatty, acids into the intestinal environment and involving of active bacteria with the digestive processes, protein synthesis and nutrient absorption in gastrointestinal tract[20].

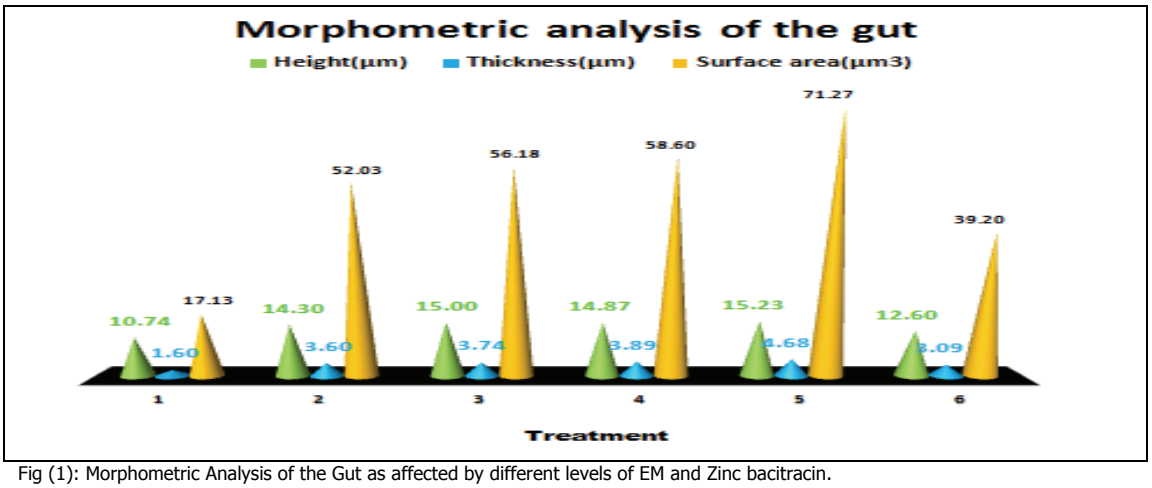

Fig (1): Morphometric Analysis of the Gut as affected by different levels of EM and Zinc bacitracin.

Table (1): Digestibility coefficient of chicks as affected by the different nutrients of the experimental treatment (Means ± SE).

Items / Treatment	Digestibility coefficient %					
	OM	DM	CP	EE	CF	NFE
T1 Basal diet (control)	81.7±1.39^{c}	79.5±1.46^{c}	86.8±0.40^{c}	76.2±0.74^{d}	33.2±1.60^{d}	77.4±0.64^{b}
T2 Basal diet + EM (2.5 ml/kg diet)	87.3±0.54^{a}	85.4±0.45^{a}	91.1±0.41^{ab}	81.1±0.67^{bc}	50.1±2.78^{ab}	82.4±0.79^{a}
T3 Basal diet + EM (5.0 ml/kg diet)	87.7±0.31^{a}	85.5±0.31^{a}	91.8±0.26^{a}	82.9±0.72^{bc}	52.1±1.63^{a}	83.3±0.98^{a}
T4 Basal diet + EM (7.5 ml/kg diet)	87.6±0.29^{a}	85.5±0.28^{a}	91.1±0.41^{ab}	83.9±0.67^{ab}	50.1±2.79^{ab}	82.2±0.66^{a}
T5 Basal diet + EM (10.0 ml/kg diet)	87.9±0.50^{a}	85.6±0.31^{a}	91.9±0.25^{a}	84.4±0.59^{a}	52.1±1.64^{a}	83.4±0.97^{a}
T6 Basal diet + Zinc bactracin (500 mg/kg)	84.7±0.49^{b}	82.5±0.46^{b}	90.2±0.38^{b}	83.5±0.83^{ab}	46.9±0.42^{bc}	80.8±0.59^{ab}
Sig	***	***	***	***	***	***

a,b,..: Means in the same column followed by different letters are significantly different at p≤0.05.

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EFFECT OF DIETARY FLAXSEED OIL SUPPLEMENTATION ON REPRODUCTIVE PERFORMANCE OF RAMS DURING SUMMER

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SUMMARY

The work was conducted on 12 healthy ossimi rams. Rams were equally divided into 3 groups (Control, Test 1 and Test 2). Rams of tested groups received 25 and 50 gm of Flaxseed oil daily for 3 months in their rations ,
Flaxseed oil as a feed additive has a lowering effect on body temperature while respiration rates elevated (P < o.o5). An increase in body gain and tests circumference were recorded. Significant elevation in the values of ejaculate volume, sperm motility, sperm concentration, total sperm count and live sperms were recorded in semen of both tested groups.

INTRODUCTION

Environmental temperature influences reproductive function in males by alteration in spermatogenesis and reduction in semen quality [2] . Supplementing of heat stressed animals with nutritional materials is essential to correct their negative balance caused by metabolic disorders [9]. Flaxseed oil cantains 58 % linolenic acid as antioxidant that enhance health of animals [4] . The objective of our work was to study effect of flaxseed oil on semen characters of ossimi rams under heat stress during summer months.

MATERIAL AND METHODS

The work was conducted on 12 ossimi rams 423 ± 2.08 days old , healthy and free from internal and external parasites . Rams were divided into 3 groups each of 4, one control and the others for tests. Experimental rams (Test 1 and Test 2) received daily 25 and 50 gm of Flaxseed oil respectively as feed additive for 3 months in their rations.
Drinking water was available and minerals were supplied all the days. Chemical analysis of ration [1], as well as the fatty acids contents of Flaxseed oil [3] were estimated. Environmental temperature, relative humidity and hours of photo periods were recorded. Rectal temperature, respiration rate, body weight and testes circumferences were measured. Physical characters of weekly collected semen were done [9]. Blood serum samples were monthly collected and analyzed for testosterone [8] . Analysis of variance was carried out [5].

RESULTS

The data of results are shown in tables 1 – 6.

Tables 1, 2: Composition of Ration and Flaxseed oil

(1)

Ration Constituents	%
Dry matter	89.20
Crude protein	16.30
Ether Extract	3.32
Crude fibre	10.91
NFF	61.65
Ash	7.82

(2)

Fatty acids types of Flaxseed oil		w/w %
Palmitic	(16.0)	8.7 ± 0.2
Stearic	(18.0)	3.3 ± 0.1
Oleic	(18:1,n – 9)	20.7 ± 0.2
Linoleic	(18:2, n – 6)	28.8 ± 0.3
Linolenic	(18:3 , n – 3)	36.8 ± 1.5

Table 3: Air temperature, Relative humidity and photo periods variations

Summer months	Air Temp. ^{0}C		R.H. %		Photo periods (hrs)
	Max.	Min.	Max.	Min.	
June 2009	40.3	24.0	71.70	35.0	22.6
July 2009	34.1	25.5	75.30	49.0	19.4
August 2009	44.0	26.1	55.00	54.0	27.0
Mean values	39.4	25.2	67.33	46.0	23.0

Table 4: Effects of flaxseed oil as feed additive on some body parameters

Parameters / Animals	Control	Tested / Flaxseed oil	
		T_1 (25 gm)	T_2 (50 gm)
Resp. rate / min.	76.95 ± 3.4[b]	81.11 ± 2.6 [a]	78.94 ± 3.3[a]
B. Temp. ^{0}C.	39.32 ± 0.3[a]	39.01 ± 0.2[b]	39.06 ± 0.2[b]
Initial B. Wt. (kg)	55.00 ± 2.33[a]	54.6 ± 2.25[a]	54.7 ± 2.27[a]
Final B.Wt. (kg)	68.30 ± 0.01[a]	69.8 ± 2.99[a]	71.4 ± 3.23[a]
Daily gain (gm)	148.9 ± 10.12[a]	169.0 ± 13.21	185.6 ± 14.11[a]
Testes circum. (cm)	29.5 ± 0.29[a]	30.75 ± 0.33[a]	30.45 ± 0.2[a]

Table 5: Effects of flaxseed oil as a feed additives on semen characteristics

Semen characters	Control	Tested / Flaxseed oil	
		T_1 (25 gm)	T_2 (50 gm)
Ejaculate Vol. (ml)	o.912 ± 0.03[b]	1.04 ± 0.04[a]	1.04 ± 0.3[a]
Sperm motitity (%)	55.11 ± 0.65[b]	63.00 ± 0.77[a]	71.76 ±0.78[a]
Sperm.Con. (10^9/ ml)	1.25 ± 0.06[b]	1.75 ± 0.04[a]	1.75 ± 0.5[a]
T.sperm output(10^9/ejac.)	1.14 ± 0.03[b]	1.81 ± 0.04[a]	1.83 ± 0.03[a]
Motile sperm/ml (x10^9)	0.69 ± 0.18[c]	1.10 ± 0.31[b]	1.25 ± 0.49[a]
Motile sperm/ejac.(x10^9)	0.63 ± 0.29[c]	1.14 ± 0.26[b]	1.31 ± 0.68[a]
Live sperms (%)	73.23 ± 0.66[b]	82.17 ± 0.88[a]	80.16 ± 1.06[a]
Dead sperms (%)	26.53 ± 0.66[a]	17.52 ± 0.66[b]	19.81 ± 0.99[b]
Abnormal sperms (%)	21.58 ± 1.06[a]	14.16 ± 0.65[b]	11.27 ± 0.64[b]

Table 6: Testosterone (ng / ml) Profile by age and Flaxseed oil

Periods	Control	Tested / Flaxseed oil	
		T_1 (25 gm)	T_2(50 gm)T_2
Pre-treatment	0.336 ± 0.012[a]	0.371 ± 0.008[a]	0.369 ± 0.007[a]
One month post treatment	0.371 ± 0.012[a]	0.389 ± 0.021[a]	0.395 ± 0.012[a]
Two months post treatment	0.410 ± 0.002[b]	0.463 ± 0.075[a]	0.471 ± 0.037[a]
Three months post treatment	0.456 ± 0.024[b]	0.515 ± 0.075[a]	0.527 ± 0.013[a]

N.8 : a,b means in the same raw followed by the same letter are not significantly different ($P< 0.05$). T_1, T_2 = Exper. Animals group 1 and 2.

DISCUSSION

It was clear from table 2 that , Flaxseed oil contains high amounts of linoleic and linolenic acids as good antioxidants. In spite of the high ambient temperature associated with high humidity during summer months (table , 3) , the addition of Flaxseed oil to ram's ration reduce body temperature and improve body parameters (table, 4), Also, Scrotal temperature of rams is regulated independently of body temperature through feedback circuit involving scrotal - thermo receptors and effectors which are related to tunica dartos muscle and scrotal sweat gland activities . The unsaturated fatty acids content in Flaxseed oil showed a great improvement to all semen characters of rams. On the other hand, the high ambient temperature has a negative influence on semen characters in rams [6].

The significant increase in testosterone concentration (table, 6 .) , may be due to the adequate amount of unsaturated fatty acids such as linoleic and linolenic acids. These unsaturated fatty acids especially lenolenic could be converted or involved in the synthesis of cholesterol which is considered the precursor materials for steroid synthesis [7]. It has been suggested that short daylight stimule testosterone secretion while long daylight inhibit it. Thus , in spite of long daylight in summer months Flaxseed oil improves testosterone in rams.

CONCLUSIONS

In the present work, the addition of Flaxseed oil to balanced feed mixtures fed to rams was found to have a minor effect on libido traits of rams, whereas it positively influences qualitative semen traits especially during times of heat stress (Summer).

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THE EFFECTS OF SUPPLEMENTATION OF EFFECTIVE MICROORGANISMS ON EGG PRODUCTION TRAITS, QUALITY PARAMETERS AND CHEMICAL ANALYSIS DURING THE LATE LAYING PERIOD IN HENS

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SUMMARY

The effects of effective microorganisms (EM) on the Egg production traits, Egg quality and egg chemical analysis of layers were studied. Five hundred and fourty Inshas layers (a local Egyptian chicken strain) were randomly divided into 6 groups with 90 layers in each group. Layers in group 1 were fed a control diet. The remaining groups received the control diet that contained 2.5, 5.0, 7.5 or 10.0 ml of EM/ kg, and 20 mg of zinc bacitracin/kg respectively. The obtained results clarified improvements in egg production (Egg number and Egg mass/ hen) and Egg quality (($P < 0.01$) of ayers when EM was added to the diets. The results also showed that, egg yolk cholesterol was significantly decreased in chicks that fed diets with different levels of EM as compared with the control diet , while egg protein percentage was significantly ($p \leq 0.05$) increased in birds fed diets with different levels of EM.

INTRODUCTION

The poultry industry has become an important economic activity in many countries. In large-scale rearing facilities, where poultry are exposed to stressful conditions, problems related to diseases and deterioration of environmental conditions often occur and result in serious economic losses. Prevention and control of diseases have led during recent decades to a substantial increase in the use of veterinary medicines. However, the utility of antimicrobial agents as a preventive measure has been questioned, given extensive documentation of the evolution of antimicrobial resistance among pathogenic bacteria. So, the possibility of antibiotics ceasing to be used as growth stimulants for poultry and the concern about the side-effects of their use as therapeutic agents has produced a climate in which both consumer and manufacturer are looking for alternatives. Probiotics are being considered to fill this gap and already some farmers are using them in preference to antibiotics [1].

Effective Micro-organisms (EM) is a microbial preparation developed by Professor T. Higa of University Of The Ryukyus in Japan. The EM is composed of different microbes that include bacteria, yeasts and/or fungi. Some of the benefits claimed to accrue from the use of EM include improved meat and manure quality, improved animal health, reduction of foul smells and absence of toxic effects on bird growth [2]. Increased egg production and egg weight and improvements in gross margins by up to 28.5 % have also been reported [2]. Use of EM in Africa is a new innovation and novel idea. There is no available literature regarding use of microbial preparations in broiler production.Therefore, this experiments was designed to investigate the possibility of using probiotic namely, (EM) effective microorganism (instead of using antibiotics) to Inshas chickens (Egyptian local strain) , and to evaluate its effects on egg production trats, quality parameters and chemical analysis of egg.

MATERIAL AND METHODS

A total number of 540 unsexed vaccinated Inshas (local Egyptian chicken strain) one day-old-chicks were weighed , wing banded and randomly divided into six experimental groups (three replicates each group) . The birds were placed in a room (floor pens) maintained at a constant temperature of 28+/-3 oC and a relative humidity of 70+/-3% .Food and water were always available *ad libtum* . The basal diet was formulated to meet the nutrient needs suggested by the NRC, 1994. Total cholesterol content was determined by colorimetric cholesterol assay according to [3]. Eggs were collected and recorded daily. Egg production was expressed as hen-day production The eggs were weighed (nearest gram)daily and immediately after collection **.** At the last week in the experiment, 30 eggs from each replicate were collected for egg quality analysis. This was measured according to the next formula presented by [4].The experimental design consisted of six dietary treatments as follows; (T1) Basal diet (control), (T2) Basal diet + EM (2.5 ml/kg diet), (T3) Basal diet + EM (5.0 ml/kg diet), (T4) Basal diet + EM (7.5 ml/kg diet), (T5) Basal diet + EM (10.0 ml/kg diet) and (T6) Basal diet + Zinc bactracin (500 mg/kg).. The results obtained were statistically analyzed using Duncan's Multiple Range Test {17}. Statements of statistical significance are based on $P<0.05$.

RESULTS AND DISCUSSION

Egg production performance

Egg Production traits: It is generally observed that laying rate of Inshas hens was low during the first 90 day Fig (1) as compared to laying rates of commercial strain.The addition of EM with different levels and Zinc bactracin improved (P ≤0.001) egg number during laying period . Egg number was significantly increased by 28% in the hens fed T5 diet as compared with control diet. There are no significant differences between T5, T4, T3 and T2 diets. Egg mass /hen value for all treatments are shown in Table (10) .Since variations in average egg weight were not as great as those of egg number, so it was logic to find that egg mass results are closely related to the records of egg number .The highest values were found in the hens fed T5, T4, and T3 diets followed by those fedT2 and T6 diets.

Egg quality: Data presented in Fig (2) show of the effect of the different dietary treatments on some egg quality traits. It is clearly evident that feeding birds on diets supplemented with different levels of EM and Zinc bactracin significantly affected on egg quality traits.This improvement may attribute to produce lactic acid which alters pH of chickens gut making it improper media for harmful bacteria such as *salmonella* and pathogenic species of *E.coli* [5], improve nutrient availability and absorption [6], produce specific antibacterial compounds such as hydrogenperoxide [7] and compete with other microbes for adhesive sites [8]. In addition, dietary supplementation of yeast during heat stress caused egg production than control as reported by [9].

Egg chemical analysis

Egg cholesterol: Egg yolk cholesterol was significantly decreased in chicks fed diets containing different levels of EM as compared with the control diet Fig (3). Generally, it could be seen that yolk cholesterol was decreased (P ≤0.001) by 32.3 % as the level of EM increased from 0 to 10 ml / kg in the diets. These results are in agreement with, [2] .This reduction may be explained as mentioned by [10] who attributed that to these bacteria and presume that, this type of bacteria convert feed cholesterol to coprostanol, which is absorbed poorly by gastrointestinal tract. However, some lactobacillus have a direct effect on cholesterol levels by assimilation and removed from the growth medium [11]. Also, similar trend was obtained by [12] with Gimmizah and Matourh strains and [13] who reported that a significant decrease in total cholesterol and insignificant decrease in total lipids at 6 weeks of age with adding lacto sacc and yea sacc in Japanese quail diet. Also, [14] found that the addition of lactobacillus acidophilus culture significantly reduced the levels of serum cholesterol.

Egg protein: Results obtained showed that egg protein percentage was significantly (p≤0.05) increased in birds fed diets with different levels of EM. The highest values of egg protein was observed in chicks fed T5 diet followed by T4 diet followed by T3 diet and also, T2 diet . While, the lowest values was found in chicks fed control or T6 diets.Statistical analysis of the results obtained proved that EM addition to chick diets significantly increased the egg protein more than Zinc bacitracin supplemention.

The increases in the previous parameters may indicate that an enhancement of immunity occurred corresponding to feeding either probiotics, prebiotics or both synbiotics as a result of improving feed conversion, absorption and utilization of nutrients. Similarly, [12] with Gimmizah and Mandarh strains and [13] with Japanese quail reported that addition of microbial probiotic caused higher level of plasma total protein as well as albumin and globulin fractions than those of control group it is lead to increase the protein in egg.

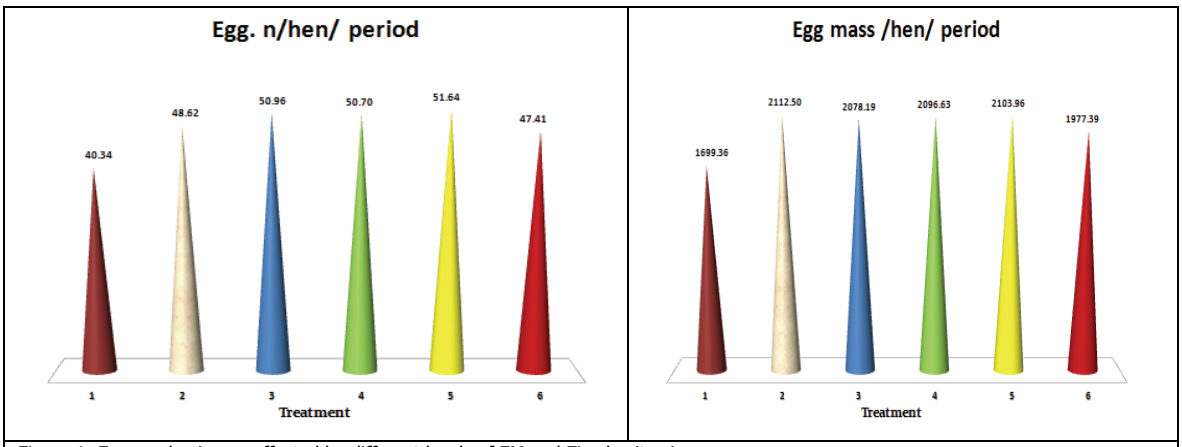
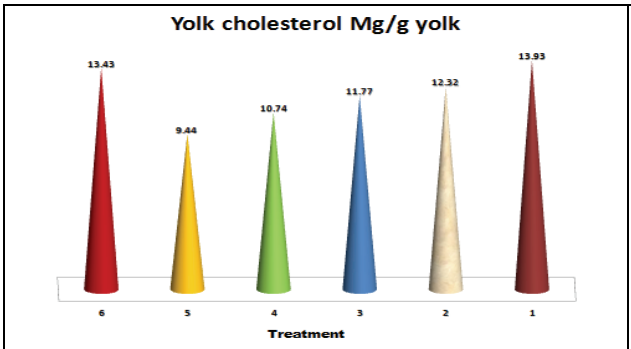


Figure 1: Egg production as affected by different levels of EM and Zinc bacitracin.



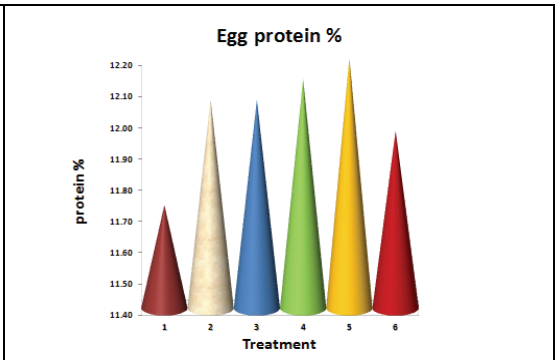
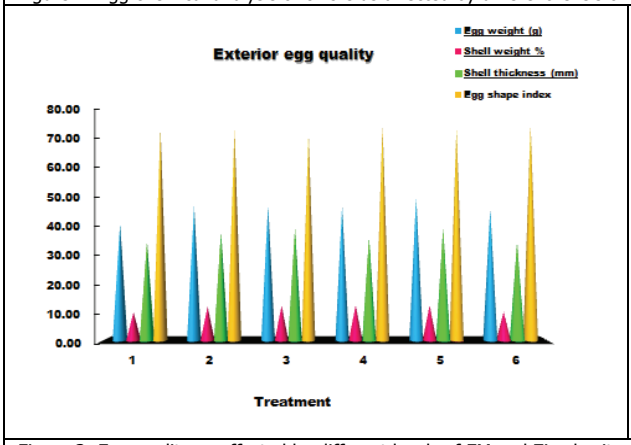


Figure 2: Egg chemical analysis of chicks as affected by different levels of EM and Zinc bacitracin.



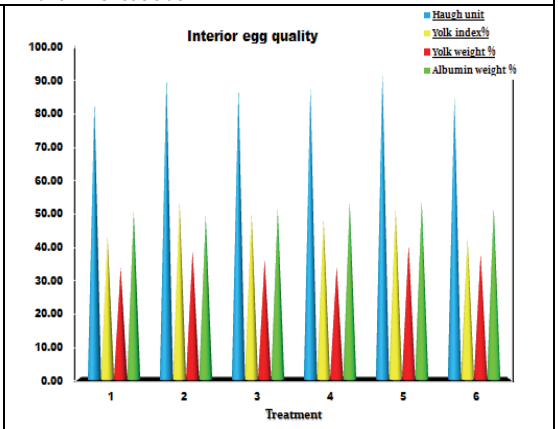


Figure 3: Egg quality as affected by different levels of EM and Zinc bacitracin.

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HISTOLOGICAL STUDY ON EFFECT OF NIGELLA SATIVA ON THE AGED OLFACTORY SYSTEM OF FEMALE ALBINO RAT (Abstract)

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ABSTRACT

Background: Nigella sativa (NS) (black seed), has an extraordinary and wide-ranging healing properties neuroprotective and antioxidant effects. Aging process is commonly associated with a decline in the chemical senses including smell.

Aim of the work: To detect a possible improving effect of NS on the histological aging changes of the olfactory system.

MATERIAL & METHODS

15 female albino rats were used and divided into 3 groups (5 animals each): Group I (control adult): 3 months old. Group II (control aged):18 months old. Group III (treated aged): 18 months old, received NS orally at a dose of 40mg/kg/day for 2 months. Specimens from the olfactory epithelium (OE), bulb (OB) and cortex (OCx) were processed for light and electron microscopy.

RESULTS

Aging in OE is associated with a reduction in thickness, loss of surface cellular processes, deep nuclear staining and vacuolations. NS treatment improved the OE thickness and nuclear staining and reduced vacuolations. Aged OB and OCx exhibited a reduction in size in mitral and pyramidal cells respectively, with dark staining of their nuclei and reduced cytoplasmic basophilia. NS treatment markedly increased the cytoplasmic basophilia in both mitral and pyramidal cells. The results are discussed in view of the light and electron microscopic results.

CONCLUSION

Use of NS, could be of value in improving the structural changes of the peripheral and central main olfactory organs which occurs in association with aging and results in deficit in the sense of smell.

EFFECT OF FEEDING DIETS CONTAINING AN ANTIBIOTIC, OR A PROBIOTIC ON GROWTH AND PATHOGENIC INTESTINAL BACTERIA IN DOMESTIC FOWLS

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Poultry Production Dept, Faculty of Agric, Kafr El-Sheikh Univ, Egypt.*

A 4-wks study was conducted to determine the effect of feeding diets containing an antibiotic (Zinc bactracin) , or a probiotic (Effective Microorganisms), on performance, and urease Pathogenic Intestinal Bacteria of Inshas chicks (a local Egyptian chicken strain) .The experimental design consisted of six experimental groups: control and 5 dietary treatments as follows; (T1) Basal diet (control), (T2) Basal diet + EM (2.5 ml/kg diet), (T3) Basal diet + EM (5.0 ml/kg diet), (T4) Basal diet + EM (7.5 ml/kg diet), (T5) Basal diet + EM (10.0 ml/kg diet) and (T6) Basal diet + Zinc bactracin (500 mg/kg). Feeding treatment was started at 4 wks of age and lasted at 41 wks of age. Characteristic investigations were including : Live body weight ; Body weight gain ; feed consumption ; efficiency of feed utilization and Bacteria Enumeration (Aerobic plate count (x106/g), *E. coli*, Salmonella, Staphylococci and Coccidia ovum). Feeding diets containing the probiotic were significantly ($P < 0.01$) increasing the average daily gain during the expermental period compared to the control . This increase was partially accounted with increased feed intake. During the expermental period, feeding the diet containing probiotic significantly reduce the counts of total viable bacteria ($P < 0.01$),, *E. coli*, salmonella, staphylococci and Coccidia ovum in caecum compared with untreated control diets.. Our study indicating that, dietary probiotic decreases pathogenic intestinal bacteria of chicks and this may be beneficial for improving animal health and growth performance.

INTRODUCTION

One way is to use specific feed additives or dietary raw materials to favorably affect animal performance and welfare, particularly through the modulation of the gut microbiota which plays a critical role in maintaining host health [1]. A balanced gut microbiota constitutes an efficient barrier against pathogen colonization, produces metabolic substrates (e.g. vitamins and short-chain fatty acids) and stimulates the immune system in a non-inflammatory manner.

In this context probiotics, prebiotics and synbiotics could be possible solutions. The main effects of these feed additives are the improved resistance to pathogenic bacteria colonization and enhanced host mucosa immunity; thus resulting in a reduced pathogen load, an improved health status of the animals [2] and a reduced risk of food-borne pathogens in foods.

The impact of biotechnology in poultry nutrition is of significant importance. Biotechnology plays a vital role in the poultry feed industry. Nutritionists are continually putting their efforts into producing better and more economical feed. Good feed alone will not serve the purpose but its better utilization is also essential. Dietary changes as well as lack of a healthy diet can influence the balance of the microflora in the gut thus predisposing to digestion upsets. A well-balanced ration sufficient in energy and nutrients is also of great importance in maintaining a healthy gut. A great deal of attention has recently been received from nutritionists and veterinary experts for proper utilization of nutrients and the use of probiotics for growth promotion of poultry.

Effective Micro-organisms (EM) is a microbial preparation developed by Professor T. Higa of University Of The Ryukyus in Japan. The EM is composed of different microbes that include bacteria, yeasts and/or fungi. Some of the benefits claimed to accrue from the use of EM include improved meat and manure quality, improved animal health, reduction of foul smells and absence of toxic effects on bird growth [3]. Use of EM in Africa is a new innovation and novel idea. There is no available literature regarding use of microbial preparations in broiler production. Therefore, this experiments was designed to investigate the possibility of using probiotic namely, (EM) effective microorganism (instead of using antibiotics) to Inshas chickens (Egyptian local strain) , and to evaluate its effects on growth and pathogenic intestinal bacteria .

MATERIAL AND METHODS

A total number of 540 unsexed vaccinated Inshas (local Egyptian chicken strain) one day-old-chicks were weighed , wing banded and randomly divided into six experimental groups (three replicates each group) . The birds were placed in a room (floor pens) maintained at a constant temperature of 28+/-3 oC and a relative humidity of 70+/-3% .Food and water were always available *ad libtum* . The basal diet was formulated to meet the nutrient needs suggested by the NRC, 1994 [13]. Body weight was determined individually to the nearest gram at four weeks intervals up to the 40 week of age. Feed intake was recorded every week by supplying a weighed amount of feed and subtracting the unconsumed portion from the total amount offered. The average feed intake of each bird was calculated by dividing the monthly consumed feed by the

number of individuals in each group during this month , considering the death if any.

At the time of slaughter test, 6 samples of ileum and caecum contents were collected and examined to define and count the pathogenic bacteria for each treatment. Fecal matter samples were collected in sterile polyethylene bags. All samples were delivered directly to the laboratory for bacterial count and definition using the procedure of [4]. The experimental design consisted of six dietary treatments as follows; (T1) Basal diet (control), (T2) Basal diet + EM (2.5 ml/kg diet), (T3) Basal diet + EM (5.0 ml/kg diet), (T4) Basal diet + EM (7.5 ml/kg diet), (T5) Basal diet + EM (10.0 ml/kg diet) and (T6) Basal diet + Zinc bactracin (500 mg/kg).. The results obtained were statistically analyzed using Duncan's Multiple Range Test {17}. Statements of statistical significance are based on $P<0.05$.

RESULTS AND DISCUSSION

Growth performance

Data presented in Fig (1) show the effect of the different dietary treatments on live body weight (LBW) at various ages.

Generally , it could be seen that the feed additives were significantly ($P \leq 0.001$) increased LBW in all treatments. The results indicated that body weight in all supplemented groups was increased ($P \leq 0.001$) by 14.4, 15, 16.9, 17.9 and 9.4% for T2, T3, T4, T5 and T6 diets,respectively, as compared with control group. Also, feed intake and feed conversion were improved by feed additives .These results may be explained the review that probiotics are natural control method that based on ensuring the bird has an adequate gut microflora counter pathogenic bacteria colonization in its digestive tract and consequently has healthy gut that results in good digestion and nutrient absorption [5].

Bacteria Enumeration

Results presented in Fig (2, 3, 4, 5 and 6) indicated that experimental diets caused severe suppression pathogenic intestinal bacteria counts. Where, there were significantly ($P<0.01$) reduction in counts of total viable bacteria, *E. coli*, salmonella, staphylococci and Coccidia ovum in caecum comparing with untreated control diet.The present results of EM dietary treatments agrees with that found by [6] who observed that frequency of Salmonella colonization was significantly reduced due to probiotic bacteria treatment. Also, [7] reported that lactobacillus was able to inhibit the growth of some pathogenic bacteria such as *E. coli* and salmonella. The antagonistic activity of lactic acid bacteria towards pathogens can be attributed to the production of bactericidal substances like bacteriocins, organic acids and hydrogen peroxide as reported by many workers for example [8]. The addition of probiotics product decreased the *E. coli* count as found by [9]. This type of bacteria produces lactic acid which alters the pH of chicken gut making it improper media for harmful bacteria such as salmonella and pathogenic species of *E. coli.* [10]. Probiotics decreased proliferation of pathogenic bacteria [11] concluded that probiotics enable the host animal to return to normal through increasing normal gut flora on the expense of pathogenic organisms. Furthermore, [12]reported that the beneficial effect of probiotics since their microbial constituents produce natural lactic acid that helps in maintaining an optimum low pH which inhibits growth of undesirable bacteria leading to optimum enzyme activity. Authors concluded that the antibacterial action produced by probiotics was probably due to a combination of factors which include organic acids (acetic and lactic acids), hydrogen peroxide and bacitracin.

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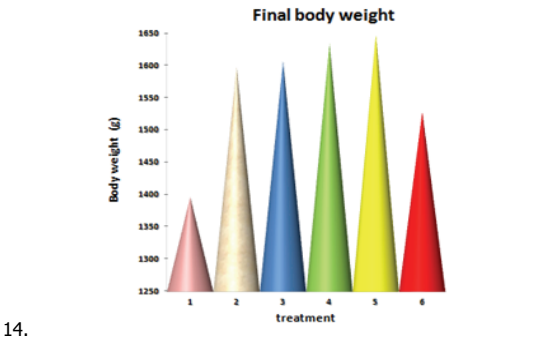

14.

Fig (1): Effect of different levels of EM and Zinc bactracin on body weight of birds .

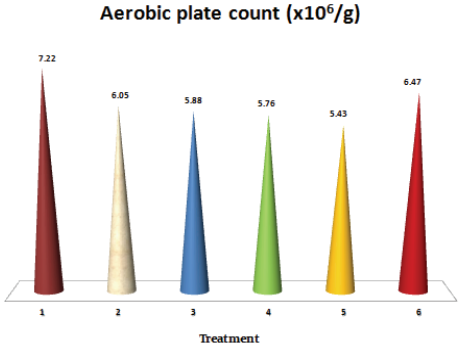

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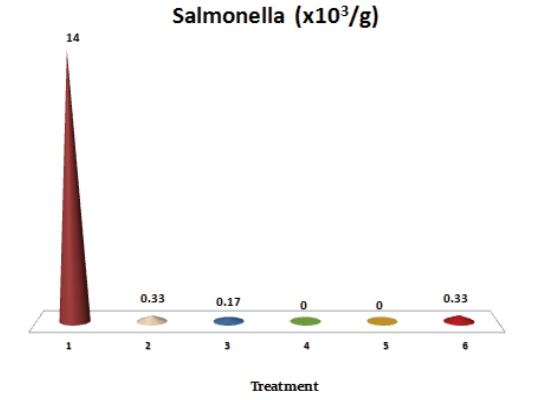
Fig (2): Effect of different levels of EM and Zinc bactracin on aerobic plate count of birds.



Fig(3):Effect of different levels of EM and Zinc bactracin on Salmonella bacteria of birds

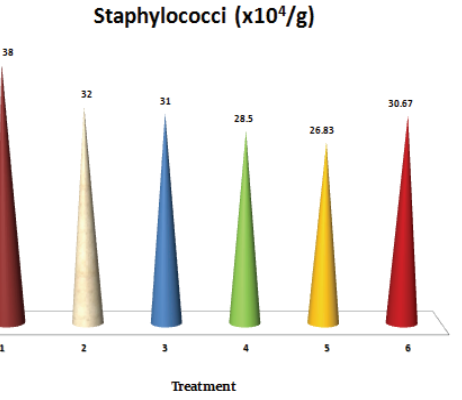


Fig (4): Effect of different levels of EM and Zinc bactracin on Staphylococci bacteria of birds.

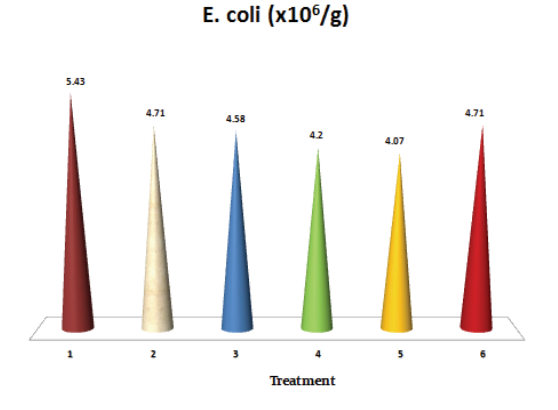


Fig (5): Effect of different levels of EM and Zinc bactracin on *E.coli* bacteria of birds.

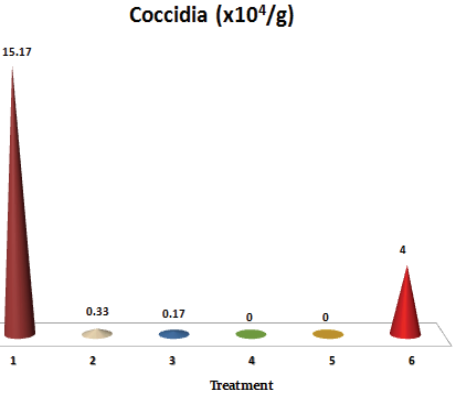


Fig (6): Effect of different levels of EM and Zinc bactracin on Coccida of birds

INVESTIGATING THE EFFECT OF PROBIOTICS ON CHICKS FERTILITY AND SEMEN QUALITY

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This study was conducted to investigate the effect of effective microorganisms (EM) probiotics on the Fertility, hatchability and Semen quality of Inshas chickens (a local Egyptian chicken strain). 540 chicks were randomly selected from 1 to 5 treatments: 1) no probiotics (control); 2))Basal diet + EM (2.5 ml/kg diet); T3) Basal diet + EM (5.0 ml/kg diet); T4) Basal diet + EM (7.5 ml/kg diet); T5) Basal diet + EM (10.0 ml/kg diet) and (T6) Basal diet + Zinc bactracin (500 mg/kg) . Feeding treatment was started at 4 wks of age and lasted at 41 wks of age . Characteristic investigations were including : Semen quality (Semen volume, Sperm motility %, Sperm cell concentration ($x10^6$/ml) and Sperm abnormal and dead); Fertility, hatchability and chick weights.
Obtained results could be summarized as following ;
1- All studied traits were affected by the feed additive treatments.
2- The studied feed additives showed significant beneficial effects almostly in all studied traits.
3- In many cases ,the most improving effect was obtained with Basal diet + EM (10.0 ml/kg diet).

INTRODUCTION

The first goal of the livestock production is the delivery of safe foods for human consumption taking into account the welfare of the animal and respect for the environment. An important field of zootechnical research is the improvement of the quality and safety of the meat. It is well recognized that pathogens, such as Campylobacter and Salmonella can be transmitted along the food chain and can be the source of human illness. In the past, antibiotics have been included in animal feed at sub-therapeutic levels, acting as growth promoters (Antibiotic Growth Promoters; AGPs).
However, worldwide concern about development of antimicrobial resistance and about transference of antibiotic resistance genes from animal to human microbiota [1] led to banning the use of antibiotics as growth promoters in the European Union since January 1, 2006 (EC 2001& 2003).
The removal of these compounds from animal diets has put tremendous pressure on the livestock and poultry farms, one of the main consequences being a substantial increase in the use of therapeutic antibiotics[2]. There is evidence that AGPs have long been effective in prevention of necrotic enteritis (NE) in poultry flocks and that the incidence of NE has increased in countries where AGPs have been stopped[3].
There is the need to look for viable alternatives that could enhance the natural defense mechanisms of animals and reduce the massive use of antibiotics [4].
Effective Micro-organisms (EM) is a microbial preparation developed by Professor T. Higa of University Of The Ryukyus in Japan. The EM is composed of different microbes that include bacteria, yeasts and/or fungi. Some of the benefits claimed to accrue from the use of EM include improved meat and manure quality, improved animal health, reduction of foul smells and absence of toxic effects on bird growth [5] . Increased egg production and egg weight and improvements in gross margins by up to 28.5 % have also been reported [5]. Use of EM in Africa is a new innovation and novel idea. There is no available literature regarding use of microbial preparations in broiler production.Therefore, this experiments was designed to investigate the possibility of using probiotic namely, (EM) effective microorganism (instead of using antibiotics) to Inshas chickens (Egyptian local strain) , and to evaluate its effects on chicks fertility and semen quality.

MATERIAL AND METHODS

A total number of 540 unsexed vaccinated Inshas (local Egyptian chicken strain) one day-old-chicks were weighed , wing banded and randomly divided into six experimental groups (three replicates each group) . The birds were placed in a room (floor pens) maintained at a constant temperature of 28+/-3 oC and a relative humidity of 70+/-3% .Food and water were always available *ad libtum* . The basal diet was formulated to meet the nutrient needs suggested by the [6] . Ejaculates were collected from individual males (9 birds / treatment) in the last week of the experiment using the method of [7].To determine semen ejaculate volume , sperm abnormality, sperm concentration , sperm viability and dead spermatozoa. Two fluorometric measurements from each male undiluted semen sample was obtained [8]. Fertility and hatchability were measured three times during the experimental period at 26, 27 and 28 wk of age . Eggs were collected for 7-day periods and were stored in a room at 18.5°C dry bulb and 70% relative humidity. They were incubated at 37.5°C and relative humidity was 60% and hatched at 37.2 °C and relative humidity was 70% in an automatic incubator . The removed eggs and eggs not

hatched in day 21 were broken to differentiate infertile eggs from those containing dead embryos.Fertility was calculated as the number of fertile eggs relative to total number of eggs set; meanwhile hatchability was calculated as the number of healthy hatched chicks relative to total fertile number of eggs.The experimental design consisted of six dietary treatments as follows; (T1) Basal diet (control), (T2) Basal diet + EM (2.5 ml/kg diet), (T3) Basal diet + EM (5.0 ml/kg diet), (T4) Basal diet + EM (7.5 ml/kg diet), (T5) Basal diet + EM (10.0 ml/kg diet) and (T6) Basal diet + Zinc bactracin (500 mg/kg). The results obtained were statistically analyzed using Duncan's Multiple Range Test {17}. Statements of statistical significance are based on P≤0.05.

RESULTS AND DISCUSSION

Semen quality

Results regarding semen quality in response to different dietary treatments investigated are shown in Fig (1, 2, 3, 4 and 5).

Semen volume

Semen volume was significantly increased by 29.1 and 21.9% in cocks fed T6 diet and other diets (T2, T3, T4 and T5), respectively as compared with those fed control diet. There are no significant differences among the chicks fed T2, T4, T5 and T6 diets.

Sperm motility %

Sperm motility % was increased (p≤0.01) from 79 to 91% as the level of EM increased from 0 to 10 ml/kg in cocks diets. Also, it improved (p≤0.01) by 17.7 % in cocks fed T6 diet as compared with those fed control diet.

Sperm cell concentration

Sperm concentration was increased (p≤0.01) by 15.2% in cocks fed T6 diet, while it was improved (p≤0.01) by 11.4% in those fed other diets (T2, T3, T4 and T5). There are no significant difference among dietary treatments.

Abnormal and dead sperms

Data presented in Table (13) showed the effect of different dietary treatments on abnormal and dead sperms. Abnormal and dead sperms were significantly decreased (p≤0.05& p≤0.01) in cocks fed EM and Zinc bacitracin diets as compared with those received control diet. Generally, semen quality of cocks fed Zinc bacitracin diet was improved more (p≤0.05 & p≤0.01 & p≤0.001) than in those kept on diets supplemented with different levels of EM. The present results were in agreement with data obtained by [9]. This improvement in semen quality may be due to improve utilization of protein and mineral absorption [9].

Fertility, hatchability and chick weight

Fig (4) shows the effects of different levels of EM and Zinc bactracin on fertility and hatchability percentages and chicken weight at hatch . The highest values of fertility and hatchability percentages were found for chicks fed diets with different levels of EM followed by those fed zinc bactracin diet. However, the lowest values were observed for chicks received control diet. This increase may be attributed to improved of semen quality of chicks. The same trend was obtained in chicken weight which increased (p≤0.001) by 6.5 to 9.7 in EM diets and incresed by 3.2 % in Zinc bactracin diets as compared with control diet.In a recent study, obligately homofermentative lactobacilli produced high antioxidant activity whereas this was highly strain dependent among facultatively and obligately heterofermentative lactobacilli [10].

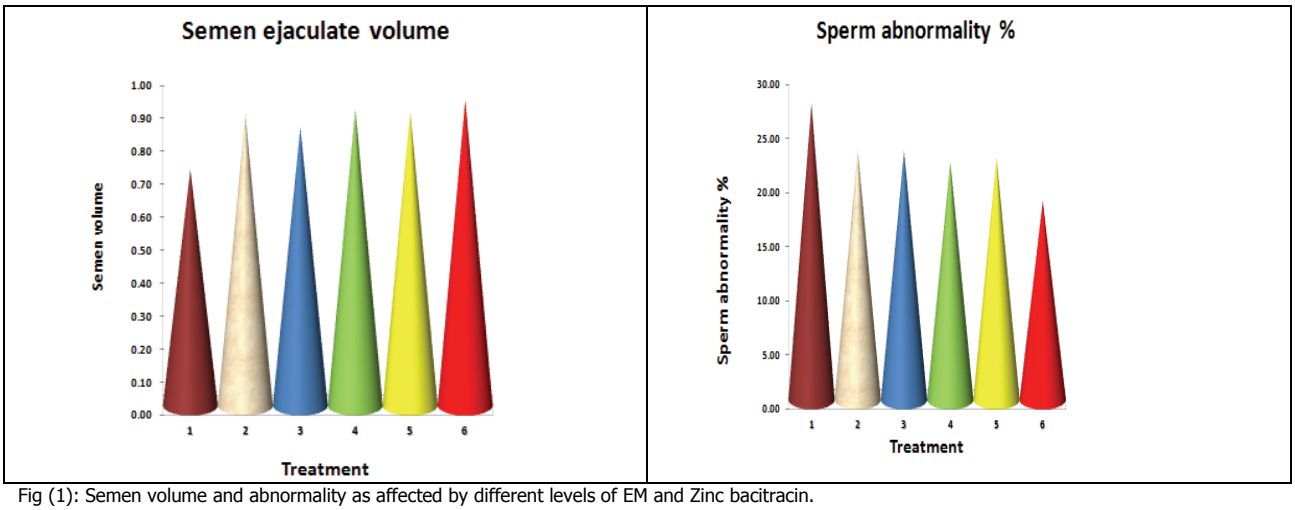

Fig (1): Semen volume and abnormality as affected by different levels of EM and Zinc bacitracin.

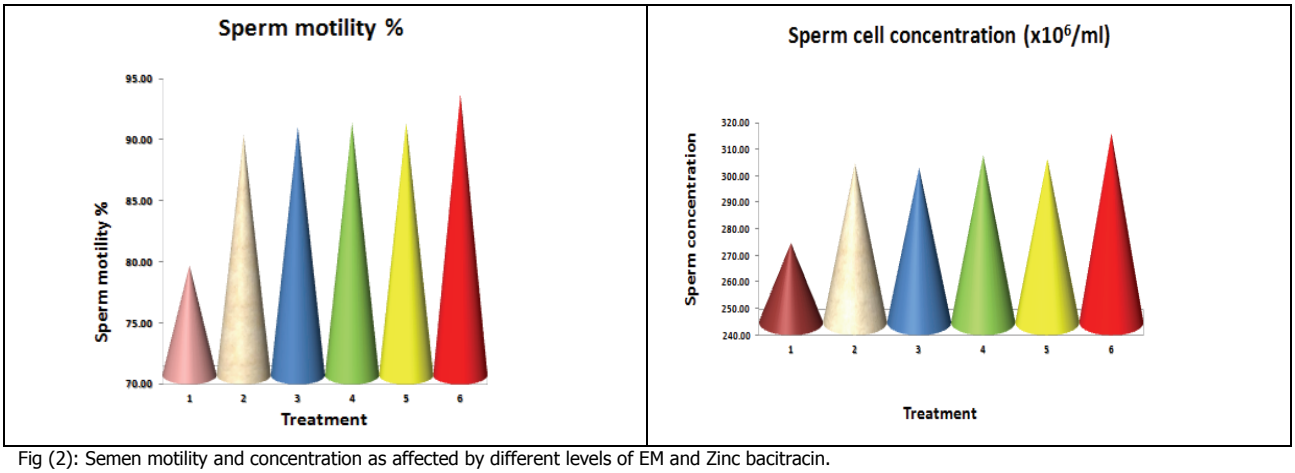

Fig (2): Semen motility and concentration as affected by different levels of EM and Zinc bacitracin.

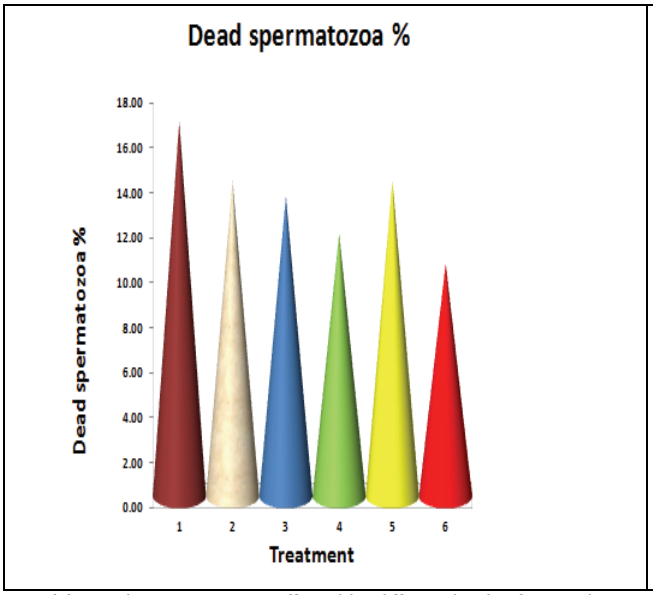

Fig (3): Dead spermatozoa as affected by different levels of EM and Zinc bacitracin.

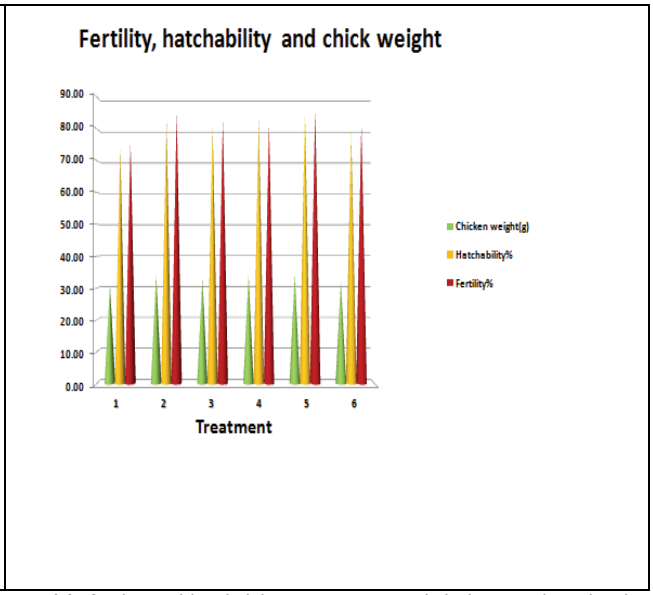

Fig (4): fertility and hatchability percentages and chicken weight at hatch as affected by different levels of EM and Zinc bacitracin.

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EFFECT OF PROBIOTIC ON GUT DEVELOPMENT OF DOMESTIC FOWLS (Abstract)

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This experiment was conducted to study the effects of effective microorganisms (EM) and Zinc bacitracin on gut development, digestibility coefficient of nutrients and intestinal histology of Inshas chicken (a local Egyptian chicken strain) . Five hundredand fourty chicks were randomly assigned to 1 to 6 dietary treatments for 41 wk. The dietary treatments were 1) control; 2) Basal diet + EM (2.5 ml/kg diet); T3) Basal diet + EM (5.0 ml/kg diet); T4) Basal diet + EM (7.5 ml/kg diet); T5) Basal diet + EM (10.0 ml/kg diet) and T6) Basal diet + Zinc bactracin (500 mg/kg). The obtained results showed that, villi height , villi thickness and villi surface area were significantly increased in birds fed EM with different levels and Zinc bactracin diets . The data on the digestibility coefficient of nutrients revealed that, all nutrients of EM diets were more efficiently digested than that of Zinc bactracin diet ($p \leq 0.01$) . While, digestibility coefficient of OM, DM, CP, EE, CF and NFE was significantly increased as compared with chicks kept on the control diet. Moreover, It was generally noticed that intestinal histology was almost following the same trend observed with gut development and digestibility coefficient .

THE EFFECT OF MYCOFIX® SUPPLEMENTATION ON THE REDUCTION OF LYMPHOCYTE DNA DAMAGE INDUCED BY DEOXYNIVALENOL IN BROILERS

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SUMMARY

Deoxynivalenol (DON) is one of the most abundant and important trichothecenes in food and feed, and is a significant contaminant due to its frequent occurrence in toxicologically relevant concentrations worldwide. The exposure to this toxin is a permanent health risk for both cereals eating humans and grains fed farm animals. As a pro-oxidative substance DON has negative influences on health and performance of chicks. However, there is a little information available regarding the effect of DON on DNA fragmentation in blood lymphocytes. In addition, the effects of the Mycofix® Plus (Biomin GTI GmbH, Herzogenburg, Austria) supplementation to deoxynivalenol contaminated broiler diets on lymphocytes DNA have not yet been demonstrated. Therefore, the aim of the present study was to evaluate the effects of Mycofix® Plus supplementation on the lymphocyte DNA damage induced by DON in chickens. Thirty two 1-d-old broiler chicks were randomly divided into four groups. The control group was fed non-contaminated diet and a second group supplemented with Mycofix® Plus. Another group of broilers was fed a diet artificially contaminated with 10 mg feed grade DON/kg diet, whereas another group was fed the DON-contaminated diet supplemented with Mycofix® Plus. The diets were provided *ad libitum* for 5 wk. At the end of the low protein feeding trial in the grower period, blood was collected and the degree of lymphocyte DNA damage was measured in plasma by Comet assay. Production parameters of DON group were significantly impaired in comparison to the control. DON significantly increased the amount of DNA damage in chicken lymphocytes by 46.8%. To our knowledge, these are the first data on genotoxic effects of moderate dose of DON on chicken lymphocytes. However, Mycofix® Plus supplementation significantly reduced the percentage of DNA damage in the tail in DON with Mycofix® Plus treated group. These results demonstrated that the diets contaminated with the mycotoxin DON at moderate levels in combination with low protein feed are already able to induce lymphocyte DNA damage in chickens. Supplementation with Mycofix® Plus protected lymphocyte DNA from toxin impact and it can be beneficial for remaining the lymphocyte DNA integrity.

INTRODUCTION

The toxic effects of *Fusarium* mycotoxins in animals and poultry include reduced growth, feed refusal and vomiting, immunosuppression, gastrointestinal lesions, and neurological and reproductive disorders [1].

DON-induced oxidative stress and mitogen activated protein kinases (MAPKs) activation leads to a phenomenon known as ribotoxic stress response, where DON binds to ribosomes and inhibits protein synthesis [2]. At dosages that partially inhibit translation (100 – 250 ng/ml), expression of proinflammatory genes such as cyclo-oxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) as well as cytokines such as interleukin – 6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNFα) is elicited [3-6]. At higher dosages, (500-1000 ng/ml and above), DON induces apoptosis, leading to immune suppression [7-8].

Adverse effects of mycotoxins on cells are also associated with the increased production of free radicals and reactive oxygen species resulting in oxidative damage of target tissues [9]. There is a very delicate balance between antioxidants and pro-oxidants in the body of young chickens. Nutritional stress factors can act as breakers of the antioxidant/pro-oxidant balance [10]. Additionally, many authors have suggested that DON and T-2 toxin-induced free radical production may be one of the mechanisms causing membrane and DNA damage, thus oxidative stress being an important factor in their toxicity [11-15].

Moreover, it was shown that DON caused DNA fragmentation in chicken spleen leukocytes [16]. Accordingly, the aim of the present research was to study the effect of DON on lymphocyte DNA fragmentation in broilers, and furthermore, to evaluate the potential of Mycofix® Plus in prevention of toxin mediated changes in low protein grower period.

MATERIAL AND METHODS

Experimental Birds and Housing

Thirty two-1d-old broilers of a commercial strain (Ross 308), procured from a commercial hatchery, were randomly divided into four groups, each comprising 8 birds. The birds were housed in four wire-bottomed pens fitted with electrical heaters during the 35 days of experimental period. The temperature started at 33 °C (from day 0 to day 3) and was gradually reduced according to normal management practice (2–3 °C/week). During the first 2 weeks, chicks were provided with 24 h of light, after which lighting was decreased gradually (2 h daily) to 20 h by the third week and remained there until the end of the trial. The permission for this experiment was granted by the Federal Ministry for Education, Science and Culture, Vienna, Austria vide letter No. BMWF-68.205/0032-II/10b/2010.

Treatment and Diets

The control group was fed starter and grower diets based on corn, soya HP, fat and a premix with vitamins, minerals, amino acids, salt, and monocalcium phosphate. Birds of each group were fed with one of the following dietary treatments;1) basal diet, 2) diet contaminated with 10 mg feed grade DON/kg feed, 3) diet contaminated with 10 mg feed grade DON/kg feed and supplemented with Mycofix plus (2.5 kg/ton of diet), 4) diet supplemented with Mycofix plus (2.5kg/ton of diet). Chicks were fed the normal starter diets from d 1 to 13 and the low protein grower diets from d 14 to 35. Feed and water were offered ad libitum. Representative feed samples were taken at the beginning of the starter and grower periods and were analyzed for nutrient content and for DON.

Traits

Chicks were weighed individually, and feed consumption for each pen was measured weekly during the 5-wk experiment. Body weight (BW) gain and feed conversion were determined, whereas weekly and cumulative feed:gain ratios were calculated. At the end of feeding trial, blood was collected and the degree of lymphocyte DNA damage in plasma was measured by Comet assay.

Lymphocyte nuclear DNA damage- Comet assay

Comet assay for cytotoxicity testing was performed according to Steenkamp et al. [17] with little modifications: An appropriate cell suspension (40 µl) of isolated lymphocytes was diluted in 400µl 0.5 % low melting point argarose (LMPA) and 100µl were pipetted on glass slides precoated with 1% high-melting point agarose. After lysis (performed in pre-chilled lysis solution) slides were placed in a Comet assay tank® (Trevigen Inc.) and incubated in pre-chilled electrophoresis buffer. Electrophoresis was performed at 25 V, 300 mA for 20 min in the comet assay tank (chilled with ice). Afterwards slides were washed in neutralization buffer and dH2O, fixed in 80 % ethanol and stained according to the instructions of the Comet assay silver staining kit® (Trevigen Inc.). Three slides were prepared for each sample. Fifty randomly chosen cells were scored per slide (total 150 cells). Comet parameters were analyzed by CometScore® (TriTek Corp).

Statistical Analysis

Statistical program SPSS (version 17; SPSS GmbH, SPSS Inc., Munich, Germany) was used for data analysis. The Kolmogorov Smirnov test was used to test the normal distribution of the data. Analysis of variance (ANOVA) was performed between the four groups followed by Duncan test to find the significance between dietary treatments. The probability values of 0.05 ($p \leq 0.05$) were considered significant.

RESULTS

Body weight gain and live weight gain

One of the main adverse effects observed after DON exposure is reduced feed intake and body weight during the low protein grower phase (Table 1). In the present experiment, feed refusal was observed during the period of week 3 to week 5. Moreover, artificial contamination of the feed with 10 mg/kg of feed grade DON also decreased the BW gain. Contamination of broiler diet with 10 mg DON/ kg diet reduced the feed efficiency by increasing the feed conversion rate (feed to gain ratio) at 3, 4 and 5 wk compared to controls.

Table 1: Effect of dietary *Fusarium* mycotoxin deoxynivalenol on performane of broiler chickens

Parameters	Dietary treatment					P
	Control	DON (10 mg/ kg)	DON Plus Mycofix	Mycofix (2.5 kg/ ton)	SEM	
Initial body weight	47.38	50.50	49.63	49.63	1.69	NS
Weight at day 35	1205.75[a]	996.25[b]	1224.88[a]	1293.38[a]	29.71	0.001
Cumulative body weight gain	1158.38[a]	945.75[b]	1175,25[a]	1242,75[a]	29.75	0.001

Means within the same row with different superscripts are significantly different (One way ANOVA followed by Duncan test, n = 8/treatment).

Lymphocyte DNA damage

DON significantly increased the amount of DNA damage in chicken lymphocytes presented as the proportion of DNA in the tail of the comet. However, Mycofix® Plus supplementation significantly reduced the percentage of DNA damage in the tail in DON treated group (Table 2).

Table 2. Lymphocyte DNA damage of broilers treated with DON or/and Mycofix

Parameter	Treatments				SEM	P
	Control	DON (10 mg/ kg)	DON Plus Mycofix	Mycofix (2.5 kg/ ton)		
% of DNA in the tail	27.74[b]	40.37[a]	24.72[b]	26.72[b]	2.09	0.016

Means within the same row with different superscripts are significantly different (One way ANOVA followed by Duncan test, n = 6/treatment)

DISCUSSION

In the present study, diets contaminated with 10 mg of feed grade DON per kg feed significantly influenced the production parameters of chickens. Reduced daily weight gain, lower body mass at the end of the experiment and increased feed to gain ratio are characteristics of DON intoxication. However, most experimental studies [18-24] with poultry show a highly variable effect of DON on performance indicating that zootechnical traits might not be a sensitive indicator of toxicity of this *Fusarium* toxin. This finding may be ascribed to the inhibition of protein synthesis by this toxin. We could hypothesize that when the birds are fed with low protein diets, the toxic effect of DON will be visible.

Lipid peroxidation may be one of the underlying mechanisms by which DON cause cell injury, DNA damage and apoptosis [11, 12, and 25]. The present study indicates that DON induced DNA damage in chicken lymphocytes measured by the comet assay and represented as a proportion of DNA in the tail of the comet. This finding is in agreement with the study of Frankic et al. [16] who found that DON caused DNA damage in spleen leukocytes when added at a concentration of 10 mg/kg feed.

In this experiment we have investigated the effect of DON on TBA-reactive substances (TBARS) in liver and we found no significant difference between groups (data not shown). The results of our study are not in full accordance with previous findings concerning the connection between DNA damage and lipid peroxidation. Since there were no prominent changes in other markers of oxidative stress (TBARS), we assume that DNA damage was mostly induced by direct action of DON, or through different epigenetic mechanisms such as formation of DNA adducts, rather than through free radical formation. On the other hand, it could also be possible that the comet assay is more sensitive than other methods used for evaluation of low levels of oxidative stress [26].

Mycofix® Plus, is a feed additive, used in order to cope with the more non-polar mycotoxins DON or zearalenone (ZON) [27]. The effects of the addition of Mycofix® Plus to DON contaminated low protein broiler diets have not yet been demonstrated. The results of the present study demonstrated that supplementation of contaminated diets with Mycofix® Plus protected lymphocyte DNA from toxin impact and it can be beneficial for remaining the lymphocyte DNA integrity.

CONCLUSIONS

The current study provided evidence that DON is genotoxic to chicken lymphocytes at concentrations of 10 mg/kg low protein feed. It could be concluded that oxidative pathways may be only of minor importance in mycotoxin-induced DNA fragmentation. However, Mycofix® Plus supplementation in low protein diet reduced the risk of DNA damage in chicken immune cells due to the action of DON.

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EFFECT OF DIETARY PROBIOTIC ON IMMUNE RESPONSE OF BROILERS TO B1 STARIN OF NEWCASTLE VIRUS

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SUMMARY

We investigated the effect of two commercially-available probiotics based on *Enterococcus faecium* and *Bifidobacterium* spp. on serological response of broilers against Newcastle Diseases Virus (NDV) vaccine. For this one-day-old, 156, Ross chicks were randomly allocated to three groups of 52 birds each. Chicks in group 1 received control mash diet during the experiment, but those in groups 2 and 3 were fed on control diet supplemented with *Enterococcus faecium* and *Bifidobacterium* spp. based on their instruction, respectively. On 9th day of age all groups were vaccinated by eye-drop with B1 strain and killed oil emulsion vaccine subcutaneously (Razi, Iran), simultaneously. Twenty blood samples were taken from each group on days 12 and 33 after vaccination. The sera were assayed for antibody against NDV by HI test.Results showed that at second bleeding, antibody titers of all groups were significantly higher than first sampling (p<0.05). At first and also second bleedings the antibody levels in groups 2 and 3 were significantly higher than first group (p<0.05), but there was no significant difference between groups 2 and 3 (p>0.05). It is concluded that diet supplemented with probiotics based on *Enterococcus faecium* or *Bifidobacterium* spp. have potential to enhance the humoral immune response of broiler chicks against NDV vaccine.

Keywords: *Bifidobacterium* spp., *Enterococcus faecium*, Humoral, Immunomodulator, Broiler chiks

INTRODUCTION

Current researches of probiotic focus on the capability of the products to increase immune system and immune response mechanism which is thought to play an important role to protect the body from any kind of pathogen infection. Studies on the effect of probiotics on the immune system involved the usage of experimental animal, especially poultry [2, 3, 5, 7, 8] because of their health importance due to their important role as a main source of human protein. It has been reported that probiotics are able to migrate from the gut to the systemic circulation. They could translocate and survive for many days in the spleen, liver and lungs. Their cell walls might have a co-stimulatory role on the induction of the systemic immune response [3, 12]. Oral administration of *Lactobacillus casei* has been reported to enhance activity of splenic NK cells and to stimulate phagocytic activity [11]. It has been reported that lactobacilli also have the effect of increasing serum anti-*E.coli* IgM levels in rodents and the production of autocoids by probiotic bacteria might have a pronounced influence on the induction of immunity, although the underlying mechanisms by which that occurs are largely unclear [6].

Due to the important roles of probiotics on impronement of immunological response, the current research work was conducted to verify and compare the stimulatory effects of two commercially available probiotics based on *Enterococcus faecium* and *Bifidobacterium* genera on humoral immunity of broiler chicks' sera.

MATERIAL AND METHODS

Chickens and diets

A total of one hundred and fifty-six (156) day-old broiler chicks (Ross 308) which consists of male sex were obtained from a commercial hatchery with a good reputation of producing disease free chicks and randomly divided into 3 groups (A, B, and C) of 52 birds each. The birds in group A received control mash diet during the experiment, but those in groups B and C were fed control diet supplemented with two commercial probiotics based on *E. faecium* and *Bifidobacterium* genera in average amount of 4×10^9 cfu kg^{-1} from first day to the end of the experiment, respectively. The control diet was a typical corn-soybean diet that was formulated to meet broiler nutrient requirements for starter (1 to 14 days), grower (15 to 28 days), and finisher (29 to 42 days) growth periods (Table 1) [10]. The basal diet was prepared every 2 wk and was stored in sacks in a cool place. Each dietary treatment had four replicate pens with 13 chicks per pen and the pens were randomized with respect to the dietary treatments. On day 9 of age, chicks in both groups were vaccinated against NDV with B1 via eye drop and a killed oil emulsion vaccine subcutaneously. All chicks were

reared under sanitary conditions and feed and water were provided *ad libitum*.

Blood sampling

On 21st and 42nd days of age, twenty chicks from each group were bled via brachial vein puncture using sterilized needle. After collecting blood from chicks, they were marked with leg bands, so that they were not reused for blood collection. The separated sera by centrifugation (1000 rpm, 5 min) were stored at -40°C until the end of the experiment.

Test of humoral immune response

Antibodies to Newcastle Disease (ND) antigen in blood sera were measured by haemagglutination inhibition (HI) test as described by Brown *et al.* [1]. ND antigen was supplied from the Razi Institute of Vaccine Production, Karaj, Iran

Statistical analysis

The obtained data were submitted to paired t-test in the case of evaluating the effect of age on antibody response of each group and also one-way ANOVA for investigation of possible statistical differences among three groups on each age using the Sigma State software (version 2.03, Systat software Inc., Point Richmond, CA, USA). Values were compared using Tukey's post hoc test when they passed the normality test and Dun's post hoc test in case of failure to pass normality. The significance of differences between mean values was set at $p<0.05$.

RESULTS AND DISCUSSION

The results of Table 2 show that in all groups, the serum antibody titers against NDV increased with a significant change from first to second sampling ($p<0.05$). At first and second bleeding, the antibody titers of B and C groups were significantly higher than group A ($p<0.05$), whereas there was no difference among groups B and C ($p>0.05$). In this work, we aimed to get a picture of the humoral immune status of broilers fed probiotic via the determination of serum antibody titers against NDV. Hence to the significant differences among probiotic treatments and control group, it could be concluded that probiotic inclusion based on *Enterococcus faecium* and *Bifidobacterium* genera had noticeable effect on systemic humoral immune status of the broilers. These findings might seem to be in accordance with the results reported by other studies, whereby probiotics resulted in an enhancement of broiler humoral immune response [4, 9]. In the studies above, though, the enhancement of the humoral immune response by probiotics was measured against specific model antigens and could therefore be regarded as an improved capacity of the humoral immune system of birds to cope with foreign antigens. The findings of our study implied that proper difference among serum antibody titers of groups fed *Enterococcus faecium* and *Bifidobacterium* genera did not occur.

CONCLUSSION

As a conclusion, evidence from this study showed the effectiveness of probiotic supplementations based on *Enterococcus faecium* and *Bifidobacterium* genera on humoral immunity against NDV. However, further work might be considered to focus on planning more comprehensive experimental designs examining mucosal cellular and humoral immunities and strain-host interactions.

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Table 1. Composition of base mash for experimental diet[1]

		Control diet	
Item	1 to 14 d	15 to 28 d	29 to 42 d
Ingredients (%)			
Corn	60.12	63.27	66.82
Soybean meal (45%)	26.76	24.81	23.81
Fish meal	7.11	5.00	2.21
Vegetable fat	3.00	4.00	4.00
Limestone	1.59	1.28	1.23
Di-calcium phosphate	0.68	0.90	1.18
Mineral and vitamin premix[2]	0.40	0.40	0.40
Salt	0.24	0.25	0.27
DL-Met.	0.10	0.07	0.07
L-Lys.	0.00	0.014	0.006
Calculated analysis (per kg of diet)			
ME (MJ)	12.4	12.7	12.7
CP (g)	220.0	200.0	180.0
Fat (g)	61.7	70.30	68.7
Met. and Cys. (g)	9.5	8.5	7.7
L-Lys. (g)	13.7	12.0	10.0

[1]Basal diets contained salinomycin Na as a coccidiosat at 60 mg/kg of feed.
[2]The mineral and vitamin premix provided the following per kilogram of diet: vitamin A 12000 IU; vitamin D3 4000 IU; vitamin E 75 mg; Menadione (vitamin K3) 9 mg; Thiamine 3 mg; Riboflavin 7 mg; Pyridoxine 6 mg; Cyanocobalamin 35 µg; Nicotinic acid 40 mg; Pantothenic acid 15 mg; Folic acid 1.5 mg; Biotin 135 µg; Ascorbic acid 100 mg; Choline chloride 400 mg; Cobalt 250 µg; Iodine 1.5 mg; Selenium 200 µg; Iron 50 mg; Magnesium 150 mg; Copper 15 mg; and Zinc 70 mg.

Table 2. Effect of probiotic supplementation on serum antibody titer[1] against Newcastle disease virus based on HI test in broiler chickens

	Treatment groups		
Days of age	A	B	C
21	5 ± 0.39^{aA}	6.1 ± 0.21^{bA}	6.05 ± 0.61^{bA}
42	5.92 ± 0.4^{aB}	6.75 ± 0.51^{bB}	6.92 ± 0.15^{bB}

[1]Values represent means ± SE for each treatment; n = 20.
[a-b] Means in rows lacking a common superscript are significantly different (P<0.05).
[A-B] Means in columns lacking a common superscript are significantly different (P<0.05).
Group A= Control treatment; Group B= Treated with *Enterococcus faecium*; Group C= Treated with *Bifidobacterium* genera

SOME CLINICOPATHOLOGICAL STUDIES ON THE EFFECT OF GARLIC AND LEVAMISOLE ON ALBINO RATS EITHER WITH INTACT OR DAMAGED LIVER

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ABSTRACT

This study aimed to investigate the hepato-protective effects of garlic and levamisole on blood pictures, liver functions of CCL_4 treated albino rats. This could be accomplished by clinicopathological and histo pathological means of investigations. **Methods** The experimental design consisted of three experimental groups: control and 2 treatments as follows ; (gr1) represented as control , (gr2) given garlic at dose 54 mg/kg b.wt. daily for 4 weeks orally by stomach tube. , (gr3) Given levamisole at dose 2.5 mg/kg b.wt. 3 times/week orally by stomach tube for 4 weeks , (gr4) Given a combination of garlic and levamisole , (gr5) Given CCL_4 S/C injection at dose 0.2 ml/kg b.wt. 3 times/week for 4 weeks, (gr6) Given a combination of CCL_4 and garlic , (gr7) Given a combination of CCl_4 and levamisole, (gr8) Given a combination of CCL_4, garlic and levamisole. At the end of 4th weeks FBE, blood and serum samples were collected for hematological and biochemical studies then, the animals were sacrificed and specimens from liver were collected. **Results** represented that administration of garlic and levamisole can ameliorate the toxic effects of CCL_4 as well as induced marked improvement in hematological and biochemical parameters and diminishing the severity of hepatic lesions in the affected rat's liver. **Conclusion,** These findings suggest that garlic and levamisle have a synergistic effect, and hepatoprotective agents against hepatotoxicity.

Keywords: CCL_4,garlic,levamisole,albino rats, liver

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INTRODUCTION

During the last few years, the International Pharmaceutical Companies began to manufacture drugs from naturally occurring herbal plants (medicinal plants). Allium sativum (Garlic) has become increasingly popular in recent years, not only because of its palatable flavour but also because of its medicinal properties(1)

Levamisole is the levoisomer of tetramisole, which was reported as an immunopotentiating agent in addition to antiparasitic and immune modulating effect. A significant cytoprotective effect of levamisole has been demonstrated (2)

Carbon tetrachloride (CCl_4) has been used as a liquid solvent, fumigant, cleaning agent, and starting material or intermediate for numerous chemical processes**(**3**).**Exposure to CCl_4 can lead to damage of a number of organ systems. Although the primary organ for CCl_4-induced toxicity in most animal species is the liver **(**4**)**

MATERIALS AND METHODS

Animals

120 clinically healthy male albino rats (two months old) (100-180 gm bwt) with average 150 gm. They were purchased from laboratory animals center, Faculty of Vet .Med, Alexandria University.

Chemical

Garlic (Tomax): in the form of tablets each tablet contain 200 mg of garlic powder ,obtained from Atos pharmaceuticals Co., Cairo, Egypt.

Levamisole: in the form of levamisole hydrochloride 7.5 gm solvent 100 ml, obtained from El-Nasr pharmaceutical, chemicals Co. Egypt .

Carbon tetra chloride (CCL_4): obtained from El-Nasr Pharmaceutical Chemicals Co. Egypt.

Experimental design

The total 120 rats were divided into 8 equal groups. Five rats from each group selected and sacrificed for collecting blood samples at 4th weeks (FBE), tissue specimens were also collected at the time of sacrification for histopathological examination.

Blood sampling, haemological &biochemical and histopathological studies

At the end of 4th weeks from FBE, two separate blood samples were collected from the retro orbital venus plexus from 5 rats from each group of animals. One sample was with anticoagulant for hematological examination encluding((RBCs)was performed according (5). Haemoglobin estimation (Hb) was performed according to(6). (PCV) was estimated according to(7) and the second sample was for serum separation for estimation of serum chemistry. Blood films were stained by Giemsa stain and the differential leucocytic count were performed and then the absolute counts were calculated according to(8).Biochemical studies included determination of the activities of Alanine amino transferase (ALT), Asparatate amino transferase (AST), alkaline phosphatase (AP), serum total protein, albumin,globulin and A/G ratio. Specimens from the liver, of all tested and control animals were collected then directly fixed in 10% neutral formalin and embedded in paraffin. Sections of 5µn thickness, stained by H&E)according to method of (9).

Statistical analysis

The results were analyzed using t-test according to (10)and MINITAB statistically Software, Copyright 1992, release 8, MINITAB Inc. Analysis of Variance (ANOVA)

RESULTS AND DISCUSSION

The picture of enthrone in rats received CCl_4 after 4 weeks illustrated significant decrease in RBCs count, HB concentration and PCV in(gr5). It was easy to notice the definite improvement in the level of RBCs count, HB concentration and PCV in rats of (gr8) as in (fig1).Garlic is known to be the richest source of sulfur compounds among all vegetable that has the ability to conjugate the toxic chemicals with glutathione and cysteine-S(11). The leukogram in rats received CCl_4 alone showed leukocytosis with lymphopenia, neutrophilia and monocytosis which reflect a picture of stress due to the effect of CCl_4 injection, while the leukogram in rats received garlic and levamisole revealed an increase in total leucocytic count, lymphocytic count, neutrophil and monocyte. This increases may be attributed to the immunostimulatory effect of garlic (12) as in(fig2). The proteinogram in(gr5) showed decreased total protein, albumin, in albumin/globulin ratio. The hypoalbuminaemia may be attributed to destructive effect of CCl_4 in the liver and it reflected the ability of liver which is the main source of albumin synthesis in the body(13). The proteinogram of(gp 8) when compared with (gr5) revealed improvement as increase in total serum proteins, globulin and ,albumin levels as in(fig 3).These results agree with that of(13). Serum aminotransferase activities have long been considered as sensitive indicators of hepatic injury **(**14**).** Therefore, the marked release of AST and ALT into the circulation indicates severe damage to hepatic tissue membranes during CCl_4 intoxication .It is easy to notice that CCl_4 clearly affect the liver and its enzymes as in (fig 4) (15). These results were confirmed by our histopathological finding in the liver which showed coagulative necroses particularly centrolobular and in the periportal zone also sever cloudy swelling, vascular and hydropic degeneration and fatty change were seen in the hepatic cell as in (fig 5). CCl_4 induces its effects through the oxidation of the unsaturated fatty acids in the cell membrane (lipid peroxidation) by trichoromethyl free radicals (CCl_3) originating in the hepatocytes via bioactivation of CCl_4 with enzymes cytochrome P450. This picture was improved in (gr8).The cytoprotective mechanism of garlic may be through its antioxidant power that prevents lipid peroxidation (16) or through inhibition of cytochrome P-450 and subsequently CCl_4 does not biotransformed into its toxic form namely CCl_3 (17).

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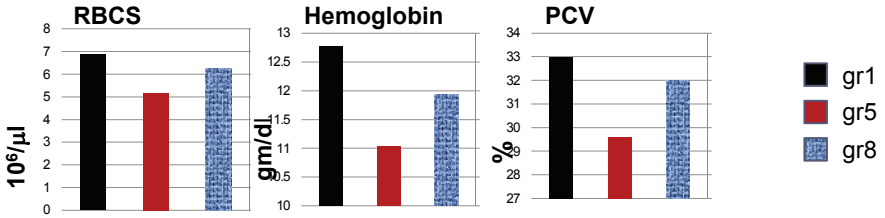

Fig 1: showed erythrogram of normal rat (gr_1),CCL_4 treated rats (gr_5) and rat received garlic & levamisole (gr_8)

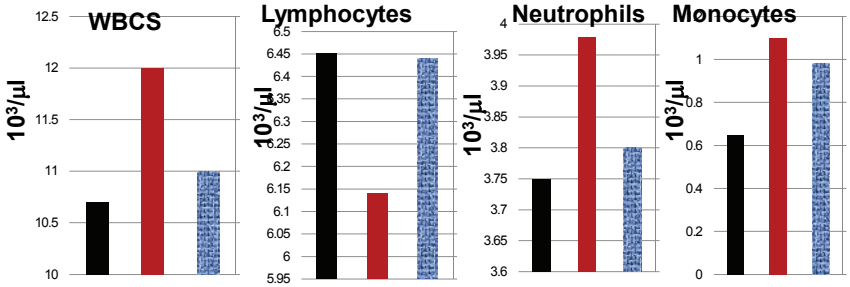

Fig 2: showed leukogram of normal rat (gr_1),CCL_4 treated rats (gr_5) and rat received garlic & levamisole (gr_8)

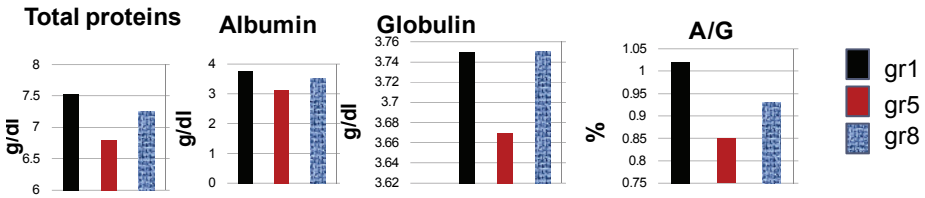

Fig 3: showed ptoteinogram of normal rat (gr_1),CCL_4 treated rats (gr_5) and rat received garlic & levamisole (gr_8)

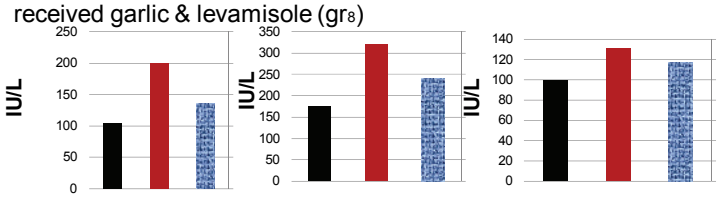

Fig4: showed serum enzyme of normal rat (gr_1),CCL_4 treated rats (gr_5) and rat received garlic & levamisole (gr_8)

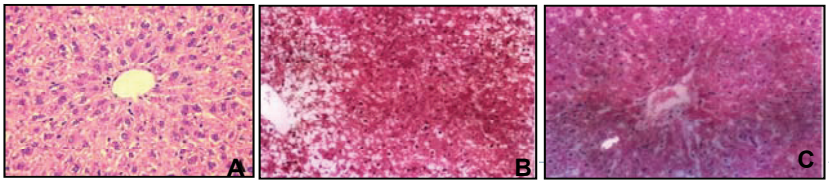

Figure5: Histopathology of liver showing the normal (gr1)architecture(A) , CCL4 treated rats (gr5)show coagulative necrosis(H&E 200)(B) and congested portal blood vessels and hepatic sinusoids(gr 8) (H&E200)(C)

A FLOW CYTOMETRIC INVASION INHIBITION ASSAY FOR THE SCREENING OF ANTI-EIMERIAL PHYTOGENICS

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SUMMARY

The assay described here is an *in vitro* screening method for anti-eimerial efficacy. Sporozoites of *E. tenella* were labeled and added to an animal cell lines together with test substances. After incubation, the cells were analyzed with a flow cytometer to detect if the tested substances impaired the sporozoites ability to invade the cells. Several coccidiostatic drugs and two known anticoccidial phytogenics were tested as a proof of concept. Among the tested drugs monensin sodium showed the lowest MIC_{50} (125 µg/L). Among the tested phytogenic samples thymol (MIC_{50} 13 mg/L) and quinine hydrochloride (MIC_{50} 3 mg/L) proved to be effective. This assay shall be used to screen for new phytogenics in the course of the development of anti-eimerial feed additives for broiler production.

INTRODUCTION

Coccidiosis is an infection of the gastro-intestinal tract caused by protozoan parasites (Coccidia). In domestic chickens this disease is caused by the genus *Eimeria*; predominantly by the species *E. tenella*. Symptoms of acute coccidiosis include diminished appetite, diarrhea and in severe cases death due to dehydration, but even subclinical infections can impair growth performance and feed conversion rate [1]. Worldwide annual damage to the poultry industry due to coccidia is estimated to be about € 1.7 billion [2]. Conventionally, coccidiosis in poultry is controlled by the prophylactic use of anticoccidial drugs in feed, but due to the spreading of drug resistances these additives may be banned in Europe by 2012 [3]. This would open the market for alternative means of coccidiosis control like vaccination, probiotics and new phytogenic feed additives [4].

The assay described here is a modified version of an invasion assay described before [5, 6]. In brief, sporozoites of *E. tenella* are labeled with the fluorophore 5(6)-carboxyfluoresceindiacetate succimidylester (CFDA-SE) and added to MDBK cells in the presence of test samples. After an incubation period the cells are washed, detached and analyzed with a flow cytometer to assess the rate of infected cells. In addition, the viability of the MDBK cells is assessed with a tetrazolium salt assay to screen for cytotoxic effects. Samples that kill extracellular sporozoites or otherwise impair their ability to enter the host cells without being cytotoxic for the cells are regarded as specifically anticoccidial. To show that this *in vitro* assay can predict *in vivo* activity several known anticoccidial drugs and phytogenics were tested.

MATERIAL AND METHODS

Cell culture: Madin-Darby bovine kidney cells (MDBK) were obtained from DSMZ (ACC174) and cultivated in DMEM/F12 medium (Biochrom) with 5% FBS (PAA), 4 mM L-glutamine, 100 U/mL penicillin 0.1 mg/mL streptomycin and 15 mM HEPES (Sigma).

***Eimeria tenella*:** Oocysts of *E. tenella* (Wis strain) were purchased from the Royal Veterinary College (London, UK) and stored in PBS (PAA) at 4 °C until used.

Coccidiostats and phytogenics: Quinine hydrochloride (AKRAS) was solved in PBS. Monensin sodium (Sigma), toltrazuril (Riedel de Haen), decoquinate (Prochema) and thymol (Sigma) were solved in 70% ethanol and diluted further to test concentrations, which only contained negligible amounts of ethanol (<0.5% v/v).

Sporozoite purification and CFDA-SE labeling: Purification was done as described by Mattig *et al* [7] with modifications. In brief, oocysts were vortexed with 0.5 mm glass beads in 1 s long bursts until more than 90% of the oocysts appeared cracked under the microscope. Then the sporocysts were incubated at 40 °C for 2 h in excystation medium consisting of 0.2% taurodeoxycholic acid (Sigma) and 2.5 mg/mL trypsin (PAA) in HBSS (PAA). Sporozoite hatching was checked microscopically. The suspension was then filtered with a paper filter (Sigma) and concentrated by centrifugation at 2000 x *g* for 8 min. Sporozoites were then labeled with 2.5 µM CFDA-SE (Sigma) for 30 min at 40 °C and washed twice with HBSS and centrifugation at 2000 x *g* for 8 min. Sporozoites were counted with a Bürker-Türk chamber and diluted with MDBK medium to 100,000 per mL.

Invasion inhibition assay: MDBK cells were harvested, seeded in 96-well plates at 10,000 cells in 200 µL per well and incubated for 24 h at 37 °C and 5% CO_2. 100 µL of supernatant were removed from every well and 100 µL of sporozoites suspension and 100 µL of test sample dissolved in MDBK medium were added to the wells.

MDBK medium without test samples was added to control wells. The plates were incubated at 40 °C and 5% CO_2 for about 24 h.

Measurement: After the incubation period the cells were washed to remove extracellular sporozoites, and a WST-1 cell proliferation assay (Roche) was performed according to the manual. Afterwards the supernatant of the wells was removed. The cells were detached with Accutase (PAA), suspended in PBS by thorough pipetting and transferred to a non-binding U-bottomed 96-well plate (Greiner). Measurements were conducted with an ACCURI C6 flow cytometer. MDBK cells were gated in a forward/sideward-scatter plot. As shown in figure 1, infected cells (containing fluorescent sporozoites) were distinguished from non-infected cells in the FL1 channel (515 – 545 nm). Invasion rates of sample and control wells were compared to calculate the relative inhibition by samples with the following equation:

Inhibition [%] = (1 – *invasion rate in sample well* / *mean invasion rate of control wells*) x 100

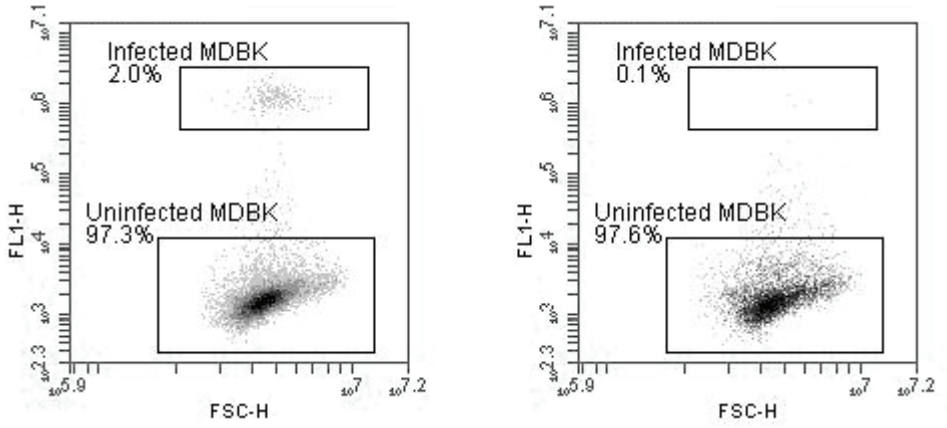

Figure 1: Example scatterplots. Left: The infected control shows a subpopulation of infected MDBK cells that can easily be distinguished from the main MDBK population by their FL1 signal. Right: Monensin treated infected MDBK cells almost completely lack this subpopulation showing that the drug inhibited sporozoite cell invasion.

The lowest concentration showing 50% inhibition or more (MIC_{50}) was determined for every sample. The results of the WST-1 assay were used to detect cytotoxic effects of samples. Concentrations with viability lower than 75% of the mean viability of control wells were excluded from the evaluation.

RESULTS

All tested compounds, with the exception of toltrazuril, showed a dose-dependent effect against *E. tenella* sporozoites (see figure 2).

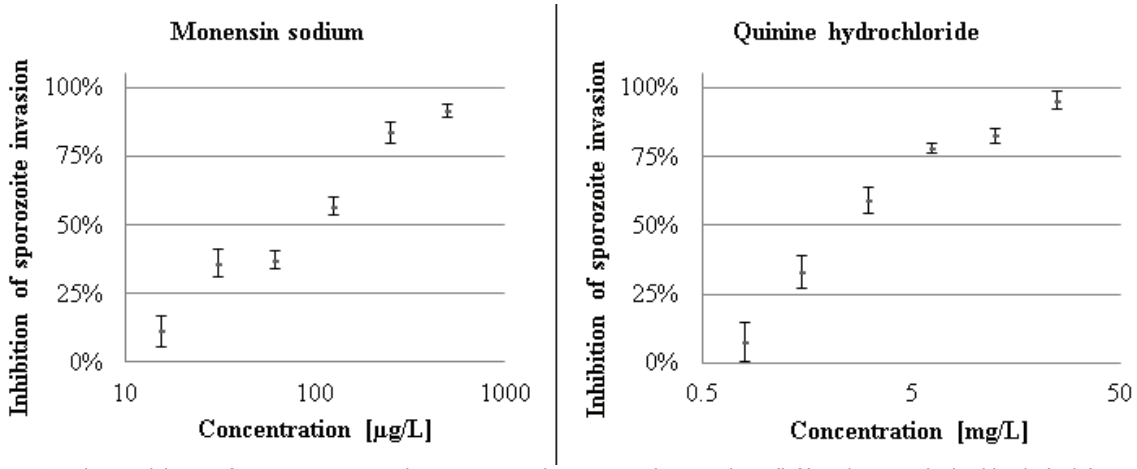

Figure 2: Relative inhibition of sporozoite invasion by monensin sodium, a coccidiostatic drug, (left) and quinine hydrochloride (right). Error bars show standard errors.

The results are presented in table 1. Among the coccidiostats, monensin sodium performed best, decoquinate showed a weaker less distinct effect in this assay, while toltrazuril failed to show a strong anticoccidial activity. Both tested phytogenic samples showed a specific effect against the *E. tenella* sporozoites.

Table 1: Results for the tested coccidiostats and phytogenic samples. * The highest tolerated concentrations of toltrazuril did not show more than 50% inhibition. ** For these samples the highest tested concentration was not toxic for the MDBK cells.

	MIC_{50} (smallest concentration showing 50% or more inhibition)	highest not cytotoxic concentration
Coccidiostats:		
Monensin	125 µg/L	> 4 mg/mL **
Toltrazuril	> 40 mg/mL*	40 mg/mL
Decoquinate	5 - 10 mg/L	20 mg/mL
Phytogenics:		
Thymol	13 mg/L	100 - 200 mg/L
Quinine hydrochloride	3 mg/L	> 200 mg/L **

DISCUSSION

Two of three coccidiostatic drugs and two phytogenics that have been reported to be anticoccidial before [8] have been found to be effective in this assay. These results suggest that the assay may be used for screening for new anticoccidial compounds. The flow cytometric evaluation enables a higher throughput of samples than previously described assays with microscopical evaluation.

The differences in efficacy among the tested coccidiostats might be explained by the different modes of action of these drugs. Toltrazuril [9] and decoquinate [10] both do not necessarily inhibit sporozoites but the development of later stages of the *E. tenella* life cycle (first and second generation merozoites), which would explain why their effect on sporozoite invasion was small compared to monensin sodium.

This suggests that substances that do not either kill free sporozoites or somehow inhibit them from invading host cells might be erroneously declared ineffective with this assay. This is an important limitation to be aware of, since the inhibition of later developmental stages is also a possible mode of action for a product.

CONCLUSIONS

It has been shown that this assay can be used to screen for anticoccidial drugs or phytogenics. This *in vitro* screening method can help to reduce the number of feeding trials necessary to find effective substances for an anti-eimerial feed additive.

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PROTECTIVE EFFECTS OF COPPER AND CHICORY ON THYROID ACTIVITY IN MOLYBDENOTIC RABBITS (Abstract)

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The thyroid is one of the most important gland in body which influenced by nutritional and medicinal factors. In this study the thyroid gland and Red blood cell profile of rabbits in experimental poisoning with molybdenum was evaluated and the effects of copper sulfate and chicory leaf extract on serum thyroid hormones of molybdenotic rabbits were detemined.

Twenty- five rabbits were divided into 5 groups. In all groups, sodium molybdate with a dose of 80mg/kg/day for 30 days was administered orally. In groups 2 and 4 chicory extract with a dose of 450 mg/kg/day and copper sulfate with a dose of 4 mg/day respectively was used orally simultaneous with sodium molybdate administration. Groups 3 and 5, after 30 days and appearance of clinical symptoms, were treated with chicory extract (450 mg/kg/day) and copper sulfate (a drench containing 0.05g and consumption of water containing 0.1 g/500ml adlibitum) respectively for 15 days. Blood samples were taken at the beginning and the end of study and serum levels of thyroid hormones and red blood cell profile were determined.

The results showed that serum levels of T_3 and T_4 and values of RBC, Hb and PCV decreased significantly in group 1, but in the other groups these values did not change significantly. Thus, chicory extract and copper sulfate can be effective on thyroid activity of molybdenotic rabbits.

EFFECT OF FERMENTED WHEAT GERM EXTRACT (FWGE) ON THE INTESTINAL MORPHOLOGY

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SUMMARY

In the pigs fed with FWGE supplemented normal diet the shortening of intestinal villi was significantly milder ($p<0.0041$) regarding both duodenum and jejunum. The thickening of villi and hyperkeratotic layer in the non glandular region of stomach, the degree of epithelial exfoliation, the lymphocytic, histiocytic and plasma cell infiltration and was milder, as well. The broiler chickens fed with diet supplemented with FWGE had a significantly higher average villus high in the duodenum, jejunum and ileum with 10-33% on day 10, 21 and 42, as compared with the control ones of the same ages. The incidence of villus atrophy accompanied by widening of the lamina propria, fusion of the villi and leucocytic infiltration in the lamina propria was higher in the intestines of control chickens, indicating a less favourable microbial environment in the intestinal content.

INTRODUCTION

In animal farming antibiotics containing feed additives are often used for nutritive, preventive, and sometimes for therapeutically purposes. Data indicate that antibiotics, administered for nutritive purposes, play a significant role in the evolving resistance of some pathogen microorganisms against antimicrobial agents. This is not only a health problem of the animals, but since antibiotics may penetrate to their tissues, thus be converted into human food, they might even threaten human consumption (Danaher et al., 2008). Resistant strains may also cause serious treatment problems in human medicine.

Due to the ban of antibiotic supplementation of farm animals' feed for growth promoting and alleviating the negative effects of the ambient microbiological load extended research has started to substitute the antibiotics in animal nutrition. Great number of dietary substances e.g. including acidifiers, probiotics, prebiotics, nutraceuticals, essential oils of herbs and spices, non digestible oligosaccharides etc. has been investigated with various successes. Among the nutraceuticals fermented wheat germ extract may present promising alternative.

The positive effects of FWGE against Mycoplasma gallisepticum infections in domestic poultry (Stipkovits et al., 2004) and the many preliminary reports on its immune modulating, antioxidant and growth promoting characteristics (Kósa and Bajcsy, 2008; Kovács et al., 2008) prompted to investigate the mode of action of beneficial effects of FWGE in controlled experiments with more species of farm animal.

The aim of this study was to reveal the morphological background connected with the significant improvement of weight gain and feed conversion efficiency in both, pigs and chickens due to diet supplemented with FWGE.

MATERIALS AND METHOD

In pigs fed with normal, not supplemented (control animals) and supplemented with 1 g/kg FWGE diet (experimental animals) histo-morphological examination of the mucous membrane in the alimentary tract (stomach, duodenum, jejunum, ileum, colon) including histometrical measurement of the villi height and width in the small intestine as well as the thickness of the hyperkeratotic layer in the non glandular part of stomach was conducted at different age as day 1 at farrowing, day 28 at weaning, day 90 at fattening and day 160 at slaughtering.

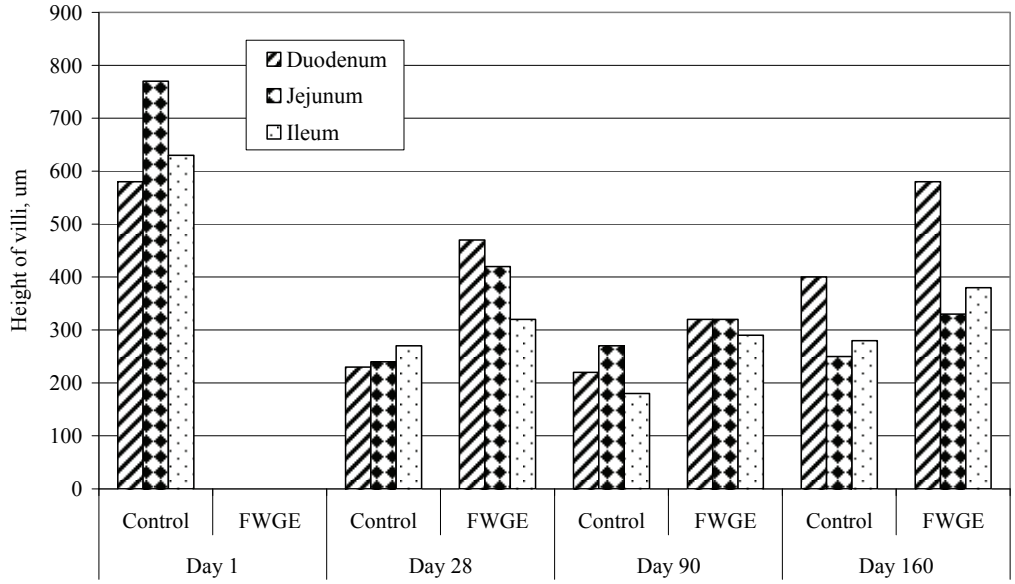
In chickens fed with normal, not supplemented (control animals) and supplemented with 1g/kg FWGE diet (experimental animals) histo-morphological examination of the mucous membrane in the small intestines (duodenum, jejunum, ileum) including histometrical measurement of height and width of the villi and that of the crypt depth was conducted at different age (day 1, 10, 21 and 42).

The histological and histometrical findings in pigs and chickens fed with normal, non supplemented and supplemented with FWGE diet were compared and the results were evaluated.

RESULTS

In the pigs fed with FWGE the shortening of intestinal villi was significantly milder ($p<0.0041$) regarding both duodenum and jejunum (Figure 1.). The thickening of the villi and hyperkeratotic layer in the nonglandular part of the stomach (Figure 2.), the degree of epithelial exfoliation, the lymphocytic, histiocytic and plasmacell infiltration and was milder, as well.

Figure 1. The impact of FWGE on the height of villi in the small intestine of pigs at different age



The broiler chickens fed with diet supplemented with FWGE had a significantly higher average villus high in the duodenum, jejunum and ileum with 10-33% on day 10, 21 and 42, as compared with the control ones of the same ages (Figure 3.). The incidence of villus atrophy accompanied by widening of the lamina propria, fusion of the villi and leucocytic infiltration in the lamina propria was higher in the intestines of control chickens, indicating a less favourable microbial environment in the intestinal content.

Figure 2. The effect of FWGE on the width of keratinized and degenerated layer in the non glandular part of the stomach in pig

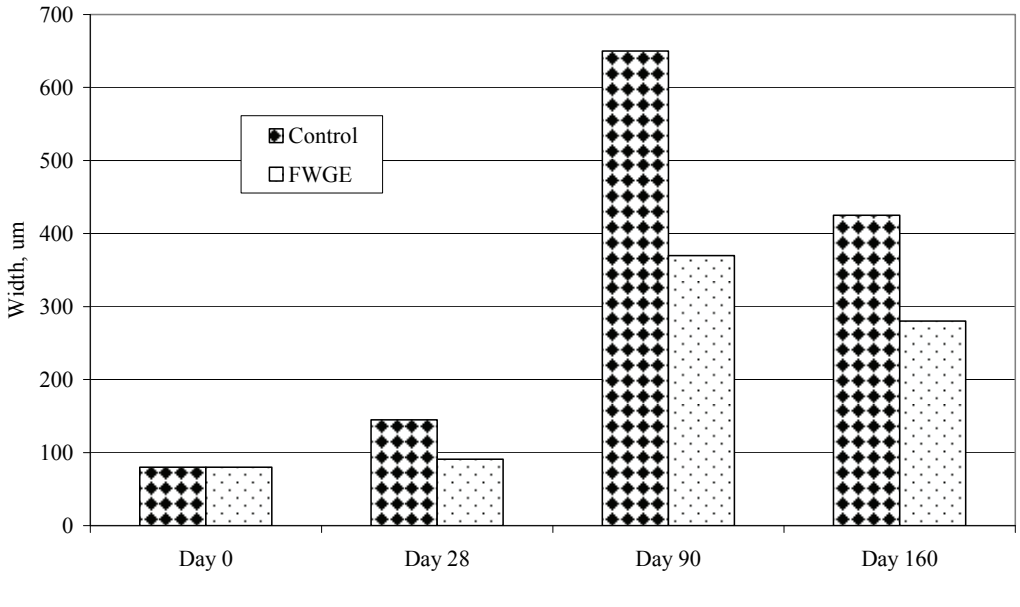
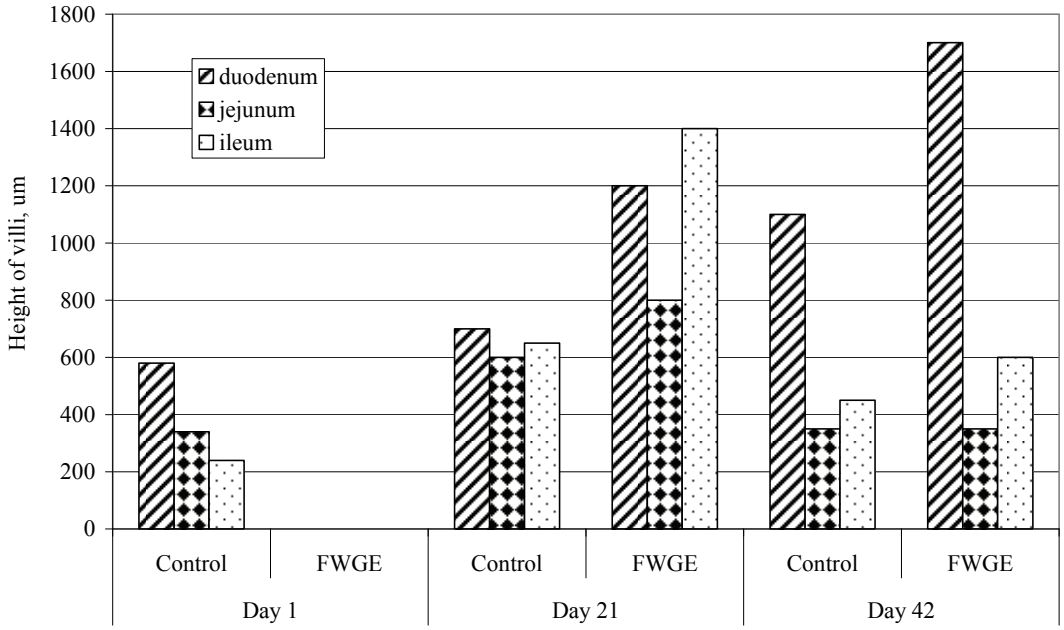


Figure 3. The impact of FWGE on the height of villi in the small intestine of broilers



DISCUSSION

These healthful and beneficial morphological changes in the gut of animals fed with FWGE could explain the significant improvement of weight gain and feed conversion due to diet supplemented with FWGE in laboratory testing and large-scale pig and poultry farms. However, further investigations are necessary to evaluate whether the increase of villus height observed in the small intestines in our experiments is caused by a more favourable intestinal microbial environment due to the FWGE or it is a direct effect of FWGE on the intestinal tissue.

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VITEX AGNUS-CASTUS EFFECTS ON INTER ESTRUS INTERVAL IN DAIRY COWS

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SUMMARY

Vitex agnus-castus is a native shrub of the Mediterranean region. It is dopaminergic and has been used as a remedy for low progesterone concentrations, corpus luteum deficiency, and etc. In women, for more than 2500 years.

In this study, Holstein dairy cows divided into control (n=7) and treatment (n=7) groups. After 21 days prescription of 50ml hydroalcoholic extract of the plant in treatment group orally, both groups synchronized by two intra muscular injection of PGF2α in 11 days apart. Prescription of herbal extract in the treatment group continued for 23 days, till to end of synchronized cycle.

During this estrus cycle blood samples collected and progesterone concentrations of separated serums were measured by RIA, moreover, ovaries and uterus of cows were examined by ultrasonography. Signs of estrus before and after treatment in two groups were recorded.

Results showed that 45 days prescription of the extract in treatment group increased average inter estrus interval versus control group (22.3 days, and 20.1 days, respectively, P= 0.05) and average serum progesterone concentration in treatment group was increased versus control group (2.88±0.63ng/ml, and 2.19±0.57ng/ml, respectively) about 32% during study cycle, but it is not statistically significant (P>0.05).

In attention to worries about probable health risks of using hormones in reproductive management in herds for meat and milk consumers and according to increasing effects of Vitex on estrus cycle and progesterone concentration, perhaps it can be a safe alternative remedy in reproduction management of dairy cows.

INTRODUCTION

All over the world, low fertility is a major limiting factor in highly managed dairy farms [1]. Various factors are associated with low fertility of the dairy cows, which one of them is luteal dysfunction and subsequent low progesterone concentration. Low progesterone levels may disturb reproductive procedures either before or after insemination [4]. The situation has been aggravated in recent years because achieving a continuous rise in milk production is associated with a gradual decline in plasma progesterone concentrations, consistent either the negative relationship between milk production and progesterone concentration [6].

Luteal deficiency after insemination could be associated with either low secretion of suboptimal amounts of progesterone or with early luteal regression, which, ultimately results in embryo death [6]. Furthermore, in these conditions hormonal treatments are used widely.

Nowadays, because of probable health risks for meat and milk consumers, pursuant usage of these various hormones for reproductive and fertility management in dairy herds, there is a growing interest in search for naturally active compounds in plants, affecting the reproductive activity. One such plant is chaste tree (*Vitex agnus-castus* Linn.), a shrub or small tree belonging to the genus *Vitex* of the *Verbenacea* family. It is native to the Mediterranean region and is found as far as western Asia. *Vitex agnus-castus* has been used to treat a variety of gynecologic conditions, Vitex agnus-castus is a native shrub of the Mediterranean region. It is dopaminergic and has been used as a remedy for low progesterone concentrations, corpus luteum deficiency in women, for more than 2500 years [3].

Over the past years, it has been used as a remedy for hyperprolactinemia, menstural irregularities, corpus luteum deficiency, fertility disorders, and poor lactation in women [3]. In veterinary practice, potentially, it has been recommended for infertility and hormonal problems caused by anovulatory cycles, luteal phase deficiency and low progesterone concentration levels, and latent hyperprolactinemia [2, 10]. The objective of present study is preliminary assessing the effects of *Vitex agnus-castus* extract on luteal function include inter estrus interval in dairy cattle.

MATERIAL AND METHODS

In this study, Holstein dairy cows divided into control (n=7) and treatment (n=7) groups. After 21 days prescription of 50ml hydroalcoholic extract of the plant in treatment group orally, both groups synchronized by two intramuscular injection of PGF2α in 11 days apart. Then Prescription of herbal extract in the treatment group continued for 23 days, till to end of synchronized cycle.

During this estrus cycle blood samples collected in days 1,5,9,13,18,22 of estrus cycle (day 0= estrus) and progesterone concentrations of separated serums were measured by RIA, moreover, ovaries and uterus of cows were examined by ultrasonography and signs of estrus before and after treatment in two groups were recorded.

RESULTS

Results showed that 45 days prescription of the extract in treatment group increased average inter estrus interval versus control group (22.3 days, and 20.1 days, respectively, P= 0.05) (Table 1) and average Serum progesterone concentration in treatment group was increased versus control group (2.88±0.63ng/ml, and 2.19±0.57ng/ml, respectively) about 32% during study cycle, but it is not statistically significant (P>0.05) (Table 2).

Table 1: Mean of Estrous Cycle Duration (days) Before and After Vitex Treatment.

	Before Treatment	After Treatment	P Value
Treatment Group	20.17	21.00	0.32
Control Group	22.29	20.14	0.05

Table 2: Mean of Serum Progesterone Concentration (ng/ml) in Treatment (vitex) and Control Groups.

Day of Estrous Cycle	Treatment Group	Control Group	P Value
1st	0.15±0.82	0.58±0.78	0.70
5th	0.88±0.82	0.65±0.78	0.83
9th	3.69±0.82	2.43±0.78	0.25
13th	5.33±0.82	4.58±0.78	0.50
18th	5.18±0.82	5.20±0.78	0.53
22ed	2.07±0.85	0.97±0.78	0.33
All Days of The Study	2.88±0.63	2.19±0.57	0.60

DISCUSSION

Studies which conducted in rats show that vitex extract effects on anterior pituitary gland, which decrease FSH hormones and increase LH therefore stimulates producing progesterone hormone [6].

Losh et al conducted a Study in 1990 on 20 female patients with secondary amenorrhea. experimental results on serum concentrations of progesterone, LH and FSH in 15 patients showed increased specific rates of progesterone and LH while FSH did not change or the rate Was slightly decreased and menstrual cycles were started in 10 women [5].

According to these studies that indicating Vitex agnus-Castus increased luteal phase progesterone in humans, primates and laboratory animals and despite the lack of significant results in our study, average serum progesterone levels increased during the estrus cycle in Vitex group and more increased average progesterone levels during the cycle in the treatment group than the control group was similar to the results of studies conducted in other species.

Based on signs of estrus, length of estrus cycle after treatment were 22.29 days in treatment group and 20.14 days in the control group (p=0.05) .Base on our study estrus cycle length in the treated group was longer than the control group.

Also, Milewicz et al in 1993 investigated the effects of Vitex agnus castus extract during the luteal phase which 37 patients (20 placebo and 17 Vitex group) subjected with short luteal phase were studied. In the final estrus cycle of vitex group were changed to normal [7].

CONCLUSION

Therefore the present results in cattle were surprisingly similar to those previously reported in humans. This significant increased estrus cycle length in vitex group compare in with control cattle was possibly due to effect of vitex on increasing luteal phase and progesterone in cattle.
perhaps according to these results vitex agnus castus extract in cattle leds to more potent corpus luteum which possibly grow faster and more lasting than the control group, and estrus cycle length showed a clear increase which it was probably due to luteotrophic effects of vitex.

In attention to worries about probable health risks of using hormones in reproductive management in herds for meat and milk consumers and according to increasing effects of Vitex on estrus cycle and progesterone concentration, perhaps it can be a safe alternative remedy in reproduction management of dairy cows.

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EFFECT OF CALCIUM PHOSPHORYL CHOLINE AND VITAMIN B12 (ROBRANTE CALIER®) IN BLOOD SERUM CALCIUM OF DAIRY COWS

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SUMMARY

Robrante calier® is an injectable pharmaceutic with antianemic and hepatic protection effects. During this study 20 ml of Robrante calier was injected subcutaneously in 20 multiparous (two or more calvings) and 5 first-calf dairy cattle immediately after parturation (treatment groups).Ten multiparous dairy cattle and 5 first-calf cows were injected with placebo and selected as control groups. Jugular blood samples were taken from all the animals in the trial as follow: immediately after parturition and after first mailking, 12 hours and 24 hours after aprturition, and 7 days after parturition The calcium concentration of serum samples was determined using commercial reagent kits, based on spectrophoyometric methods. Mean serum calcium concentration of animals of all groups were analysed by using Student's *t*- test, and a value of $P< 0.05$ was considered to be significant.

The maen serum calcium concentration in multiparous dairy cow in injected group during 12 and 24 hours after calving were 11.1± 0.99 and 10.37± 0.75 respectively, however in the control group the calcium concentration during those times were 9.7± 0.52 and 9.41± 0.57 resapectively. Calcium concentration of serum samples 7 days after calving in treatment and control groups of multiparous dairy cattle were 10.86± 0.62 and 9.51± 0.57 respectively. Statistic analyses showed significant increase in calcium concentration in sera of treatment group during 12 hours, 24 hours and 7 days after parturition as compared with the control group ($P< 0.05$). Biochemical analysis of serum samples of first- calving dairy cattle in the present study showed that the mean serum calcium concentration of injected cows during different times after parturition were higher than control cows but the difference was not statistically significant.

The results of present study showed that active components of Robronte calier significantly increased the serum calcium concentration of dairy cattle during critical times after parturition.

INTRODUCTION

Efficient milk production continues to require the dairy cow to experience the gestation and parturition each year. The transition from pregnant, non- lactating to lactating is too often a disasterous experience for the cow. Most of metabolic disease of the dairy cows – milk fever, ketosis, retained placenta and displacement of abomasum – occur in the first two weeks of lactation [1,5]. In early lactation the amount of energy required for maintenance of body tissues and the milk production exceeds the amount of energy the cow can obtain from her diet [5]. Robrante calier® (Laboratorios Calier, S.A., Barcelona, Spain) is an injectable solution with antianemic and hepatic protection effects. The active substances in Robronte calier® is specifically indicated on those stress situations which cause imbalances from metabolic or energy type. Robrante calier® contains calcium phosphorylcholine chloride, caseine peptide and vitamin B12. The calcium phosphorylcholine choloride not only provides calcium to the organism, but also choline chloride which is a very important from a biochemical point of view. The phosphorus compound save energy by bringing acetyl choline to the organism. Its restorative effect is completed by adding vitamin B12.

MATERIAL AND METHODS

To determine the effect of Robrante calier on serum calcium concentration of dairy cattle 20 multiparous and 5 first- calf cows were injected with Robrante calier® as soon as possible after parturition. Ten multiparous dairy cattle and 5 first- calf dairy cows were injected with placebo and selected as control group. Four Jugular blood samples were taken from each cows of injected and noninjected control groups. Blood samples were taken as follows: 1- immediately after parturition and after colostrum expulsion, 2- 12 hours and 3- 24 hours after parturition and 4- 7 days after parturition. Blood samples were transferred to the laboratory and sera were isolated by centrifugation and kept at – 20 °C until analysis. The calcium concentration of serum samples was determined using commercial reagent kits (Zist Shimi Co. Tehran, Iran), based on spectrophoyometric methods. Mean serum calcium concentration of groups were analysed by using Student's t- test, and a value of $P< 0.05$ was considered to be significant.

RESULTS

Results of biochemical analysis of serum samples were summerized in tables 1 and 2.

Table 1. Mean values of serum calcium concentration (mg/dl) of multiparous dairy cows during different times after parturition (Mean± SE).

Time / Group	No	At the parturition	12 hours after parturition	24 hours after aprturition	7 days after parturition
Treatment	20	8.30± 0.46	10.66± 0.99*	10.37± 0.75*	10.86± 0.62*
Control	10	8.62± 0.21	9.69± 0.22	9.41± 0.57	9.51± 0.57

* Significantly different ($P< 0.05$).

Table 1. shows mean serum calcium concentration of multiparous dairy cattle (treatment and control groups). Statistic analysis didn't found any significant difference between mean serum calcium concentration of treatment and control groups at the parturition time.

A significant increase in serum calcium concentration of multiparous dairy cows of tretment group were found during 12 hours ($P= 0.02$), 24 hours ($P= 0.03$) and 7 days ($P= 0.005$) after parturition as compared with the control group.

Table 2. Mean values of serum calcium concentration (mg/dl) of first- calving cows during different times after parturition (Mean± SE).

Time / Group	No	At the parturition	12 hours after parturition	24 hours after aprturition	7 days after parturition
Treatment	5	8.79± 0.59	11.09± 0.92	11.80± 1.34	10.87± 0.98
Control	5	9.04± 0.64	10.68± 1.52	11.36± 1.25	9.94± 0.68

As can be seen in table 2 the serum calcium concentration of treatment group of first- calving dairy cattle at the aprturition time was lower than in the control group but the difference was not significant. Although, mean serum calcium concentration of first- calving dairy cows of treatment group during different times after parturition was higher as compared with the control group, but student's *t*-test didn't show any significant difference between them.

DISCUSSION

Three basic physiologic functions must be maintained during the periparturient period if the disease is to be avoided. These are: 1. adaptation of the rumen to high energy density lactation diets to reduce the degrre of negative energy balance experienced by the cow; 2. maintenance of normocalcemia; and 3. reducing the degree of immunosuppression that occurs around parturition [1]. Both metabolic and infectious disease incidence are greatly increased whenever one or more of these physiological functions are impaired [5]. The onset of lactation places such a large demand on the calcium homeostasic mechanisms of the body that most cows develop some degree of hypocalcemia at calving [2,4]. Cows developing milk fever have higher cortisol concentrations than do non- milk fever cows [3, 4].

The results of present study showed that active substances of Robronte calier significantly increased the serum calcium concentration of dairy cattle during critical times after parturition. Since the calcium concentration of Robrante calier is not too much (100 ml of Robrante calier contains 5 grams calcium phosphorylcholine chloride) it seems that the increasing effect of this product on serum calcium concentration is brought by improving energy metabolism (by phosphorycholine chloride content) and hepatic function (by caseine- peptides and vitamin B12) as well as antistress effect in recently parturient multiparous dairy cattle.

CONCLUSION

The results of the present study showed that Robrante calier is a very potent drug in increasing of serum calcium concentration of parturient dairy cattle and its use immediately after calving significantly increases serum calcium concentration in multiparous dairy cows.

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INFLUENCE OF DIFFERENT DIETARY FIBER LEVELS AND ENZYMES ON GROWTH PERFORMANCE OF BROILER CHICKS

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SUMMARY

A total number of 320 one day old of mixed Hubbard chicks were randomly allotted into four collections, each subdivided to two groups, 40/each. Chicks were experimented to investigate the effect of four dietary levels of crude fiber (3.5, 5.0, 6.5, and 8.0%) without or with enzymes in a factorial arrangement 4 x 2 during growing-finishing period. All the single groups were fed on the diets free enzyme, while the enzyme was added to the diet of the paired groups. The best BWG and FCR were recorded by the groups fed diets having 3.5 and 5.0% CF with enzyme supplementation (2232.94 g & 1.89) and (2116.47 & 1.90) respectively, while the worest values recorded by the groups fed diets having 8.0% CF with or without enzyme(1759.41g & 2.37) and (1748.82 g &2.51) respectively. There were no significant differences in the dressed carcass and proventriculus percentages among all the treated groups. The spleen and gizzard percentages in the birds fed diets having 8% CF with or without enzyme supplementation were significantly increased, while the liver percentages of the same groups were significantly decreased compared with the control. The results obtained concluded that addition of enzyme improved growth performance of broilers up to 6.5 % crude fiber level.

INTRODUCTION

There is great interest in the use of enzymes for improving digestibility and metabolizable energy of poultry feeds. Use of this biotechnology has not only improved the efficiency of using conventional feed ingredients, but has also allowed nutritionists to use unconventional feed ingredients that are cheaper and more readily available [9]. One way of counteracting the possible anti-nutritive effects of high fiber contents in the feeding-stuffs and consequently improving their nutritive value, is to supplement them with enzymes. Poultry produce a number of enzymes, including amylases to digest starch, proteases to digest protein and lipases to digest fats. However, they do not produce enzymes to digest fibers in feeds. The main potential of enzyme addition to feed appears for digestion of substances that an animal is intrinsically incapable of digesting These enzymes can open up the complex feed cell walls, allowing the animals own enzymes to digest the enclosed nutrients [8]. The interaction effect of dietary crude fiber levels and enzyme supplementation on performance carcass yield was evaluated by many researchers as [14, 2, 16, 19, 7, 12]. The inclusion of enzyme in high fiber diet (6.5%) based on copra meal significantly increased body weight and live weight gain of broilers until 6 weeks of age [22]. The current work was conducted to study the possibility of feeding diets containing different crude fiber levels (3.5, 5.0, 6.5, and 8.0%) with or without enzymes on the performance and carcass traits of broilers during a rearing period from 21-49 days of age.

MATERIAL AND METHODS

The current work was conducted at the Department of Animal & Clinical Nutrition, Faculty of Veterinary Medicine, Assiut University. **Birds and management:** A total number of 320 one day old of mixed Hubbard chicks obtained from a local commercial source, leg banded and were randomly allotted into four collections, each subdivided to two groups, each of 40 chicks. Birds were submitted to standard management commonly adopted in commercial broiler production. Chicks were vaccinated against the common poultry diseases according to the conventional program used for broiler chicks. The prophylactic measures were taken to control diseases and to increase the viability of the birds. **Experimental design:** Chicks were experimented to investigate the effect of four dietary levels of crude fiber (3.5, 5.0, 6.5, and 8.0%) without or with enzymes in a factorial arrangement 4 x 2 on growth performance and carcass traits of broilers.

Diets and feeding: In the starting period (0-3 weeks), the birds in all 8 groups were allotted to dietary treatments based on commercial iso caloric–iso nitrogenous starter pelleted diet. The grower–finisher experimental pelleted diets were formulated to contain 18.8% crude protein, 3000 kcal/kg ME, and four levels of crude fiber 3.5, 5.0, 6.5 and 8.0 % (Table1). The chemical composition of the experimental diets were done according to [4] official methods, while energy value were cited from [18]. In the growing-finishing period, the first two groups were fed ad-libitum on broiler grower-finisher experimental diets containing 3.5% crude fiber level (without or with enzyme), while the second, third and fourth collections (groups from 3 to 8) raised on fiber

levels (5.0, 6.5 and 8.0%). All the single groups were fed on the diets free enzyme, while the enzyme was added to the diet of the paired groups. The experiment extended for 4 weeks duration in one feeding phase, growing-finishing (4-7 weeks of age). Chicks were subjected to feed ad-libitum with free access to fresh water throughout the experimental period. Birds were checked twice daily and the weight of dead birds was used to adjust the average feed consumption and body weights.

Table 1: The physical and chemical composition (%) of the experimental diets

Ingredients	Crude Fiber level (%)			
	3.5%	5.0	6.5	8.0
Physical composition				
Corn Grain	57.06	38.76	30.23	25.54
Soybean Meal (44%)	30.77	29.68	29.65	28.40
Wheat Bran	0.82	10.99	10.00	10.00
Berseem hay	-	2.00	7.04	10.97
Date seeds	-	2.00	5.00	8.93
Molasses	5.00	5.00	5.00	5.00
Sunflower Oil	2.80	7.58	9.00	9.00
Ground Limestone	1.00	1.71	1.70	0.73
Dicalsium Phosphate	1.73	1.42	1.50	0.50
Common Salt	0.40	0.40	0.40	0.40
Vitamin Mineral Premix*	0.30	0.30	0.30	0.30
DL-Methionine	0.10	0.14	0.17	0.21
L-Lysine HCl	0.02	0.02	0.01	0.02
Chemical composition and energy value				
Calculated ME (Kcal/kg diet)	3000	3000	3000	3000
Crude protein	18.8	18.8	18.8	18.8
Crude fiber	3.47	4.96	6.53	8.02
Ether Extract	5.35	9.67	11.14	11.33
Methionine. + Cystine	0.72	0.72	0.72	0.72
Lysine	1.00	1.00	1.00	1.00
Calcium	0.9	0.9	0.9	0.9
Available phosphorus	0.35	0.35	0.35	0.35

* HIPRA VIT® supplied per kilogram of diet : Manganese, 0.02%; Zinc, 0.02%; Iron, 0.01%; Copper, 0.0025%; Iodine, 0.0003%; Selenium, 0.00003%; Folic acid, 0.69 mg; Choline, 386 mg; Riboflavin, 6.61 mg; Biotin, 0.03 mg; Vitamin B6, 1.38 mg; Niacin, 27.56 mg; pantothenic acid, 6.61 mg; thiamine, 2.20 mg; manadione, 0.83 mg; Vitamin B12, 0.01 mg; Vitamin E, 16.53 IU; Vitamin D3, 2,133 ICU; and Vitamin A, 7,716 IU.

Growth performance and feed conversion

The average amount of feed intake by each bird was calculated by dividing the weekly consumed feed by its respective number of birds in each group at this week. Regarding the development of the body weight and weight gain, the birds were individually weighed every week and the live weight changes were taken as the criteria of the effect of the different treatments, and as a measure for growth.

C**arcass traits**

Five randomly selected birds from each group were slaughtered at the end of the experimental period for carcass traits. Feed but not water was removed from birds 12 hours prior to slaughtering. Dressed carcass weight and the weights of some internal organs of birds were recorded at the end of the experiment.

Statistical analysis

Experimental crude data subjected to several statistical analyses, from which the means and standard errors was calculated and differences were testing for significance by the two-way analysis of variance using the general linear models procedure of Statistical Analysis System [21], and differences ($P<0.05$) among treatments were tested using Duncan's multiple range test [11]

RESULTS

Birds fed diets containing 6.5% CF with enzyme supplementation achieved nearly the same weight gain and feed conversion as control group fed on diet having 3.5% CF without enzyme supplementation. While groups fed diets having 8.0% CF supplemented with or without enzyme recorded significantly ($p>0.05$) lower weight gains than that recorded by the control one (Table 2). At the end of the experimental period, the best BW gain was recorded by the groups fed diets having 3.5%CF with enzyme supplementation (2232.94 g), while the worst gain was recorded by the groups fed diets having 8.0% CF either supplemented with enzyme or not (1748.82 g) and (1759.41 g)respectively.

Table 2: Growth performance of broilers as influenced by level of crude fiber and enzyme supplementation

CF level	3.5%		5.0%		6.5%		8.0%	
Enzyme	-	+	-	+	-	+	-	+
Group No.	1	2	3	4	5	6	7	8
Initial weight (g)	782.35 ±25.8 [b]*	743.53 ±12.63 [c]	781.18 ± 23.65 [b]	810.0 ± 18.39 [a]	829.41 ± 11.03 [a]	800.59 ± 12.96 [a]	802.35 ± 22.48 [a]	815.88 ± 19.61 [a]
Final weight (g)	2852.94 ±63.81 [b]	2976.47 ± 57.38 [a]	2888.24 ± 74.08 [b]	2926.47 ±76.59 [b]	2738.24 ± 65.52 [c]	2870.59 ± 53.39 [b]	2561.76 ±50.46 [d]	2564.71 ± 69.90 [d]
Total body weight Gain	2070.59 ± 49.06 [c]	2232.94 ± 49.45 [a]	2107.06 ±65.74 [b]	2116.47 ±63.84 [b]	1908.8 ±61.27 [d]	2070.00 ±56.70 [c]	1759.41 ± 46.02 [e]	1748.82 ± 62.20 [e]
Feed consumption	4229.4	4223.5	4370.6	4023.5	4482.4	4470.6	4170.6	4382.4
FC index	2.04	1.89	2.07	1.90	2.35	2.16	2.37	2.51

* Figures in the same row having the same superscripts are not significantly different ($P<0.05$)

There were no significant differences in the dressed carcass percentage among groups related to the interaction between the crude fiber level and enzyme supplementation. The results of the proportional weights of some internal organs in all the treated groups showed significant differences. The spleen and gizzard percentages in the birds fed diets having 8% CF with or without enzyme supplementation were significantly increased, while the liver percentages of the same groups were significantly decreased compared with the control (Table 3).

Table (3): Carcass traits of broilers as influenced by level of CF and enzyme supplementation

CF level	3.5%		5.0%		6.5%		8.0%	
Enzyme Supp.	-	+	-	+	-	+	-	+
Group No.	1	2	3	4	5	6	7	8
Dressed Carcass *(%)*	72.1± 1.11 [a*]	71.7± 0.82 [a]	72.2± 0.95 [a]	72.3± 1.01 [a]	71.8± 1.13 [a]	71.7± 0.92 [a]	72.1± 0.91 [a]	72.0± 1.02 [a]
heart	0.48± 0.04[a]	0.49± 0.02 [a]	0.51± 0.06 [a]	0.50± 0.02 [a]	0.47± 0.05 [a]	0.47± 0.02 [a]	0.52± 0.04 [a]	0.51± 0.04 [a]
liver	2.26± 0.05 [b]	2.37± 0.05 [a]	2.22± 0.06 [b]	2.25± 0.06 [b]	2.23± 0.07 [b]	2.34± 0.05 [a]	2.13± 0.05 [c]	2.16± 0.03 [c]
spleen	0.153± 0.01 [b]	0.139± 0.01 [c]	0.148± 0.01 [b]	0.147± 0.02 [b]	0.162± 0.01 [a]	0.164± 0.04 [a]	0.168± 0.03 [a]	0.166± 0.03 [a]
gizzard	1.48± 0.05 [bc]	1.47± 0.07 [bc]	1.51± 0.07 [b]	1.51± 0.07 [b]	1.55± 0.04 [b]	1.54± 0.05 [b]	1.63± 0.05 [a]	1.62± 0.04 [b]
Proven-triculus	0.42± 0.01 [a]	0.43± 0.02 [a]	0.44± 0.03 [a]	0.38± 0.01 [b]	0.44± 0.01 [a]	0.43± 0.03 [a]	0.43± 0.01 [a]	0.38± 0.02 [b]
Dressed Carcass *(%)*	72.1± 1.11 [a]	71.7± 0.82 [a]	72.2± 0.95 [a]	72.3± 1.01 [a]	71.8± 1.13 [a]	71.7± 0.92 [a]	72.1± 0.91 [a]	72.0± 1.02 [a]

* Figures in the same row having the same superscripts are not significantly different ($P<0.05$)

DISCUSSION

The results of BWG and FCR agreed with those reported by [1] who found depression in BW and FCR of broiler chicks with 9% dietary crude fiber. Poor growth performance of broiler chicks fed on a high fiber diet was reported by [3]. The depressing effect of high CF on weight gain could be attributed to that most NSPs in water produce a very viscous solution. Any increase in digesta viscosity causes an increase in thickness of the unstirred water layer adjacent to the mucosal villi, consequently, there is reduced solubilization and uptake of most nutrients. The increased digesta viscosity also influences the gut microflora and there is an indication that their overgrowth may, in fact, add to the overall deleterious effects [17].

The improvement effect of enzyme supplementation on the body weight gain and feed conversion of broilers was recorded by many researchers [23, 20]. One explanation of this improvement in weight gain is that the dietary inclusion of enzymes to broiler diets can improve feed digestibility and nutrients absorption by reducing the foregut digesta viscosity [5]. The results of interaction between crude fiber level (3.5, 5.0, 6.5 and 8.0%) and enzyme supplementation were agreed with those recorded by [13] and [16]. Broilers fed diet containing 5% CF without enzyme supplementation had poorest feed conversion ratio [20], while enzyme supplementation improved the FCR of birds fed diet having 5.0% CF. The improvement in growth performance due to enzyme supplementation with high fiber diets may be attributed to increase in digestion and absorption of all nutrients and not simply to the starch alone [6]. The addition of cell wall degrading enzymes may release nutrients coated by non starch polysaccharides (NSP) contained in the feed and favour their digestion [10].

Similar results of carcass traits were recorded by [12, 13, 7, 20 & 23] who found that no significant differences were detected due to interaction between different levels of crude fiber with poly-enzyme addition in all carcass characteristics. It was also reported that carcass characteristics were not affected by the enzyme supplementation [16, 2]. The increased size of the gizzard may be as a result of handling bulky feeds. It has been speculated whether the beneficial effect of insoluble fiber on digestion is a result of increased gizzard activity [15].

CONCLUSIONS

Birds fed diet supplemented with enzyme scored the best results than that fed un-supplemented one. Addition of enzyme improved feed conversion ratios and growth performance of broilers fed on pelleted diets up to 6.5% CF level.

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CHICKEN HETEROPHIL PETIDES: POTENTIAL ANTIBIOTICS AND BASIS FOR NEW FEED ADDITIVES

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SUMMARY

Heterophils in broilers are the counterpart of mammalian neutrophils. They constitute the angular stone of the innate immune system against invading microorganisms with highly potential antimicrobial equipment. The non-oxidative mechanism is well developed and depends mainly on cationic antimicrobial peptides localized within cytoplasmic granules.

Heterophils were obtained during the exudative phase of induced inflammatory response by intraperitoneal injection of 3% starch soluble then purified and homogenized. The cytoplasmic granules obtained by centrifugation gradient were extracted and their contents were tested against standard strains of *S. aureus* and *E. coli* by using an ultrasensitive radial diffusion method.

Even with the very small amount of loaded materiel and its very low protein concentration its antibacterial activity is conserved and an inhibition zone of 0.6 cm was observed against *E. coli* and an inhibition zone of 0.68 cm against *S. aureus.* The heterophils crude granule extract showed striking antibacterial activity even at very low concentration either against Gram positive or Gram negative bacteria (20µg/ml) and the one-dimensional AU-PAGE showed great cathodal mobility and confirm the cationic nature of these bioactive molecules.

These results confirm the existence of very efficient non-oxidative mechanism and molecules with potent antibacterial activity where the cationic antimicrobial peptides are the most powerful molecules of this antibacterial activity. With their striking activity and the ability to kill microorganisms even with very small concentrations may constitute a new antibiotic class and the basis for new feed additives.

INTRODUCTION

Chicken heterophils, called also pseudoeosinophils, are the counterpart of neutrophils in mammals; with rod shaped or spherical cytoplasmic granules; they are the most numerous blood leucocytes and constitute the first line of defense against invading microorganisms, where the initial response of the body to microbial invasion and the events in the first few hours are crucial in determining the outcome of infection [1]. The heterophils lack myeloperoxidase, an essential enzyme of the respiratory burst [2-6], and their antimicrobial activity depends mainly on non-oxidative mechanism. The cationic antimicrobial peptides located in the cytoplasmic granules are the most active molecules of the non-oxidative killing activity of heterophils. The cationic antimicrobial peptides have a wide killing activity; they are active against enveloped viruses, Gram-negative and /or Gram-positive bacteria, fungi, parasites and even cancer cells [7]. The acute exudative phase of the inflammatory response, induced either by artificial irritants or infectious agents or their fractions such lipopolysaccharids, in avian tissues is characterized by the predominance of the heterophils [6]. Many researchers in the field of non-oxidative killing activity have been attracted by the avian heterophils due to their antimicrobial activity which depend primarily on oxygen-independent mechanisms [6] and were useful in studying oxygen independent killing activity.

MATERIAL AND METHODS

Cell isolation: "ISA 15" broilers chicks, obtained from a local hatchery the day of hatching, were reared on cages with *ad libitum* access to water and antibiotic free feed with appropriate granulometry. Intraperitoneal injection (i.p.) of irritant solutions is the most used method to get high proportion of heterophils. The interval between the i.p. injection and lavage of the peritoneal cavity with heparinized or non-heparinized buffer solution vary from 6 hours to 24 hours [2,7]. The animals were sacrificed and the peritoneal cavity was washed with PBS: pH=7.2 and the heterophils were collected and washed twice with PBS.

Granule isolation and extraction: For best release of cytoplasmic granules, the heterophils were sonicated in an ice bath to prevent molecules denaturation, with an ultrasonic homogenizer (BANDELIN sonopuls HD 200) with

a power of KE76/D; the granules were pelleted at high speed centrifugation (27000 x g) for 30 min in a cooled centrifuge. The granule extraction was carried out overnight in 5% acetic acid for 16 hours with a magnetic stirrer at 4°C. Followed by high speed centrifugation 27000 x g and the peletted materiel was reextracted.

Antibacterial activity: After extraction the supernatant was dialysed overnight against 1 % glacial acetic acid using dialysis tubing with a MW cutoff of 1000 Da and magnetic stirrer at 4°C. The granule extract was concentrated by lyophilisation. The lyophilized material was resuspended in 0,01 % glacial acetic acid and protein content was determined. The antibacterial activity was tested against standard strains of bacteria *E. coli* ATCC 25922 and *staphylococcus aureus* ATCC 25923 by using an ultrasensitive radial diffusion method.

AU-PAGE: AU-PAGE of granule extract was carried using disc gel electrophoresis with reverse current (cathode on bottom).

RESULTS

The crude extract of heterophils' cytoplasmic granules tested against standard strains by ultrasensitive method where the wells, with a diameter of 3 mm, loaded with 5µl of the solution to be tested. The antibacterial activity against *E. coli* ATCC 25922 with adjusted protein concentration showed a marked antibacterial activity (0.7 - 1.1cm) and the diameter of the inhibition zone is quietly visible even the small amount of the tested materiel.

In another experiment, the total protein was adjusted to 20µg/ml and the wells were loaded with 5µl of the solution to be tested against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. Even with the very small amount of loaded materiel and its very low protein concentration its antibacterial activity is conserved and an inhibition zone of 0.6 cm was observed against *E. coli* ATCC 25922 and an inhibition zone of 0.68 cm against *S. aureus.*

DISCUSSION

The heterophils crude granule extract showed striking antibacterial activity even at very low concentration either against gram positive or gram negative bacteria (20µg/ml) and the AU-PAGE showed great cathodal mobility and this confirm the presence of non-oxidative mechanisms and potent antibacterial molecules like cationic antimicrobial peptides [8].

CONCLUSIONS

Chicken heterophils are major blood leukocytes with remarkable antibacterial activity of their cytoplasmic granules this study confirm the antibacterial effect of their cytoplasmic granules on either Gram negative or Gram positive bacteria. This antibacterial killing activity of heterophils is an oxygen independent mechanism where AU-PAGE of their granule extract showed their cationic nature. The cytoplasmic granules are equipped with powerful molecules against bacteria and responsible for the main activity of heterophils. Chicken heterophils, by their potent antibacterial activity, play a key role in the innate immune system and constitute very efficient and protective shield against invading microorganisms.

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EFFECT OF DIETARY INCLUSION OF DE-OILED DISTILLER´S GRAINS ON THE PERFORMANCE PARAMETERS OF TURKEY POULTS

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SUMMARY

A trial was conducted to investigate the effect of dietary incorporation of different levels of de-oiled DDGS on the performance parameters of turkey poults. Five hundred and twenty eight male two weeks old poults were randomly divided into six groups each containing 88 poults (control, 5%, 10%, 15%, 20% de-oiled DDGS and 20% DDGS). Each treatment group was further subdivided into eight replicates of 11 poults per replicate. The diets were formulated on digestible amino acid basis. The experimental diets were fed from the 2nd to the 6th week of age. The results revealed that turkey poults fed on diets containing different inclusion levels of de-oiled DDGS or 20% DDGS not differ significantly in body weight, feed efficiency, average daily gain and daily feed intake in comparison to the control. It was concluded that up to 20 % of de-oiled DDGS or DDGS can be incorporated in starter turkey diets (2-6 wks of age) as an alternative protein source.

INTRODUCTION

With the current expansion of ethanol production to satisfy the high demand for clean, and an enviromental friendly source of energy, the production of distiller's dried grains with solubles (DDGS) which is a byproduct of ethanol production from corn will also increase proportionately. Most of the increase in ethanol production capacity is expected to come from new dry grind corn plants. Furthermore, there is a great interest in developing new processing technology for DDGS to increase its nutritional value, producing new products for market diversification and increasing the profitability of ethanol producers. The modified DDGS include high protein DDGS **(1, 2, 3, 5)**, elusieve (de-fibered) DDGS (**6)** and solvent or mechanically extracted (De-oiled) DDGS **(7).**

The main adventage of extraction of oil from distiller's grains is the reduction in the effective cost of ethanol production from grains based bio-refinary as it allow for production of multiple commercially valuable products from the grains (ethanol production, solvent extraction of crude oil from DDGS and even further trans-estrification of crude oil to produce biodiesel and glycerine can occur in one facility).

MATERIAL AND METHODS

Ingredients and Diets

Diets were formulated on a digestible amino acid basis to be isocaloric. Corn, SBM, poultry meal, DDGS and de-oiled DDGS were assayed for digestible amino acid basis using cecectomized roasters. True metabolizable energy (TME) was determined with young turkeys (6-8wks of age). Chemical analysis (dry matter, crude protein, fat, fiber and amino acids) of the ingredients were conducted prior to start of the trial. Inclusions of poultry by product meal (PBM) were limited to 7% to keep phosphorus from becoming excessive. The ME content of the diets was 2890 Kcal/Kg diet. The level of digestible methionine+cystine, digestible lysine, and digestible threonine were 1.007, 1.526, and 0.961% respectively. Diet protein level was established by restricting the use of supplemental threonine. There were 6 dietary treatments each treatment containing 8 replicates with 11 poults/ replicate pen. The diet treatments are provided in Table 1.

Table 1.

Treatment	Diet Description
1	Control
2	As 1 + 5% De-oiled DDGS
3	As 1 + 10% De-oiled DDGS
4	As 1 + 15% De-oiled DDGS
5	As 1 + 20% De-oiled DDGS
6	As 1 + 20% DDGS

Experimental Birds/ Housing

Five hundred seventy-six male poults(Nicholas 88 x 700), were received from a commercial hatchery.The poults were sexed, beak trimmed, three toe clipped, injected and cocci vaccinated at the hatchery. On arrival, they were randomly distributed to different pens (12 poults/ pen) then group weighed and fed on crumbled commercial starter diet for 2 weeks. Pens measured 6x8 ft and were bedded with wood shavings. At two weeks of age, the poults were wing banded, individually weighed and randomly distributed by weight to 48 pens (11 turkeys/ pen) to assure equivalent starting weight. The duration of the experimental trial was from 2^{nd} to 6^{th} weeks of age. Turkey body weight and feed consumption was determined at 2^{nd}, 4^{th} and 6^{th} weeks of age.

Exp. Design Analysis

The experimental design was randomized block design with eight replicate pens per treatment. Pen was the experimental unit. Analysis of variance was conducted to determine treatments effect on gain, feed intake and feed conversion. Mean testing (Least Significant Difference) was conducted to determine if performance differences existed for turkeys fed the control diet series with no DDGS, diet series with varying levels of deoiled DDGS and diet series of DDGS.

RESULTS

The TMEn value of de-oiled DDGS was 2410 kcal/kg diet. The true amino acid digestibility coefficients for de-oiled DDGS was found to be nearly the same as DDGS, except for the amino acid lysine which was decreased in de-oiled DDGS (56%) versus (75.2%) in DDGS. The crude fat content of de-oiled DDGS is about three times lower than DDGS. Total phosphorus and calcium content of de-oiled DDGS and DDGS are nearly the same. Sodium and chlorine content in de-oiled DDGS is about 20% lower and 45% higher than DDGS, respectively.

The poult performance results as shown in Figure 1 revealed that turkey poults fed on diets containing different inclusion levels of de-oiled DDGS or 20% DDGS had body weight, average daily gain and daily feed intake that are not significantly different from that of turkeys fed the control diet. Some minor differences existed among treatments for feed efficiency. Feed efficienty of Treatment 3 was significantly greater ($P<0.05$) than Treatments 2 or 4. The results indicate that poult performance from 2^{nd}-6^{th} weeks of age was not negatively affected by dietary inclusion of gradually increasing levels of de-oiled DDGS up to 20% or 20 % DDGS.

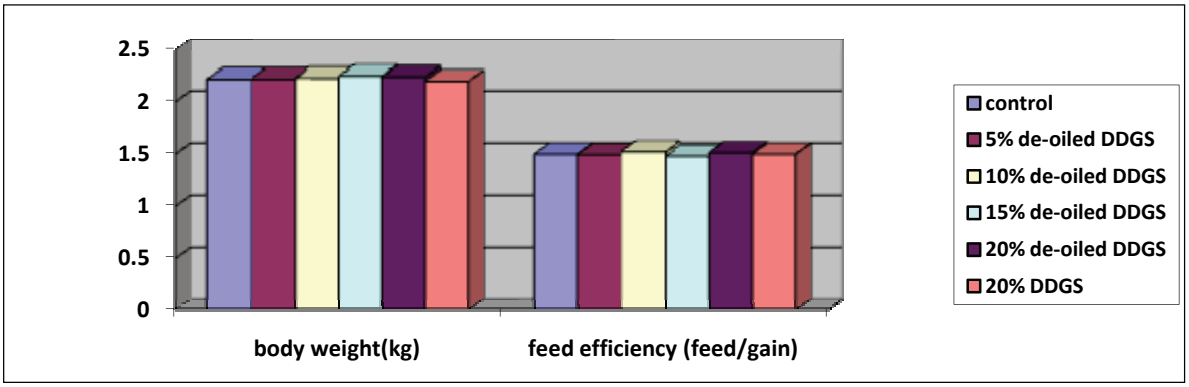

Figure 1. Turky body weight and feed efficiency (2^{nd}- 6^{th} wk of age)

DISCUSSION

De-oiled DDGS is an intermediate protein source, as it contains 31.0 % CP in comparison to 44.1%, 48.5 % and 26.8 % in high protein distiller's grains (HPDDG), soybean meal and DDGS respectively. Its protein quality like other corn byproducts is limiting in lysine, tryptophan and arginine. The amino acid content of de-oiled DDGS is intermediate; it is higher than corn and DDGS, but lower than other modified distiller's grains such as HPDDG and soybean meal. The reduced lysine digestibility may be due to additional heat treatment during oil extraction process in extractor, desolventizer and dryer. The energy value of de-oiled DDGS of 2410 kcal/kg diet is lower than other ethanol byproducts due to extraction of about 80% of its oil content. The reduced energy content of the de-oiled DDGS resulted in more supplemental fat use as the inclusion level of product increased. While the inclusion of de-oiled DDGS had no negative effects on poult performance, it should be noted that diets were formulated on a digestible amino acid basis and to be isocaloric. Lack of such adjustments might result in reduced in performance; alternatively, such adjustments also increase the cost of the diet.

CONCLUSIONS

1. No significant differences were observed in the performance parameters among different dietary treatments when compared to the control.
2. De-oiled DDGS can be used in turkey diet as an alternative protein source.
3. The economic efficiency of using de-oiled DDGS in turkey diets depends upon the prices of DDGS and animal fat.

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PRODUCTION OF SUCROSE LAUREATE-STABILIZED WATER-SOLUBLE PHYTOSTEROL NANODISPERSIONS

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SUMMARY

This work concentrated on the production, characterization and effect of temperature, of the optimized sucrose laureate stabilized water-soluble phytosterol nanodispersions using emulsification-evaporation technique. Preliminary study was previously done to recognize the independent parameters that would potentially affect the interested independent parameters. Response surface methodology (RSM) was employed to model and optimized the independent parameters namely phytosterol, sucrose laureate concentrations, homogenization cycles, and homogenization pressure. Response variables, mean particle size (PS) and % phytosterol retention (%Ph) were well fit by a quadratic model. The PS, pH value and %Ph of the optimized nanodsipersions were 4.4 nm, 6.45 and 90%. The PDI value was 0.550 at monomodal distribution. The optimized nanodispersions were visually translucent. The PS of the optimized phytosterol nanodispersions was stable in response to heat treatment and chilling treatment but not to freezing treatment. However, the PS of the phytosterol nanodispersions that underwent freezing treatment could be secured by adding small amounts of cryoprotectants, such as sucrose and glucose. The knowledge gained from study will serve as a model for development of water-soluble functional lipids for use as human food or animal fed. Increase solubility of functional lipid may increase dosage efficacy, reduce cost and overdose related toxicity.

Keywords: Phytosterols/ sucrose laureate/ nanodispersions/ response surface methodology.

INTRODUCTION

Phytosterol are hypocholesterolemic agents prominent for their efficacy in improving the serum cholesterol profile and reducing the risk of cardiovascular diseases. The water-insoluble and hardly lipid-soluble nature of the phytosterols makes incorporation into foods problematic. Only a very small amount of pure phytosterols can be incorporated into the oil phase of a food product or significant crystallization may occur. Esterified phytosterols water reported to have increased solubility in fats (Ostlund, 1999), are preferable for fortification in food industry. However the incorporation of pure and/or esterified phytosterols is limited to high-fat food products only. Consumer intended to benefit from the serum-cholesterol-lowering effects of phytosterols have had to consume high-fat foods despite the health risk of a high-fat diet. Some recent studies have shown that phytosterols in emulsified form, which have increased water solubility, show higher efficacy in serum-cholesterol lowering (Meguru *et al*., 2001; Shin *et al*., 2005). While conventional microemulsion had improved the water-solubility of the phytosterols, nanotechnology is opening another window of opportunity to further enhance the solubility, functionality, esthetical property and stability of the phytosterols dispersion system. Phytosterols prepared in a water-dispersible form enables their application into a wider range of food products. Development and optimization of the production of stable nanoparticlulate phytosterols with low polydispersity is critical for enhancing the response dosage.

MATERIAL AND METHODS

Vegapure® FTE (minimum 99% phytosterols) was a gift from Cognis (Duesseldorf-Holthausen, Germany). Sucrose laureate (L-1695) were kindly contributed by Mitsubishi-Kagaku Food Corp (Tokyo, Japan). HPLC grade hexane (purity ≥ 98.8%), ethanol (> 99%), dichloromethane, toluene (99.8%), potassium hydroxide, sodium chloride and sodium sulfate were purchased from Merck (Darmstadt, Germany). Standards for β-sitosterol (> 95%), stigmasterol (95%), stigmastanol (97.4%), campesterol (65% crystalline), brassicasterol and 5α-cholestane (internal standard), were supplied by Sigma-Aldrich (St Louis, MO, USA). N-O-bis-(trimethylsilyl)

trifluoroacetamine (BSTFA) was purchased from Supelco (Bellefonte, PA, USA). Headspace 20 ml vials and PTFE/silicon septa aluminium caps were obtained from Agilent Technologies (Wilmington, DE, USA). Preparation of phytosterol nanodispersions and all related analysis were previously described by Leong *et al.* (2010).

RESULTS AND DISCUSSION

The mean particle size, pH value and phytosterol retention of the optimized sucrose laureate-stabilized water-dispersible phytosterol nanodispersions were 4.4 ± 0.7 nm, 6.45 ±0.12 and 89.8 ± 2.6%, respectively. The PDI value was 0.550 ± 0.030 at a monomodal distribution. The optimized phytosterol nanodispersions were visually translucent. The hexane and ethanol residues found in the nanodispersions were 48.2 ± 3.3 µl/l and 930.3 ± 7.9 µl/l, respectively. Complete removal of organic solvents from aqueous-based food products can be complicated and sometimes impractical or impossible. Hexane is commonly used as a solvent in food processing and the extraction of vegetable oil. The detectable hexane residue in common soy protein, soy oil and soy meal has been shown to range from 20 to 1000 ppm (Woerfel & Erickson, 1995). The use of ethanol in food processing is acceptable only if the final ethanol content is less than 0.5% and more preferably less than 0.1%. Most Halal-certifying bodies allow a small amount of ethanol (0.1% or less, or occasionally up to 5%) in the final food products (Riaz & Chaudry, 2004). It is interesting to note that the net surface change of the optimized phytosterol nanodispersions was negative, with a zeta potential recorded as –33.5 ± 3.5 mV, although the sucrose laureate was a non-ionic emulsifier. Similar findings were reported for Tween 20- and sucrose laureate-stabilized β-carotene nanodispersions (Yin *et al.,* 2009), corn oil emulsion prepared with Tween 20 (Mun *et al.,* 2007) and palm oil emulsion prepared with a non-ionic emulsifier (Ahmad *et al.,* 1996). The cause of these observations could not be exactly clarified, but it was suggested that the spontaneous adsorption of the hydroxyl ion from the aqueous phase to the emulsifier head group at the interface was responsible for the negative surface charge (McClement, 2005).

Effect of thermal treatment on the optimized phytosterol nanodispersion

The mean particle size (Table 1) and the particle size distribution profile of the optimized phytosterol nanodispersions did not change significantly after 24 hours of chill treatment (at 4 and 10 °C), at room temperature (25°C), and with heat treatment at 50 °C. Heat treatment at 75 °C and autoclave treatment at 121 °C for 30 min and 15 min, respectively, increased the mean particle size and shifted the particle size distribution profile slightly to the right, indicating a growth in particle size. However, the mean particles size remained nanosized and monomodal after the heating and chilling treatment. These results are in broad agreement with Oh *et al.* (1995) and Kunieda & Yamagata (1993), who reported that emulsion systems prepared with sucrose esters were heat-insensitive. It is known that the hydrophilic sucrose esters are rather heat-resistant (Szuts *et al.,* 2007), consistent with the higher heat stability of phytosterol nanodispersions stabilized with sucrose laureate.

Table 1: Mean particle size of the optimized phytosterol nanodispersions after thermal treatment

Treatment temperature (°C)	Treatment time	Mean particle size (nm)[A]
121	15 min	9.9[a]
75	15 min	5.1[b]
50	24 hr	3.5[c]
25	24 hr	3.8[c]
10	24 hr	4.1[c]
4	24 hr	4.1[c]

[A] Means within the column with different superscripts are significantly different ($p < 0.05$).

After the freeze-thaw at -4 °C and –20 °C without excipients, the mean particle sizes increased significantly to 263 and 264 nm, respectively (Table 2), and the particle distribution profiles were polymodal. Other studies also found that the freezing process caused aggregation and breakage of dispersed nanoparticles (Zhang *et al.,* 2008). Freezing and dehydration generally generate many stresses that partially, and often completely, destabilize an emulsion system (Abdelwahed *et al.,* 2006). Many factors could account for the instability due to freezing. The freezing process caused crystallization of the water phase in the nanodispersions, resulting in insufficient free water available to fully dehydrate the emulsifier present at the interface and, hence, promoting particle-particles interaction (Carvajal *et al.,* 1999). Other studies explained that the crystallization in the water phase forced the particles to cluster together and promote particle-particle interaction (Hartel, 2001), whereas the emulsifiers absorbed into the ice-crystal interface reduced the amount of emulsifier available to stabilize the particles. However, the mean particle sizes of the phytosterol nanodispersions were effectively preserved after the freeze-thaw treatment at -4 and -20 °C with the presence of 5%

Table 2: Mean particle size of the optimized phytosterol nanodispersions before and after the freeze-thaw treatment

Treatment temperature (°C)	Excipient	Mean particle size (nm)[A]	
		Before freezing	After freeze-thaw

- 4	Non	3.8[a]	263[a]
	5% glucose	4.2[a]	3.8[b]
	5% sucrose	4.1[a]	4.6[b]
-20	Non	5.1[a]	264[a]
	5% glucose	4.3[a]	4.1[b]
	5% sucrose	4.2[a]	4.4[b]

[A] Means within the column with different superscripts are significantly different ($p < 0.05$).

w/v sucrose or glucose. Sucrose and glucose, both chemically innocuous food compounds, were among the popular cryoprotectants used to freeze-dry nanoparticles (Abdelwahed *et al.,* 2006; Zhang *et al.,* 2008). The cryoprotectants helped prevent particle aggregation and protected the particles against the mechanical stress exerted by the ice crystals during freezing. The protective mechanism may arise because the additional cryoprotectant increased the viscosity of the dispersion system due to the interaction between the hydroxyl group of the cryoprotectant and the water molecules, which can suppress the ice crystallization or lead to the formation of imperfect ice crystals, which could limit the mechanical damage to the nanoparticles (Hancock & Zografi, 1997).

CONCLUSION

This study successfully demonstrated the production of sucrose laureate-stabilized water-dispersible phytosterol nanodispersions with small particle size (<10 nm) and high phytosterol retention (>90%). The prepared phytosterol nanodispersions were stable to heat treatment and freezing with the presence of cryoprotectant. The produced phytosterol nanodispersions were expected to have a better bioavailability and greater health effect as compared to the micron-sized emulsion. The knowledge gained from this study is important to the development of water-soluble functional lipid used as human food or animal fed.

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EFFECTS OF DONATING AND INHIBITING OF NITRIC OXIDE (NO) ON MOTION PARAMETERS OF RAM EPIDIDYMAL SPERMATOZOA

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SUMMARY

In this study, we reinvestigat the effects of NOC- 18 and TRIM on motion parameters of epididymal ram sperm in vitro with different and higher concentrations. After incubation of epididymal sperm samples for 45 and 90 min in the presence of NOC-18 (0.001, 10, and 10000 µM) and TRIM (0.5,50,5000 µM), motion parameters were evaluated by computer-aided sperm analysis. To compare with our previous study, a significant decrease in BCF (at 45 min) and ALH, in the NOC-treated sperm was observed when compared to controls. There was significant increase of VCL, VSL, VAP, (at 45 min and not at 90min), and STR (at 90 min) of sperm in NOC-treated groups at a concentration of 10000 µM as compared to controls. Different concentrations of TRIM appeared significant ($p<0.05$) in reducing in, VSL, VCL, VAP, , LIN, and STR of sperm compared to controls. There was also a significant ($p<0.05$) decrease in WOB (in 50,5000 µM of TRIM) and MAD (at 45 min, all concentrations of TRIM; 90 min, only in 0.5 and 50 µM of TRIM). BCF and ALH parameters of sperm (all concentrations of TRIM) were significantly ($p<0.05$) increased as compared to their controls. In conclusion, confirming our previous study, higher concentrations of NO donors and NO inhibitors could affect the sperm motility. Although, exogenous NO does not increase sperm motility, high concentrations of nitric oxide may produce toxic substances which reduce the spermatozoa motility parameters.

INTRODUCTION

Nitric oxide is produced by various cells in different organs including smooth muscle cells, mesangial cells, neurons, platelets, hepatocytes, macrophages, fibroblasts, and epithelial cells. Nitric oxide regulates smooth muscle cell tone, platelet aggregation and adhesion, cell growth, apoptosis, neurotransmission, and injury and infection-induced immune reactions (Lincoln et al. 1997; Pacher et al. 2007). Because these processes are also associated with the biology, physiology, and pathophysiology of various reproductive processes, it is highly likely that NO plays an important role in reproduction (McCann et al. 1999). Nitric oxide is synthesized universally from L-arginine and molecular oxygen by an enzymatic process that utilizes electrons donated by NADPH. Nitric oxide synthase (NOS) enzymes convert L-arginine to NO and L-citrulline via the intermediate N-hydroxy-L-arginine. Nitric oxide is generated either by the constitutively expressed enzymes NOS-1 and NOS-3 or the induced enzyme NOS-2. NOS-1 and NOS-3 are activated in response to physiological stimuli that trigger an intracellular Ca2+ signal; they produce NO rapidly and transiently at low levels. NOS-2 is not expressed in resting cells but is induced by immunological stimuli such as bacterial lipopolysaccharide (LPS) or cytokines such as IL-1, TNF-α, or IFN-γ (Michel and Feron 1997; Coleman 2001; Hassanpour et al. 2009). NO has been recognized as a molecule that importantly regulates the biology and physiology of the reproductive system and not surprisingly, there is now strong evidence supporting a role for NO in modeling sexual and reproductive functions in mammalian species. Following the recognition of NO as a mediator of penile erection (Burnett 2006), NOS protein and activity have been demonstrated in both male and female reproductive organs (Burnett et al. 1995; Telfer et al. 1995) suggesting an involvement for NO in the physiology of reproduction. Evidence has been reported that NO can also be generated by spermatozoa. Immunoreactivity for NOS has been observed in mouse, human (Herrero and Gagnon 2001), and bull sperm (Meiser and Schulz 2003). The aim of the present study is to characterize motility parameters of ram epididymal sperm after 45 and 90 min incubation with NOC-18 (nitric oxide donor) and TRIM (nitric oxide synthase inhibitor) to compare with our previous study with higher concentrations of these substances.

MATERIAL AND METHODS

Preparation of cauda epididymal spermatozoa from ram

Twenty-four cauda epididymides from healthy adult ram were retrieved in the abattoir and transported to the laboratory on ice in less than 2 h. The cauda epididiymal sperm was obtained as previously described by Blash et al. (2000) and transferred into 35 mm Petri dishes containing 2 ml of equilibrated Hepes-TALP (114 mM NaCl, 3.1 mM KCl, 0.3 mM NaH2PO4, 2.1 mM CaCl2, 0.4 mM MgCl2, 2 mM NaHCO3, 0.2 mM sodium pyruvate, 10 mM sodium lactate,
10 mM Hepes,5 mg/ml BSA, and 0.7 mg/L Pen/Strep). All dishes containing samples were incubated in a humidified atmosphere of 5% C02 in air at 38.5°C. All samples were analyzed by computer assisted sperm analysis (CASA). Only specimens with progressive motility >60% were used in the experiment.

Preparation of sperm suspensions

Semen samples were obtained from cauda epididymides and prepared as described above, providing 4×200 µ1 aliquots of each sample containing 60×106 cells. To three aliquots, 0.0005, 0.5, and 500 µM of NOC-18 as nitric oxide donor or 0.1, 10, and 1000 µM of TRIM as nitric oxide synthase inhibitor was added (Sigma, UK, L-6638); to the remaining aliquot, equal volume of HEPES-TALP was added as a control. All were incubated for 90 min at 38°C in 5% C02.

Measurement of motion parameters

Sperm motility parameters were measured by CASA (WLJY-900, China) with the following settings: image collection speed of 20 frames per second; analysis time per frame, less than 15 s; sperm velocity that can be analyzed, 0–180 µm/s; number of vision fields that were selected, six per samples; magnifying power of microscope (object lens), 10×; measurements were performed in Makler chambers 20 µm depth. Sperm motility parameters were analyzed at two time intervals (45 and 90 min) following incubation with different concentrations of NOC-18 and TRIM.
The studied motion parameters can be defined as follows: straight line velocity (VSL), which represents the average velocity measured in a straight line from the beginning to the end of one track in micrometers per second; curvilinear velocity (VCL), which is the average velocity measured over the actual point-to-point track followed by the cell in a micrometers per second; average path velocity (VAP), which corresponds to the average velocity of smoothed cell's pathway in micrometers per second; amplitude of lateral head displacement (ALH) in micrometers; beat cross frequency (BCF) is the frequency at which the sperm cell's head crosses
the sperm cell's average pathway in Hertz; linearity (LIN), which estimates linearity of a curvilinear path in percentage; straightness (STR) estimates the proximity of the cell's pathway to a straight line with 100% corresponding to the optimal straightness; wobble (WOB), which is the measure of oscillation of the actual path about the average path; and mean angular displacement (MAD), which is the time average of absolute values of the instantaneous turning angle of the sperm head along its curvilinear trajectory in degree (Verstegen et al. 2002).

Statistical analysis

All data are presented as means±SEM. The statistical analysis was carried out using SPSS 14.0 software (SPSS Inc., New York, USA). The control and treatment groups were compared at each time interval using one way repeated measurement analysis of variance. Differences were considered significant at a $p<0.05$.

RESULTS

Effect of incubation with NOC-18 on sperm motion parameters

A significant decrease of BCF (45 min) and BCF,ALH and MAD (90min) was observed in sperm in the NOC-treated groups when compared with controls. There was significant increase of VCL (45 and 90 min), VSL (45 and 90 min), VAP (45 and 90 min), ALH (45 and 90 min), MAD (90 min), and STR (90 min) of sperm in NOC-treated groups at a concentration of 10000µM with compared to controls. Linearity and WOB parameters did not significantly change. Additionally, there was no significant variation of sperm parameters at a concentration of 10 µM in the treated groups compared to their controls (Table 1).

Effect of incubation with TRIM on sperm motion parameters

Exposure of spermatozoa to different concentrations of TRIM (0.5, 50, and 5,000 µM) resulted in a significant reduction ($p<0.05$) in VSL, VCL, VAP, ALH, LIN, and STR after 45 and 90 min incubation at all concentration of TRIM compared to their controls. There was also a significant ($p<0.05$) decrease in WOB (after 45 and 90 min, in 5,000 µM of TRIM) and ALH as well as MAD (45 min, all concentrations of TRIM; 90 min, only in 0.5 and 50 µM of TRIM) compared to their controls. On the other hand, BCF parameters of sperm (45 and 90 min, all concentrations of TRIM) were significantly ($p<0.05$) increased when compared to their controls (Tables 2) which was very similar to our previous result in another study that we have done before.

Table 1: Means±SE of sperm kinematic parameters data in different concentrations of NOC-18
[a,b,c] Means with the different indices between groups (within the same times) are significantly different for $p<0.05$

Con (µM)	VCL(µm/s)	VSL(µm/s)	VAP(µm/s)	MAD(degree)	ALH(µm)	BCF(Hz)	LIN(%)	WOB(%)	STR(%)
After 45min									
Ctrl	79.5±4.2[a]	50.7±4.3[a,b]	53.9±3.1[a]	41.2±2.1	1.6±0.1[a]	4.4±0.2[a]	59.9±3.0	74.0±1.8	81.5±2.3
0.001	80.3±2.2[a]	59.1±2.3[a]	63.5±2.2[a]	44.6±1.3	1.8±0.1[a]	4.0±0.1[b]	64.2±1.2	75.9±0.8	84.2±0.8
10	71.4±3.2[a]	51.8±3.5[a,b]	56.8±3.5[a]	39.8±1.7	1.7±0.1[a]	4.4±0.1[a,b]	63.4±2.0	73.7±1.3	82.3±1.3
10000	61.1±3.5[b]	41.8±3.5[b]	48.6±3.4[b]	39.6±1.6	1.9±0.1[b]	4.8±0.1[a]	59.6±1.8	71.9±1.3	78.9±1.2
After 90min									
Con (µM)	VCL(µm/s)	VSL(µm/s)	VAP(µm/s)	MAD(degree)	ALH(µm)	BCF(Hz)	LIN(%)	WOB(%)	STR(%)
Ctrl	69.4±3.7[a]	46.8±3.6[a]	52.3±3.4[a]	41.2 ±1.8[a]	1.4±0.1[a,b]	4.2±0.1[a,b]	52.3±1.9[a]	71.2±1.2	78.3±1.4[a,b]
0.001	79.4±2.9[a]	52.6±2.6[a]	58.9±2.5[a]	45.3±2.1[a]	1.2±0.1[a]	4.4±0.1[a]	59.4±1.5[a]	72.3±1.2	82.1±0.9[a]
10	71.3±2.0[a]	46.9±1.7[a]	53.6±2.0[a]	47.9±2.1[a]	1.5±0.1[a,b]	4.4±0.1[a]	55.1±1.0[a]	71.0±0.7	79.6±1.3[a,b]
10000	54.3±6.4[b]	32.8±5.6[b]	39.4±5.3[b]	58.6±3.9[b]	1.9±0.2[b]	5.1±0.2[b]	61.6±3.2[a]	72.3±1.0	74.1±3.8[b]

Table 2: Means±SE of sperm kinematic parameters data in different concentrations of TRIM
[a,b,c] Means with the different indices between groups (within the same times) are significantly different for p<0.05

Con (µM)	VCL(µm/s)	VSL(µm/s)	VAP(µm/s)	MAD(degree)	ALH(µm)	BCF(Hz)	LIN(%)	WOB(%)	STR(%)
After 45min									
Ctrl	69.5±2.4[a]	48.2±2.2[a]	51.9±2.2[a]	52.9±1.7	1.7±0.1[a]	4.3±0.1[a]	55.8±1.3[a]	71.2±1.0	73.9±2.0[a]
0.5	54.2±2.7[b]	30.5±2.0[b]	39.1±2.0 [b]	36.4±1.8	1.3±0.1[b]	5.2±0.1[b]	50.0±0.9[a,b]	69.7±0.6	72.6±0.9[b]
50	51.9±2.6[b]	27.5±2.3[b]	34.6±2.3[b]	32.7±1.5	1.1±0.1[b]	5.3±0.1[b]	46.9±1.1[b]	64.5±0.8	71.7±0.9[b,c]
5000	51.2±4.9[b]	23.6±3.0[b]	29.8±2.9[b]	31.1±5.2	0.9±0.1[b]	5.6±0.1[b]	41.3±1.3[c]	65.0±1.5	64.1±3.1[c]
After 90min									
Con (µM)	VCL(µm/s)	VSL(µm/s)	VAP(µm/s)	MAD(degree)	ALH(µm)	BCF(Hz)	LIN(%)	WOB(%)	STR(%)
Ctrl	54.2±4.5[a]	36.0±3.8[a]	41.9±3.6[a]	36.2±3.2[a]	1.4±0.1[a]	5.2±0.1[a]	56.8±2.0	71.9±1.6[a]	72.9±1.8
0.5	41.3±1.3[b]	22.1±1.4[b]	29.5±1.3[b]	26.1±1.1[b]	1.1±0.1[a,b]	5.6±0.1[b]	47.6±1.6	73.4±1.2[a]	65.2±1.6
50	45.7±2.2[b]	24.6±2.3[b]	31.5±2.1[b]	27.2±1.2[b]	1.1±0.1[a,b]	5.5±0.1[b]	48.5±1.6	73.4±0.9[a]	66.3±1.8
5000	42.6±2.0[b]	17.6±0.9[b]	27.6±0.8[b]	33.0±2.6[a]	1.0±0.1[b]	5.8±0.1[b]	40.1±0.8	66.4±1.3[b]	60.1±1.7

DISCUSSION

Continuing our previous study, this time the effects of NOC-18 as a nitric oxide donor and TRIM as nitric oxide synthase inhibitor with higher concentrations were evaluated on ram sperm motion parameters. The effects of NOC-18 on sperm motility are dose-dependent in vitro and low concentrations of NOC-18 increasingly effect sperm motility while high concentrations of NOC-18 conversely decrease sperm motility. Srivastava et al. (2006) determined that NO is synthesized from L-arginine by the enzyme NOS present in spermatozoa, and they suggested a possible role of NO and NOS in arginine action. In vitro studies have shown that low concentrations of exogenous NO enhance the motility of mouse (Herrero et al. 2003) and human (Zhang and Zheng 1996) spermatozoa and medium/high concentrations of NO decrease sperm motility (Rosselli et al. 1998). It is tempting to speculate in our study that small amounts of NO are generated by NOC-18 neutralize free radicals which inhibit sperm motility. In contrast, excessive generation of NO by high concentration of NOC-18 can cause sperm toxicity, as well as reduce sperm motility by contributing to the formation of peroxynitrite, a highly toxic anion of peroxidation (Levonen et al. 2001). Our previous study also showed that SNP as a NO donor could increase sperm motility at low concentration. In contrast, sperm motility declined at higher concentration (Hassanpour et al. 2007); these findings are similar to results presented by Rosselli et al. (1998), Nobunaga et al. (1996), and Weinberg et al. (1995), which also strongly suggests that exogenous NO is beneficial at low concentration for ram sperm motility and is harmful at high concentration. In this study, TRIM, as a nitric oxide synthase inhibitor in a dose-dependent mechanism, decreased progressive motility and increased immotile sperm. These data provide evidence that motility of ram spermatozoa is regulated autocrinally by nitric oxide. A study by Lewis et al. (1996) also suggested the presence of eNOS and nNOS in human spermatozoon that regulates (increases) sperm motility in an autocrine fashion, and they demonstrated that as compared to normozoospermic samples, the expression of eNOS and concentrations of NO generated were lower in asthenozoospermic samples suggesting that decreased endogenous NO may influence sperm motility and hence, fertilization. In our previous study, L-NAME, as nitric oxide synthase inhibitor, also dose-dependently decreased progressive motility and increased immotile sperm (Hassanpour et al. 2007). In conclusion that physiology of sperm motility in rams is affected by nitric oxide, although, exogenous NO does not increase sperm motility, higher concentrations of NO to compare with the results of our previous study, may initiate toxic conditions resulting in decreased sperm motility.

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EFFECTS OF 8-BR-CGMP AND 8-BR-CAMP ON RAM EPIDIDYMAL SPERM MOTILITY IN VITRO

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SUMMARY

This study evaluated the effects of exogenous cyclic GMP (cGMP) and cyclic AMP (cAMP) on motion parameters of epididymal ram sperm in vitro. After incubation of epididymal sperm samples for 45 and 90 min in the presence of 8-Br-cAMP (0.5, 5, and 50 mM) and 8-Br-cGMP (0.01, 0.1, and 1 mM), motion parameters were evaluated by computer-aided sperm analysis. At 0.5 mM concentration of 8-Br-cAMP, there was a significant increase in amplitude of lateral head displacement (ALH; at 45 min), curvilinear velocity (VCL; at 90 min), and mean angle of deviation (MAD; at 90 min) of sperm while a decrease in the beat cross frequency (BCF; at 45 min) in cAMP-treated groups was observed. At 5 mM 8-Br-cAMP, there was a significant increase in VCL (at 45 and 90 min), straight line velocity (VSL), average path velocity (VAP),MAD, ALH, linearity (LIN), and straightness (STR; at 45 min) of sperm but decreasing BCF (at 45 and 90 min) and wobble (at 90 min) in cAMP-treated groups compared to controls. At 50 mM of 8-Br-cAMP, a significant elevation in the BCF of sperm was observed while reductions in MAD , ALH, STR, and LIN was noted in the cAMP-treated groups at 45 and 90 min of incubation. The effects of 8-Br-cGMP on sperm motility were not significant. In conclusion, low concentrations of exogenous cAMP but no cGMP improves the motion parameters of ram epididymal sperm which confirms the previous studies with different concentrations of cGMP and cAMP analogs.

INTRODUCTION

Cyclic GMP (cGMP) was described as a biological product in 1963, but for many years, it was not considered as a potential secondary messenger. There are several reasons for this, including its relatively low concentration in the tissues (Revelli et al. 2002). It is now clear, however, that cGMP is a key signaling molecule in many tissue functions, such as retinal phototransduction, intestinal secretion, smooth muscle relaxation, platelet activation, and neurotransmission (Hurley 1998). Guanylate cyclases, the ubiquitous enzymes that catalyze the conversion of GTP to cGMP, are expressed in both soluble (sGC) and particulate, membrane-bound (mGC) isoforms. These isoforms coexist in most cells, where their relative amount depends on the type and physiological state of the tissue (Revelli et al. 2002). The mGC is a cell surface receptor enzyme that contains an extracellular receptor domain and an intracellular catalytic domain separated by a single transmembrane domain. The soluble isoform of guanylate cyclase and its product cGMP have been detected by immunohistochemistry in the peritubular lamina of seminiferous tubules and in the blood vessels of human testis (Middendorff et al. 1997). Cyclic AMP (cAMP) has long been recognized as an important secondary messenger in the control of cell function through signal transduction pathways in a variety of cells and tissues, in which it acts through the activation of protein kinase A (Brautigan and Pinault 1991). Changes in cyclic nucleotide levels, particularly of cAMP, are involved in regulating various signaling pathways in all cells, and these, in turn, modulate diverse physiological responses. Adenylyl cyclases (ACs) are the enzymes responsible for the intracellular production of cAMP, an important second messenger relevant in a number of biological processes in various cell types. Several isoforms of ACs have been described that are generally expressed in many cell types (Esposito et al. 2004; Taussig and Gilman 1995). In testes, at least two categories have been described: first, the membrane-associated ACs that are regulated by G protein-associated receptors (Adeoya-Osiguwa and Fraser 2002; Baxendale and Fraser 2003; Gautier-Courteille et al. 1998) and second, the structurally distinct soluble Mn2+_ dependent form that is modulated by bicarbonate and is insensitive to G protein modifiers (Esposito et al. 2004). The aim of this study is to investigate time/concentration dependent effects of exogenous cGMP and cAMP on the parameters of ram epididymal sperm motion in vitro.

MATERIAL AND METHODS

Preparation of cauda epididymal spermatozoa from ram

Twenty cauda epididymides from healthy adult ram were retrieved from the abattoir and transported to laboratory on ice in under 2 h. The cauda epididymal sperm was obtained as previously described by Blash et al. (2000) and transferred to 35-mm Petri dishes containing 2 ml of equilibrated Hepes-Tyrode's Albomine lactate pyrovate (HEPES-TALP) (114 mM NaCl, 3.1 mM KCl, 0.3 mM NaH2PO4, 2.1 mM CaCl2, 0.4 mM MgCl2 , 2 mM NaHCO3, 0.2 mM sodium pyruvate, 10 mM sodium lactate, 10 mM HEPES, 5 mg/ml bovine serum albumin, and 0.7 mg/L Pen/Strep). All dishes containing samples were incubated in a humidified atmosphere of 5% CO2 in air at 38.5°C. All samples were analyzed by computer-assisted sperm analysis (CASA). Only specimens with progressive motility >60% were used in the experiments.

Preparation of sperm suspensions

Semen samples were obtained from cauda epididymides and prepared as described above to give 4×200 µ1 aliquots of each sample containing 60×106 cells. To three aliquots were added 0.05, 0.5, and 5 mM of 8-Br-cGMP as cGMP analog or 0.1, 1, and 5 mM of 8-Br-cAMP as cAMP analog (Sigma, UK), and to the remaining aliquot, equal volume of HEPES-TALP was added as a control. All were incubated for 90 min at 38°C in 5% CO2.

Measurement of motion parameters

Sperm motility parameters were measured by CASA (WLJY-900, China), with the following settings: image collection speed: 20 frames per second; analysis time per frame, less than 15 s; sperm velocity that can be analyzed, 0–180 µm/s; number of vision fields that were selected, six per samples; magnifying power of microscope (object lens), ×10. Measurements were performed in Makler chambers 20 µm depth. Sperm motility parameters were analyzed at two time intervals (45 and 90 min) following incubation with different concentrations of 8-Br-cGMP and 8-BrcAMP. The sperm motility was assessed as: rapid progressive motility (class A), slow or sluggish progressive motility (class B), nonprogressive motility (class C), and immotility (class D) all in percentages. The studied motion parameters can be defined as follows: straight line velocity (VSL), which represents the average velocity measured in a straight line from the beginning to the end of one track in micrometers per second; the curvilinear velocity (VCL), which is the average velocity measured over the actual point-to-point track followed by the cell in micrometers per second; the average path velocity (VAP), which corresponds to the average velocity of smoothed cell's pathway in micrometers per second; the amplitude of lateral head displacement in micrometers (ALH); the beat cross frequency (BCF) is the frequency at which the sperm cell's head crosses the sperm cell's average pathway in hertz; the linearity (L1N) which estimates linearity of a curvilinear path in percentage; the straightness (STR) estimates the proximity of the cell's pathway to a straight line with 100% corresponding to the optimal straightness in percentage; the wobble (WOB), which is the measure of oscillation of the actual path about the average path; the mean angular displacement (MAD), which is the time average of absolute values of the instantaneous turning angle of the sperm head along its curvilinear trajectory in degree (Verstegen et al. 2002).

Statistical analysis

All data are presented as means ± standard error of the mean. The statistical analysis was carried out using SPSS 14.0 software (SPSS Inc., New York, USA). The control and treatment groups were compared at each time interval using one-way repeated measurement analysis of variance. Differences were considered significant at $p<0.05$.

RESULTS

Effect of incubation with 8-Br-cAMP on sperm motion parameters

At 0.5 mM concentration of 8-Br-cAMP, there was a significant ($p<0.05$) increase in ALH (at 45 min), VCL (at 90 min), and MAD (at 90 min) of sperm in the cAMP-treated groups while there was a significant ($p<0.05$) decrease in BCF (at 45 and 90 min; Table 1). At 5 mM concentration of 8-Br-cAMP, there was a significant ($p<0.05$) increase in VCL (at 45 and 90 min), MAD, ALH, LIN, and STR (at 45 min) of sperm but there was a decrease in the BCF (at 45 and 90 min), and WOB (at 90 min) in cAMP treated groups with compared to control (Table 1). At 50 mM concentration of 8-Br-cAMP, there was significant ($p<0.05$) elevation in the BCF of sperm while reductions in the VCL,VSL,VAP, MAD, ALH, STR, and LIN in cAMP treated groups at 45 and 90 min of incubation were observed when compared to controls (Table 1).

Table 1:Means (±standard error) of sperm kinematic parameters data in different concentrations of 8-Br-cAMP

Con (μM)	VCL	VSL	VAP	MAD	ALH	BCF	LIN	WOB	STR
Ctrl	72.037±4.042[a]	44.173±4.407[a]	50.661±4.158[a]	48.290±1.096[a]	1.603±0.115[a]	4.509±0.125[a]	57.346±2.614[a]	69.736±1.621	80.466±1.682[a]
0.5	74.767±4.317[a]	44.993±3.198[a]	51.843±3.112[a]	51.034±3.628[a]	1.791±0.0983[a]	4.549±0.120[a]	55.870±1.332[a]	68.991±0.785	79.239±1.531[a]
5	75.054±3.815[a]	43.737±2.040[a]	50.931±2.120[a]	51.744±3.139[a]	1.749±0.0738[a]	4.530±0.105[a]	54.783±1.007[a]	67.900±1.004	78.747±1.001[a]
50	46.301±5.180[b]	21.931±3.625[b]	30.060±3.391[b]	33.739±4.327[b]	1.170±0.122[b]	5.471±0.158[b]	43.137±2.197[b]	70.074±1.532	62.824±3.747[b]

Effect of incubation with 8-Br-cGMP on sperm motion parameters

Incubation of sperm with 0.01, 0.1, and 1 mM of 8-Br-cGMP resulted in significant ($p<0.05$) variations in the kinematic parameters of sperm after 45 and 90 min when compared to controls (Table 2).

Table 2:Means (±standard error) of sperm kinematic parameters data in different concentrations of 8-Br-cG

Con (μM)	VCL	VSL	VAP	MAD	ALH	BCF	LIN	WOB	STR
Ctrl	65.639±4.418[a]	39.064±4.284[a]	46.016±4.056[a]	43.590±2.255	1.607±0.087[a]	4.834±0.123[a]	54.273±2.178[a]	69.916±1.267	76.190±1.780[a]
0.01	64.301±3.958[a]	36.643±3.276[a]	43.321±3.108[a]	44.703±2.996	1.551±0.113[a]	4.776±0.137[a]	52.756±1.259[a]	68.561±0.825	75.840±1.444[a]
0.1	59.246±7.096[ab]	33.103±5.312[a]	40.481±5.238[ab]	39.750±4.416	1.516±0.133[a]	5.024±0.197[a]	51.237±1.958[a]	70.166±1.094	72.566±2.442[a]
1	44.011±5.693[b]	20.889±4.121[b]	29.106±3.877[b]	30.543±4.753	1.090±0.134[b]	5.507±0.185[b]	42.867±2.685[b]	71.514±1.241	61.336±3.985[b]

DISCUSSION

It has been shown that cAMP is involved in the signaling pathways that regulate sperm motility (Leclerc et al. 1996; Visconti et al. 1995) as well as sperm capacitation (Galantino-Homer et al. 1997). In fact, increased levels of intracytosolic cAMP have been demonstrated to enhance sperm motility and viability by increasing the rate of glycolysis and fructolysis and by enhancing the oxidation of lactate or pyruvate to CO2 (Dimitriadis et al. 2008; Zhang and Zheng 1996). Yanagimachi (2008) reported that the asymmetrical, high amplitude beats of the sperm flagellum and the capacitation process are dependent on the intracellular cAMP levels. These findings are consistent with those of MacLeod et al. (1991) who demonstrated that the majority of the cAMP-dependent protein kinases in the rat spermatozoa are located within the flagellum. In our study, we confirmed that exogenous cAMP significantly improves most of the kinematic parameters at low concentrations, suggesting that this molecule probably influences sperm function, especially sperm motility more than cGMP. On the other hand, we showed that high amounts of cAMP could suppress the kinematic parameters. These data confirmed our previous study with different concentrations of 8-Br-cAMP (Table 1).

Atrial natriuretic peptide and nitric oxide (NO) strongly affect sperm motility, capacitation, and acrosomal reactivity. They, therefore, stimulate sperm metabolism and promote the ability of the sperm to approach the oocyte, interact with it, and finally, fertilize it (Revelli et al. 1999, 2002). Hence, the guanylate cyclase-activating system seems to be an important regulatory feature in mammalian reproduction. Several tissues in the genital tract of both sexes may produce guanylate cyclase agonists capable of interacting with gametes, and furthermore, the spermatozoon itself can produce the powerful sGC activator NO in response to substances physiologically present in the female genital tract. Important sperm characteristics are affected by guanylate cyclase activation, and a complex system of intracellular pathways is activated by its agonists. Sperm motility appears to be affected by guanylate cyclase (Dimitriadis et al. 2008; Revelli et al. 2002). In the present study, like the results from our previous study, our data showed that exogenous cGMP improves sperm motility at low concentrations (Table 2) but these improvements are not statistically significant.

CONCLUSIONS

In conclusion, exogenous cAMP but not cGMP influences motion parameters of ram epididymal sperm. These effects of exogenous cAMP are completely concentration dependent. At low concentrations, exogenous cAMP improves kinematic parameters of sperm but high concentration suppresses these parameters.

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RESEARCH ON OPTIMIZATION OF THE ARTIFICIAL INSEMINATION MODEL FOR CATTLE CREATED ON MURES COUNTY LEVEL, CENTRAL EUROREGION

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SUMMARY

Profound social-economic transformations in the last 20 years have led to a gradual change in the structure of livestock ownership, the last agricultural enterprises in which the state owned the majority capital have been dismembered or passed into private hands by the year 2002. Currently, almost all the cattle is in private property, the size of the private cattle farms depending on the number of the cows they have in exploitation is downright demoralizing, especially if we consider this indicator among the EU countries, and it is them that we should be competing with. A first group of issues concerns the evolution of cattle based on species and breeds, the structuring approach of cattle farms, in the second group of problems we took into consideration the characterization of the main zoo-economical features of the cattle breed raised and exploited under the specific conditions of Mures County and their stage of improvement through artificial insemination.

Reproduction activity is ensured by freshening and artificial insemination, the artificial insemination network has increased as statistical weight since 2000 until 2010, being ranked above 60%. As far as the semen is concerned, it comes almost exclusively from SC Semtest SA Tg. Mures, of which 59% from tested bulls, 41% of the steers that are still being tested.

We must constantly make a selection based on performance so that in a relatively short period of 1 to 3 years the operators that do not meet the standards be eliminated. This fact results in a number of shortcomings, as well as in artificial insemination average / year / operator per county or in the results of the top performing ones, which are barely accepted and compared to the artificial insemination operators from the European Union.

INTRODUCTION

Currently, almost the entire cattle herd is in private property, the size of private farms according to the number of existing dairy operations is downright demoralizing, especially if we consider this indicator among the countries of the EU, that in fact we should be competing with [1]. Reproduction activity is ensured by mating and artificial insemination, artificial insemination network has increased as statistical weight since 2000 until 2010, being ranked above 60% at country level and in Mures County above 95%.

Thus, in view of improving breeding characteristics, the artificial inseminations are applied on a wide scale because they represent the biotechnology with maximum efficiency, using a small number of males, but of a great value. The productive levels or productive performances are the result of the interaction between their genetic potential and the environmental conditions, the latter including economic and environmental conditions [5].

Morphological classification of different types of sperm is an important component of modern sperm evaluation; however, current analysis methods are very subjective and vary greatly between operators. The artificial insemination using preserved sperm is a common mean of management of the cattle farmer nowadays. [4]

Artificial insemination operators were suddenly privatized, without practically being created another system of membership of another type, like associations or other organizations or professional bodies. It is the lack of communication, of information, of professional support, and the lack of investment along with the other arguments presented that prevent the development of this activity and the accomplishment of actual progresses.

MATERIAL AND METHODE

Profitable breeding of an animal species that provides man with the necessary food products, such as cattle breeding, requires obtaining these products at superior productive parameters and a lower cost price. That is why experts in the field are permanently concerned to discover new modern systems, ensuring a balance to both goals.

The study of this work was carried out based on official data of the Office for Zootechnical Improvement and Reproduction Mures, on data collected from BVN Semtest Tg.Mures using centralized data from farms and farm activities that use artificial insemination.

The analysis has involved the performance of the following activities: planning stages, gathering the necessary information, verification of recorded data, data processing and interpretation, calculation of key economic and technical indicators, drawing conclusions and determining measures to improve the activity of work insemination.

In the second group of issues I have taken into account the characterization of artificial insemination operators' performances and the possibilities of their improvement.

RESULTS

The results of this research are presented in two tables and a graph. The evolution of artificial insemination in the period 2000-2010 is shown in Figure 1; here it can be observed that the nucleus effective has increased in the period 2000-2005, then it decreased presently being under the level of the year 2000, artificial insemination at the same time saw a peak in 2005 then following a period of decline.

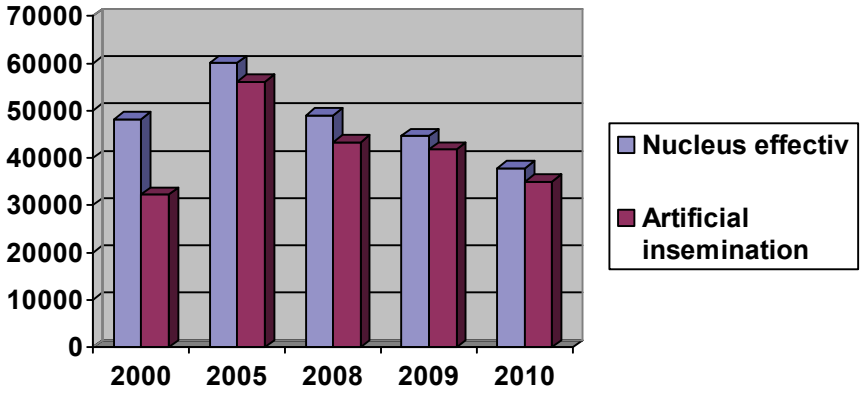

Figure 1. Evolution of artificial insemination at cattle in Mures County (in the period 2000-2010)

According to the presented data that analyzes the distribution of frozen semen, they delivered in 2010, on breeding stock classes, by Semtest-BVN Tg.Mures, the situation is presented in Table 1.

Table 1 Sales semen-BVN Semtest Tg-Mures, in Mures County in 2010, ranked by global index of improvement (VAG)

	V.A.G B.G.	>130	126 - 130	121-125	116-120	110-115	106-110	101-105
Associates	534	0	0	0	304	120	110	0
Farms	3.431	145	1.696	40	920	305	275	50
Operators	18.874	30	947	10	8.854	2.323	6.204	506
TOTAL	22.839	175	2.643	50	10.078	2.748	6.589	556

According to Semtest- BVN Tg.-Mures 2011

In order to find out the trend of these expenditures it was performed a study, regarding a period of six years in Harghita County, and the economic results are presented in Table 2 (for comparison - the cost of 1 kg of nitrogen and 1 km - were charged in 2005, in the period 2006 - 2010 these values were not significantly changed) [3]

Table 2 Evolution of the liquid nitrogen specific consumption per total and per artificial insemination

Year	Total liquid nitrogen(l)	First artificial insemination	Specific consumption per first artificial insemination (l)	Cost per first artificial insemination-lei
2000	65.050	24.377	2.67	2,67
2001	61.313	24.410	2.51	2,51
2002	41.300	25.531	1.62	1,62
2003	29.160	27.032	1.08	1,08
2004	26.700	29.580	0.90	0,9
2005	16.817	32.565	0.52	0,52
2006 - 2010	16.500	35.000	0.47 – 0,50	0,47 – 0,50

DISCUSSION

In the current stage we seek full completion of the entire flow of artificial insemination technology, with particular reference to the distribution of frozen semen, of cryogenic agent and the necessary materials.

The organization of insemination services must ensure to the recipient cattle farmer direct access to the source of frozen semen with high performances to achieve maximum genetic progress possible.

Acquisition of this activity in the private system will lead also to a need of financial support that includes both current operating expenses and those related to investments.

The places of authorized cattle artificial insemination, remained constant in Mures County in the period 2008-2010, that is 194 places. The authorized operators of cattle artificial insemination have diminished steadily: 2008 -188, 2009 -168, 2010 -151. The statistical weight of cattle artificial insemination in Mures County is 99.4% of which cows are -98.33%, and young cattle -99.49% in 2009 [2].

CONCLUSIONS

The objectives regarding cattle improvement are various. Semtest Tg. Mures aims to achieve European Union standards, so that the ratio between the breeds of milk, beef and mixed ones to be 1/1/8, in the area being bred 10% milk breeds, 10% beef breeds and 80% mixed breeds. This program is intended to be achieved by 2015 [3].

The calves obtained from artificial insemination with semen from improved tested bulls are over 98%. We used only frozen semen from bulls with improved value over 115.

But at county level there is a problem that should cause worry, the significant increase of artificial insemination with frozen semen from beef breeds in 2009 to 16.4%, 2010 to 27.6%, which will seriously diminish the substitution of reform cows. It is necessary: optimization and efficiency by creating and implementing a new efficient model of AI in cattle and quantifiable results in improving cattle breeds in our country.

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COMPARATIVE RESEARCHES REGARDING SPERM MORFOMETRIC VALUES IN BOAR AND BULL

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SUMMARY

It is well known that morphological type classification of spermatozoa is an important component of the modern semen evaluation; however, the current methods of analysis are subjective and highly variable.

Starting from the fact that the morfometric researches are not too many and also not very diversified we have proposed the comparative assessment of bull and boar semen with the aid of morphometry in two species raised with economic purposes, for assuring the sustainable animals farm development .

Having in view this proposal there were made samples from 100 boar and bull ejaculates. There was setting off animal groups depending on different factors and there were used random images of the samples with the aid of a specialized soft. For the special sperm files there were determined the indices: total length of spermatozoa, head length, head width and tail length. The analyzed data were statistically processed, being recorded the main population parameters: the average, its error, the standard deviation, the coefficient of variability.

The results recorded in the present paper were compared with the ones in the Romanian and world special literature. Finally, upon all the results' interpretation regarding the morfometric features analyzed within the special sperm files excepting the tail length, all the values were higher in adult reproducers.

INTRODUCTION

In the last two decades, following the perfection of the technical devices and investigation equipment in the field of biology there were gone up the studies over the biologic features in the different species of animals. [2,6].

The present study wishes to be a real help of the comparative reproductive researches among economic purpose animal species.

MATERIAL AND METHODS

Having in view this proposal there were made notices and measurements on 100 boar and bull ejaculates. They were grouped by the two studied species, swine and cattle and by age categories: young and adult boars and also young and adult bulls. There were used random images of the samples previously obtained and the computerized analyzes were made with the aid of Integrated Sperm Analyse System ISAS Projectes I Serveis R+D S.L. For each special sperm file there were determined the indices: total length of spermatozoa, head length, head width, tail length. The analyzed data were statistically remade, being recorded the main population parameters: the average, its error, the standard deviation, the coefficient of variability. After these there were also established the absolute and relative differences among the different categories of age and species.

RESULTS

The primary data were grouped by species (boars and bulls) and age category (young and adults) and statistically remade and the results regarding the measured sizes (sperm head length, head width, tail length and total sperm length) are shown in the following tables. One of the analyzed sizes was the length of the spermatozoa head, the values depending on species and age are shown in table 1.

Table 1 Spermatozoa head length depending on species and age

Species	Age category		$\overline{X} \pm s_{\overline{X}}$
boar	Young boars		9,11 ± 0,30
	Adult boars		9,44 ± 0,44
	Difference	Absolute	0,33
		Relative	3,50
bull	Young bulls		8,77 ± 0,27
	Adult bulls		9,61 ± 0,14
	Difference	Absolute	0,84
		Relative	8,74

We notice that between the two analyzed species appeared differences as: the highest value of spermatozoa head length was recorded in adult bulls this being as average 9,61±0,14 microns. On the second place are recorded the adult boars with an average length of the spermatozoa head as 9,44 ± 0,44 microns.

In the same table we may notice that within each species there are also differences between the two age categories, which show the superiority of the older males. Thus, between the adult boars and the young boars the difference was 0,33 microns (3,50%) and between adult bulls and young bulls 0,84 microns (8,74%).Another measurement carried out at the spermatozoa head level was its length, which values depending on species and age are presented in table 2.

Table 2 Spermatozoa head width depending on species and age

Species	Age category		$\overline{X} \pm s_{\overline{X}}$
Boars	Young boars		5,25 ± 0,37
	Adult boars		5,57 ± 0,25
	Differences	Absolute	0,32
		Relative	5,74
Bulls	Young bulls		5,48 ± 0,42
	Adult bulls		5,78 ± 0,63
	Differences	Absolute	0,30
		Relative	5,19

Also regarding this feature, it may notice the same hierarchy as in the head length. The adult bulls spermatozoa had recorded the highest value of the head: 5,78 ± 0,63microns, they were followed by the adult boars with 5,57 ± 0,25 microns.

The reported differences in the two age categories emphasize the higher value of the head width in adult animals, expressed as percentage they are higher too, varied between 5,74% (in swine) and 5,19% (in bulls). It was measured the spermatozoa tail length, and the average values and their differences are presented in table 3.

In this size too, there have appeared differences, which situated the species in the same decreasing order– cattle, swine– and ages – adult animals, young animals. In youth, the average value of the tail length was 41,60 ± 2,33 microns in young boars and 54,91 ± 1,15 microns in young bulls.

As it was expected in the total length of spermatozoa too, there were emphasized differences between species and age category, these being presented in table 4. From the adult animals, the bulls were the ones with the highest value of the total spermatozoa length of 66,45 ±0 ,45 microns and then the boars with 52,31 ± 0,57 microns. Regarding the age, we may notice that in swine, the difference between young males and adult ones represented 2,79%, and in bulls the difference was higher, thus 4,04% .

Table 3 Spermatozoa tail length depending on species and age

Species	Age category		$\overline{X} \pm s_{\overline{X}}$
Boars	Young boars		41,60 ± 2,33
	Adult boars		42,65 ± 1,25
	Difference	Absolute	1,05
		Relative	2,46
Bulls	Young bulls		54,91 ± 1,15
	Adult bulls		56,75 ± 0,73
	Difference	Absolute	1,84
		Relative	3,24

Table 4 Spermatozoa total length depending on species and age

Species	Age category		$\overline{X} \pm s_{\overline{X}}$
Boars	Young boars		50,85 ± 0,23
	Adult boars		52,31 ± 0,54
	Difference	Absolute	1,46
		Relative	2,79
Bulls	Young bulls		63,76 ± 1,21
	Adult bulls		66,45 ± 0,45
	Difference	Absolute	2,69
		Relative	4,04

DISCUSSION

The first cell morph metric determinations in spermatozoa in Romania were made by A.T. Bogdan and C. Cristea in 1975. The results offered by these studies reveals the fact that in boar, the length of the spermatozoa head varied between 3,986 ± 0,037 microns and 4,642 microns, but the tail length from 39,912 ± 0,785 microns to 47,188 microns.[1,3] Comparatively, our researches recorded higher values in head length, for almost two times, but lower values in tail length.

Tiberiu Feredean, in the book „Reproduction in swine" considered the sperm cell a unique cell by its particularities (motility, fertility, development capacity and division), with a specific structure and a large autonomy, the author quoting values of the morph metric features in boars, as: total length of the 54,58 microns, with limits between 49,2-62,4 microns, head length 8,51 microns, head width 4,21 microns[4,5] . Within each species, the length of the tail in young males spermatozoa was lower with 1,80% (swine) and 3,33% (cattle).

L. M. Thurston, in 1999, in Maryland, S.U.A., at the IV-th international conference regarding the boar sperm preserving presented a study where with the aid of ASMA (Automatic Sperm Morphology Analysis System) remarked significant differences concerning the different sizes of spermatozoa, in Landrace males. [4,5].
-tail length (P=0,770);
-head width (P=0,736);
-head length (P=0,615).

Regarding the tail length, the author mentioned above noticed that there are three subpopulations: 10-22 microns, 22-73 microns and 73-130 microns. In Landrace boars there are more spermatozoa with a tail length within 73-130 microns. Comparatively these values, the researches in our study reveal that the spermatozoa are framed into the second subpopulation parameters.

CONCLUSIONS

Following the study, it may conclude that the spermatozoa dimensions depending on age and species presented different values, emphasizing the superiority of the bull sperm. There are differences regarding the age of the reproducers, so in the adults there were recorded higher values than in younger males, the differences expressed as percentage between the ages categories, no matter the species varied between 2, 46-8, 24%.

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ANALYZE OF REPRODUCTION ACTIVITY IN A PRIVATE DAIRY FARM IN SOUTH OF ROMANIA

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SUMMARY

The reproduction activity process represents the basis of cattle development, their livestock increasing, quality improvement, milk and meat yield and economic efficiency increasing.

The researches in the present paper were carried out in a private dairy farm located in the south of Romania, on a 300 Holstein Frisian cows livestock, in different lactations, raised in industrial system, in free-stall barns and milking parlor.

There were analyzed the main reproduction activity indicators in a dairy cows unit: the age at first calving, number of inseminations per one pregnancy, the length of service-period, calving interval and dry period length. The primary data were statistically processed, being calculated the main statistic population parameters: average, its error, standard deviation, coefficient of variability. There were recorded the data following every pregnancy, from the first one to the third and there was established the moment of the best results. Also, there was calculated the average for each indicator, after its evolution at the unit level.

These values were compared with the average values recorded for all the animals included in the Official Milk Control at the country level.

The obtained results represented key-points in technologic flow optimization in the analyzed unit, having in view the sustainable agriculture development principles.

INTRODUCTION

The management of the reproduction activity became a science nowadays, because only with this very good economical results can be obtained in a dairy cows exploiting unit, some specialists consider the mammary gland an annex gland of reproductive apparatus.

MATERIAL AND METHODS

The researches in the present paper were carried out in a private dairy farm located in the south of Romania, on a 300 Holstein Frisian cows livestock, in different lactations, raised in industrial system, in free-stall barns and milking parlor.

There were analyzed the main reproduction activity indicators in a dairy cows unit: the age at first calving, number of inseminations per one pregnancy, the length of service-period, calving interval and dry period length. The primary data were statistically processed, being calculated the main statistic population parameters: average, its error, standard deviation, coefficient of variability. There were recorded the data following every pregnancy, from the first one to the third and there was established the moment of the best results. Also, there was calculated the average for each indicator, after its evolution at the unit level.

RESULTS

The main results recorded and analyzed within the farm are given in six tables. Table number 1 shows the age of introduction to reproduction both in heifers (born in the farm) and in adult cows (imported as heifers). It is noticed that the age of introduction to reproduction in the farm has been raised with three months. We consider that this result is due to the different environment conditions in the farms.

Table 1: Age of introduction to reproduction of heifers and cows

Specification	X +/- s_X	S	V%
Heifers (months)	21,80 +/- 0,55	3,02	13,85
Adult cows (months)	18,13 +/- 2,21	1,50	12,07

The second table shows the age at the first calving in the two studied categories. We notice the variation of the age at first calving from 27,43 +/- 2,21 in cows to 31,08 +/- 0,55 in heifers, while maintaining the trend of the first indicator, keeping constant the length of the pregnancy.

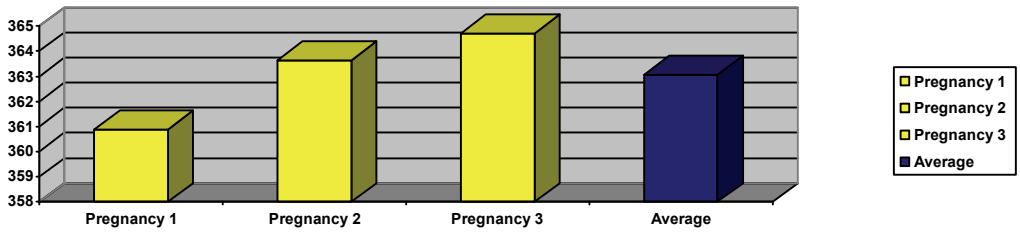
Table 2: Age at the first calving of heifers and cows

Specification	X +/- s_X	S	V%
Heifers (months)	31,08 +/- 0,55	3,02	13,85
Adult cows	27,43 +/- 2,21	1,50	12,07

The length of the calving interval has also been analyzed during three successive pregnancies. We remark the very small variations and unit framing into the animal science concept one year-one cow-one calf [3], a compulsory condition to obtain economic efficiency in a dairy cow exploiting unit.

Table 3: Calving interval in cows

Specification	X +/- s_X	S	V%
Pregnancy 1	360,89 +/- 0,79	6,64	2,38
Pregnancy 2	363,63 +/- 0,81	6,81	2,43
Pregnancy 3	364,71 +/- 0,68	5,67	2,04
Average	363,08 +/- 0,76	6,37	2,28



In table four it is shown the length of the service-period. As it is known, service –period represents the length in days from calving to the moment of the fecund insemination [1,4]. It may notice an average value of the service-period during three pregnancies of 84,06 +/- 1,08 days, the highest value being recorded in the third pregnancy.

Table 4: Service period length in cows

Specification	X +/- s_X	S	V%
Pregnancy 1	82,29 +/- 0,80	6,68	8,12
Pregnancy 2	83,86 +/- 1,20	10,01	11,94
Pregnancy 3	86,04 +/- 1,26	10,54	12,24
Average	84,06 +/- 1,08	9,07	10,76

Tables five and six shows the number of artificial inseminations used to obtain a pregnancy.

In adult animals, this indicator has been analyzed during three successive pregnancies, the recorded average is 1,78 +/- 0,23, with variations between 1,70 +/- 0,22 in the first pregnancy and 1,90 +/- 0,25 in the third. In heifers, this indicator recorded an average of
1,30 +/- 0,11.

Table 5: Number of inseminations/ pregnancy

Specification	X +/- s_X	S	V%
Pregnancy 1	1,70 +/- 0,22	1,82	1,07
Pregnancy 2	1,74 +/- 0,23	1,93	1,10
Pregnancy 3	1,90 +/- 0,25	2,07	1,19
Average	1,78 +/- 0,23	1,94	1,25

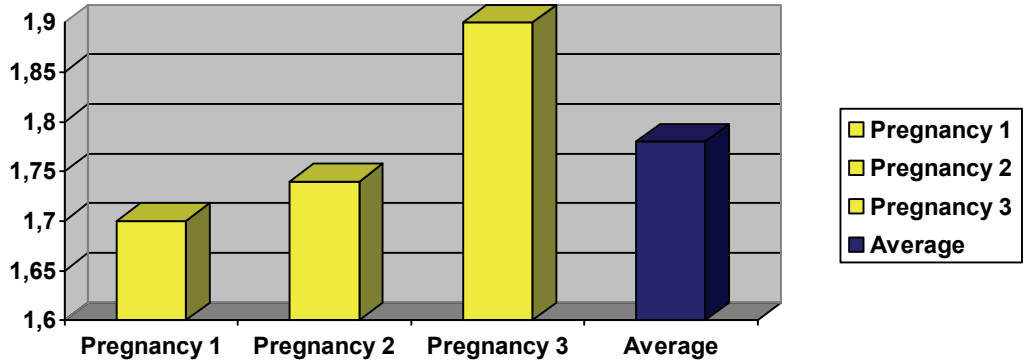


Table 6: Number of inseminations/ pregnancy in heifers and cows

Specification	X +/- s_X	S	V%
Heifers	1,30 +/- 0,11	0,60	45,84
Cows	1,78 +/- 0,23	1,94	108,95

DISCUSSION

Based on the researches during the three pregnancies in the studied farm, it has been noticed that the average values recorded in the farm and the ones reported in the Official Production Control are alike. The average number of artificial insemination per pregnancy in Romania (2009) was 1,6doses per pregnancy and for the first insemination 1,3 doses per pregnancy. The use of artificial insemination is a mandatory biotechnical procedure for obtaining satisfactory economic results in dairy cows breeding.

CONCLUSIONS

The analyzed units obtain very good reproductive indicators, above the recorded average national registered. The value of the calving interval under 365 days shows a very proficient reproduction management, taking into consideration the analyzed livestock of 300.The moment of introduction to reproduction and the age at the first calving of the Frisian females born in Romania are three months superior than the ones born in Germany (they were imported).

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GENETIC POLYMORPHISM OF *GHRH* AND *GHRH-R* GENE IN SOUTH ANATOLIAN AND EAST ANATOLIAN RED CATTLE

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SUMMARY

The present study was undertaken to determine genotypes and polymorphisms of growth hormone releasing hormone (*GNRH*) and growth hormone releasing hormone receptor (*GNRH-R*) gene in Southern Anatolian Red (SAR) and Eastern Anatolian Red (EAR) cattle. In this study, 50 cattle for each of SAR and EAR were used. Frequency of *GNRH*/*Hae*III polymorphisms allele A, which is related to milk yield and composition, in SAR and EAR were found close to those determined in high-producing European dairy breeds. Selection of SAR and EAR cattle for *GHRH* and *GHRH-R* alleles that are associated with production traits might be helpful for improving the yield characteristics of these breeds.

INTRODUCTION

GNRH is found on the 13th chromosome in cattle and *GNRH* contains five exons divided with four introns (1). With the effect of GH, growth hormone releasing hormone receptor (GNRH-R) causes intracellular cAMP increase by stimulating somatotrop proliferation and that way GH gene expression (2). Cattle GNRH-R is composed of 423 amino acids (3). This gene was determined to be located on the 4th gene (4). *GNRH-R* has a complicated genomic structure containing more than 10 exons (5). In this study, it was aimed to detect polymorphisms of *GNRH* and *GNRH-R*, which are claimed to be effective on milk yield and composition, growth and carcass yield, in Southern Anatolian Red (SAR) and Eastern Anatolian Red (EAR) cattle.

MATERIAL AND METHODS

In the study 50 SAR and 50 EAR were used. The genomic DNA extraction from the whole blood samples was obtained using the standard salt-out method (6). The Primer sequence used for the *GHRH*/ *Hae*III site: F: TTC CCA AGC CTC TCA GGT AA ve R: GCG TAC CGT GGA ATC CTA GT. The 539 bp site of *GHRH* was digested with 10 U *Hae*III (Fermantas Life Sciences, Canada) restriction enzyme to differentiate A and B alleles and the digestion products were run through 2% agarose gel. The Primer sequence used for the *GHRH-R* /*Eco*57I site: F: ACG CCA CCC TCT TTC ACC AG and R: CAT CCT GGG TGC TTC TTG AAG. The 850 bp site of GHRH-R was digested for 16 hours in $37C^0$ with 10 U *Eco*57I (Fermantas Life Sciences, Canada) restriction enzyme to differentiate A and B alleles and the digestion products were run through 2% agarose gel. Direct counting was used to estimate genotype and allele frequencies of *GHRH* and *GHRH-R* variants. The chi-square tests (χ^2) was used to check whether the populations were in Hardy-Weinberg equilibrium using PopGene32 software (7).

RESULTS

Genotype frequency of *GNRH*/*Hae*III was in agreement with Hardy-Weinberg equilibrium. Homozygote AA and BB frequency values found in SAR were found higher than expected ($P<0.05$). In EAR cattle, however, no significant difference were observed between expected and observed frequency values. Genotype frequency of *GNRH-R*/*Eco*57I polymorphisms that obtained from SAR cattle were not in compliance with Hardy-Weinberg equation while that of EAR cattle were in agreement with this equation.

DISCUSSION

Marek *et al*. (8) and Szatkowska *et al*. (9) asserted that homozygote AA genotype for *GNRH*/*Hae*III polymorphism could be connected with amounts of milk, milk fat and milk protein and percentages of milk fat and milk protein. Szewczuk *et al*. (10) investigated the *GNRH*/*Hae*III polymorphism and milk production traits in Polish Holstein-Friesian breed cattle and concluded that BB genotype might be relevant to milk fat amount and percentage. In previous studies, it was reported that A allele frequency which was claimed to be related to production traits was between 0.10 and 0.70 in high-producing European dairy cattle (8,9,10). In our results, both breeds tested were found close to high-producing dairy cattle of Europe in terms of *GNRH*/*Hae*III polymorphisms. Connor *et al*. (4)

asserted that such increases in the relevant traits could be achieved by GNRH-R and *GNRH-R* could be an important candidate gene controlling the growth and carcass traits. In a study on *GNRH-R*/*Eco*57I polymorphism, Connor *et al*. (4) reported that A allele frequency was found as 100% in Gelbvieh breed cattle and in *Bos indicus* cattle while it was 0% in Hereford and Angus breed (*Bos taurus*). In the present study, A allele frequency for *GNRH-R*/*Eco*57I polymorphisms was found as 0,53 in SAR and as 0,45 in EAR cattle.

CONCLUSIONS

A allel frequencies of *GNRH*/*Hae*III polymorphisms found in SAR and EAR breeds were close those frequency values found in high-yielding European dairy cattle breeds. Frequency of A allele of *GHRH*/*Hae*III polymorphisms which is considered to be related to growth and carcass traits was found higher in SAR than those in EAR cattle.

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GENETIC VARIABILITY OF NATIVE POLISH MOUNTAIN SHEEP OF COLOURED VARIETY (Abstract)

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One of the main principles of the FAO's Global Strategy for the Management of Farm Animal Genetic Resources is to preserve the biodiversity of farm animals by monitoring and conservation of native breeds threatened with extinction. Polish mountain sheep of coloured variety is an important part of the Podhale landscape and pastoral culture of this region. Due to its cultural values and small size of flocks this breed was included in the Polish Genetic Resources Conservation Programme. To monitor genetic variation of such small breeds there are used commonly class I genetic markers like blood groups and proteins polimorphism.

The material of research were blood probes of 108 Polish Mountain Sheep (POG) of coloured variety. Erythrocyte antigens were determined using 16 standardized test reagents: anti-Aa, Ab, Bb, Bc, Bd, Be, Bf, Bg, Bi, PLB-17, Ca, Cb, Da, Ma, R and 0. Polymorphic variants of transferrin and haemoglobin were determined by horizontal starch gel electrophoresis. Statistical analysis included calculating the frequency of alleles at different loci using direct gene counting, and calculating the degree of heterozygosity and the effective number of alleles per locus. Based on the observed and expected number of haemoglobin (HBB) and transferrin (TF) genotypes, genetic equilibrium was evaluated according to Hardy-Weinberg's law. Significant differences were determined by chi-square test.

The total number of alleles was 58 and thirty-six of them were observed in the most polymorphic B system. Among them the highest frequency was found for allele $B^{fPLB-17}$ whereas the $B^{bfPLB-17}$, $B^{biPLB-17}$, B^{cdf}, B^{cf}, $B^{cfgPLB-17}$, $B^{cgPLB-17}$, $B^{dfiPLB-17}$ and B^{di} alleles occured with frequency below 1%. The HBB^{B} and TF^{C} alleles were most frequent at the hemoglobin and the transferrin locus respectively. AB was the most frequent genotype at the HBB locus whereas genotype CD at the TF locus. The mean effective number of alleles ($\overline{E}$) was 3.5 and the mean degree of heterozygosity $\overline{H}$ was 0.544. The investigated population was in genetic disequilibrium for the haemoglobin locus.

The achievement of breeding progress is conditional on genetic variation, which can be expressed by the effective number of alleles (E), degree of heterozygosity (H) and total number of alleles. All these indicators show sufficiently variation of analyzed POG population. The data obtained on differences in the marker loci could be useful when deciding about further directions of POG sheep breeding while providing a starting point for further monitoring of genetic variation in this small population of sheep.

A GROSS MORPHOLOGICAL STUDY OF GENITAL ORGANS FROM FEMALE ZEBU CATTLE IN AND AROUND JIMMA TOWN (SOUTH-WEST ETHIOPIA)

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SUMMARY

Out of 147 female genital tracts collected and examined, abnormalities were recorded in 56(38.1%).The most common abnormalities encountered were uterine abnormalities (25.2%), followed by the ovarian(10.9%) and vaginal abnormalities (2%). Among the uterine abnormalities the three most frequent lesions, observed in cows were; endometritis (4.81%), mucometra (4.1%) and cervicities (2.7%). Similarly the most frequent ovarian abnormalities were; ovario- bursal adhesions (6.1%), ovarian cysts (2.7%) and ovarian hypoplasia (2.1%).

Keywords: Ethiopia – Genital tract - Female Zebu cattle – Abnormalities

INTRODUCTION

The productivity of cattle largely depends on their reproductive performance. Cows that rarely deliver a live calf are not worth keeping [5,6]. In Ethiopia several previous studies using abattoir material have shown that ovarian, uterine and vaginal abnormalities have been found to be the major reproductive abnormalities in different parts of the country [8,1].However no similar investigation has been conducted in western part of the country such as Jimma. The present study was undertaken in order to; identify and estimate the prevalence of female cattle reproductive tract abnormalities and to determine the anatomical and functional status of the ovaries of slaughtered female cattle in study area.

MATERIAL AND METHODS

A total of 147 female zebu cattle reproductive tracts were collected and examined at Jimma slaughter house and in the near by districts. Visible abnormalities that can be found on the vagina, cervix, uterine horns, oviducts, the ovarian bursa and the ovaries were examined and registered. The activities of the ovaries in each reproductive tract were determined on the presence or absence of ovarian structures (follicles or corpus luteum) [2].

RESULTS

Out of 147 reproductive tracts examined 38.1% (n=56) showed one or, more types of abnormalities which are summarized in the following table:

Table 1: Summary of Abnormalities of the total examined Animals.

Abnormalities	Parity	Total (n=147)	
	Cows (n=92)	Heifers (n=55)	
Total uterine Abnormalities	3 30.4% (28/92)	1 16.4% (9/55)	2 25.2%
Endometritis	7.6% (7/92)	-	4.8%
Mucometra	6.5% (6/92)	-	4.1%
Cervicitis	4.3% (4/92)	-	2.7%
Pyometra	2.2% (2/92)	1.8% (1/55)	2%
Hydrosalpinx	2.2% (2/92)	1.8% (1/55)	2%
Hydrometra	2.2% (2/92)	1.8% (1/55)	2%
Cyst in uterine lumen	1.1% (1/92)	-	0.68%
Tortuous cervical canal	1.1% (1/92)	5.4% (3.55)	2.7%
Hypo plastic cervical ring	1.1% (1/92)	5.4% (3.55)	2.7%
Pyosalpinx	1.1% (1/92)	-	0. 68%
Total ovarian abnormalities	9.8% (9/92)	12.7% (7/55)	10.9%
Ovario-bursal adhesion	6.5% (6/92)	5.4% (3/55)	6.1%
Ovarian cysts	3.3%(3/92)	1.8% (1/55)	2.7%
Ovarian hypoplasia	-	5.4% (3/55)	2%
Total vaginal abnormalities	2.2% (2/92)	1.8% (1/55)	2%
Vaginitis	2.2% (2/92)	1.8% (1/55)	2%
Total	42.4% (39/92)	32.7%(18/55)	38.1%

In this study the most frequently detected abnormalities were on; uterus, ovary, and vagina with; 25.2% (n=37), 10.9% (n=16), 2% (n=3) respectively. Uterine lesions with 25.2% prevalence rate were found to be the most frequently detected abnormalities in reproductive tracts of examined animals. Among the uterine abnormalities, endmetrities, mucometra and cervicitis were found to be the most frequent and account for 4.8%, 4.1% and 2.7% respectively. The second most frequent abnormalities of examined reproductive tracts was ovarian abnormalities with a prevalence rate of 10.9% (n=16). Ovario-bursal adhesion and ovarian cysts were detected at 6.1% and 2.7%, respectively.

Based on the presence and absence of current corpus luteum on the ovaries of non-pregnant 75.5% (n=111) animals; 62.2% (n=69) and 37.8% (n=42) were found to be cycling and non-cycling respectively. In the cycling, the current corpus luteum was noted in the right, left and both ovaries with the prevalence rate of 51% (n=35), 33.8% (n=23) and 14.7% (n=10), respectively. The average volume of the ovaries of non-pregnant animals was found to be 4.32 cm^3± 1.08 and 2.48 cm^3± 1.67 for right and left ovary, respectively.

DISCUSSION

In the present study, 38.1% of total examined animals were found with one or more abnormalities of reproductive tract, which is in agreement with [8,7] in Addis Ababa and [2] in Peru who reported 34.3%, 37% and 41.1% prevalence rates, respectively. But, it is higher than that of [3] in Canada, [1] in Bahirdar and [9] in North Ethiopia, who reported 27.7%, 27.7%, and 22.8%, respectively. This discrepancy in the prevalence rate of abnormalities could possibly be attributed to the difference in breed, sample size, management and geographical factors. In the present study, uterine abnormalities were found to be most frequent genital abnormalities in both cows and heifers with a prevalence rate of 25.2% in a total of 38.1% (n=56) abnormalities observed in examined animals. Ovarian abnormality was the second most frequently detected lesion with a prevalence rate of 10.9%. Ovario-bursal adhesion was observed with a prevalence rate of 6.1% which is in line with the findings of [1,4], who reported 5.5% and 6.85% respectively. The prevalence of uterine abnormalities was significantly higher ($p<0.05$) in cows than in heifers but there was no significant difference ($p>0.05$) of the prevalence of ovarian abnormalities between cows and heifers. This could be attributed to the parity status of the animals. However, the prevalence of both hypo-plastic cervical ring and tortuous cervical canal were higher in heifers than in cows suggesting that heifers with such abnormalities may not conceive and thus could be considered as a cause of infertility. The prevalence of mucometra 4.1% was similar with the findings of [7, 3] who reported 3.4% and 5%, respectively, but it was lower than that of [1] who reported 2.5%. This variation could be due to difference in sample size, breed and management conditions.

In general, the prevalence rate of abnormalities of reproductive tracts was significantly different ($p<0.005$) between body condition and party status of the animals. The abnormalities were higher in medium body condition cows. These abnormalities were not significantly different ($p>0.05$) among different age groups of examined animals.

CONCLUSIONS

High prevalence rate of genital abnormalities were observed both in cows and heifers suggesting that they are the major causes of fertility problems which result in reduction in the productivity of female cattle. Reproductive tract diseases should be diagnosed as soon as their occurrence and an appropriate therapy should be commenced.

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THE COMPLEX ETIOLOGY ON BOVINE MASTITIS AND THE IMPORTANCE OF THE MICROBIOLOGICAL DIAGNOSTIC

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SUMMARY

Mastitis remains the most frequent cause of infectious disease to the dairy industry. Bacteriological culture is very important and routinely used for the diagnostic. Its results are often used as basis for treatment or culling decisions and an essential part of a mastitis control program, because it is possible to know if the isolated microorganisms are infectious or contagious. The objective of the present study was to determine the pathogens distribution in the intrammamary infection (IMI) in some Brazilian dairy farms. The objective of the present study was to determine the pathogens distribution in the intrammamary infection (IMI) in some Brazilians dairy farms. Eighteen dairy farms located in different regions of São Paulo state, Brazil were enrolled. Criteria for selection of dairy herds included the farms with at least 30 cows in lactation. Milk samples were obtained aseptically based on the California Mastitis Test (CMT) positive reaction. All samples from score 3 were collected for microbiological exam. From each milk sample it was cultivated 0.01 mL in duplicate on sheep blood agar base 5% and MacConkey. The bacterial growth was observed each 24 hours, during three days. Isolates were identified according to National Mastitis Council rules (3). A total of 5,775 milk samples were examined by the CMT being 28.6% positive for subclinical mastitis. From these it was isolated 1,310 microorganisms, as follows: *Corynebacterium bovis* in 414 (31.6%) samples, coagulase-negative staphylococci (CNS) in 280 (21.3%), *Streptococcus* spp in 258 (19.6%), coagulase-positive staphylococci in 218 (16.6%), *Streptococcus agalactiae* in 32 (2.4%), *Escherichia coli* in 37 (2.8%), *Candida* spp in 12 (0.9%) and others in 59 (4.5%) samples. The results show the importance of *Corynebacterium bovis* and staphylococcal isolates, both contagious, in the IMI dairy cows, beyond another microorganisms. It is important to know the etiological aspects on mastitis to determine the source of infection and the adoption of control measures.

INTRODUCTION

Mastitis is one of the most common infectious diseases of dairy cattle and cause economic losses to dairy farms and also to the dairy industry (1). Conventional microbiological methods have been the "gold standard" for identifying mastitis pathogens from milk. Bacteriological culture is very important and routinely used for diagnostic purposes and its results are often used as basis for treatment or culling decisions as an essential part of a mastitis control program, because it is possible to know if the isolated microorganism are infectious or contagious.

Staphylococcus aureus among others pathogens is a contagious agent of mastitis. Specifically to this pathogen it is important to identify the infected cows and heifers to avoid the pathogen spread in the herd, for successful implementation of a mastitis control program (5). This situation can be the same when the aim is the control of another contagious bacteria such as coagulase negative staphylococci (CNS) or *Corynebacterium bovis* that are also of particular importance because its highly infectious characteristic and the possibility to spread for cow to cow during milking. This study was aimed to determine the pathogen distribution in the intramammary infection in some Brazilian dairy farms.

MATERIAL AND METHODS

Eighteen dairy farms located in different regions of São Paulo state, Brazil, were enrolled. Criteria for selection of dairy herds included the farmer interest and farms with at least 30 cows in lactation. Milk samples were obtained aseptically based on the California Mastitis Test (CMT) (4) positive reaction. All samples from scores 1 to 3 were collected for microbiological exam. 0.01 mL of each positive milk sample was cultivated in duplicate on sheep blood agar base 5% and agar MacConckey. The bacterial growth was observed each 24 hours during three days. Isolates were identified according to the procedures of the National Mastitis Council (3), including phenotypical characteristics as size, hemolysis and pigment production; morphological aspects by the Gram staining and biochemical profile.

RESULTS

In this study 28.6% milk samples were positive for subclinical mastitis by the CMT. From these it was isolated 1,310 microorganisms, as follow: *Corynebacterim bovis* in 414 (31.6%) samples, coagulase-negative staphylococci (CNS) in 280 (21.3%), *Streptococcus* spp in 258 (19.6%), *Streptococcus agalactiae* in 32 (2.4%), *Escherichia coli* in 37 (2.8%), *Candida* spp in 12 (0.9%) and others pathogens in 59 (4.5%) samples.

DISCUSSION

It were verified that *Corynebacterium bovis* is the most prevalent pathogen, followed by CNS, *Streptococcus* spp and coagulase-positive staphylococci. In Brazil *Corynebacterium bovis* has been widely incriminated as pathogen in the IMI (2). In the present study, CNS was the second common isolated bacterial group. Another feature of results from this study was the low prevalence of coliforms and *Streptococcus agalactiae*.

According other studies more than 135 different microorganisms have been isolated from bovine intramammary infection (7) showing it's multiple and complex etiology, that reinforces the importance of the bacteriological culture in the diagnosis of the IMI (6).

The microbiological diagnosis must be seen as one important phase of one control program for mastitis and for milk quality program, because it can give information about the source of infection.

CONCLUSIONS

The results show the importance of *Corynebacterium bovis* and the staphylococcal isolates, both contagious in the IMI dairy cows. It is important to know the etiological aspects on mastitis specially to determine the source of infection and consequently to establish the control measures to avoid mastitis and to obtain better quality milk.

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STAPHYLOCOCCUS AUREUS, *STREPTOCOCCUS AGALACTIAE* AND *ESCHERICHIA COLI* IN MILK: DOES MULTIPLEX PCR WORK LIKE MICROBIOLOGICAL ISOLATION IN SAMPLES OBTAINED FROM BULK TANKS?

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SUMMARY

Milk quality has been a frequent concern issue in Brazil where some problems still persist like low milk production, cattle genetic limitations, animal's inadequate nutrition, management problems, low production conditions in milk farms, labor's low quality and high mastitis prevalence in animals. Normative Instruction n. 51 elaborated by Brazilian Ministry of Agriculture and Supply (1) started to be attended in July 2005 and is a technical group of rules for milk business, objecting the improvement of milk quality. Considering this, there is a necessity for quick and high sensitivity and specificity diagnosis methods, with low cost and feasibility, for high number of milk samples' process. The aim of this study was to standardize the Multiplex PCR (mPCR) test for *S. aureus, S. agalactiae* and *E. coli* detection in bovine milk samples obtained from bulk tanks, and to evaluate this method as a possible tool to be used in milk quality control programs. For this study, 20 milk farms of different regions of Sao Paulo state were visited. Evaluated farms had milking machine system, bulk tank and a minimum number of 30 lactating cows. Milk samples were collected from bulk tanks for mPCR and microbiological culture. *S. aureus*, *S. agalactiae* e *E. coli* were isolated in 30%; 10% and 40%, respectively, and detected by mPCR in 0; 10% and 35%, respectively, in bulk tank milk samples. mPCR presented 78,3% accuracy; 37,5% sensitivity and 93,2% specificity and detection limit of 100 picograms of each target DNA. These data are similar to the obtained in other studies using mPCR in bulk tank milk samples. In conclusion, the present mPCR protocol showed high specificity for *S. aureus*, *S. agalactiae* and *E. coli* detection, working like the microbiological culture. However, the low sensitivity value, despites the excellent detection limit level, indicate that this mPCR can be used as an additional diagnosis in the routine methods for bovine mastitis and milk quality's monitoring.

INTRODUCTION

Bovine mastitis is an important issue in dairy herds especially in Brazil where environment and management characteristics usually establish infection conditions to cows. Bacteriological culture is routinely used for the diagnostic, due its applicability and accuracy. Molecular diagnosis, like Polimerase Chain Reaction (PCR) and multiplex PCR (mPCR) can also be applied in milk samples, in order to detect major mastitis pathogens. The aim of the present study was to compare an mPCR protocol with the routine bacteriological culture on *S. aureus*, *S. agalactiae* and *E. coli* detection in milk samples obtained from bulk tanks in Brazilian dairy farms.

MATERIAL AND METHODS

Milk samples (250 mL each) from 20 bulk tanks in Sao Paulo cities [Botucatu (four), Nova Odessa (two), Areiópolis (one), Pardinho (one), São Pedro (two), Lençóis Paulista (one), Porto Feliz (one), Itatinga (three), Lins (one), Agudos (one), Araras (one), Santa Rita do Passa Quatro (one) and Conchas (one)] were collected. All farms had milk machines and at least 30 cows in lactation. For bacteriological culture, an aliquot of 0.01 mL of milk was cultivated in duplicate on sheep blood agar base 5% and MacConkey (7). The bacterial growth was observed each 24 hours, during three days. Isolates were identified according to the procedures of the National Mastitis Council. For mPCR protocol, the primers SAU1 and SAU2; SAGA1 and SAGA2; SIP3 (F) and SIP4(R), Ecoli1 and Ecoli2 were used (Table 1). For the DNA extraction, Milk Bacterial DNA Isolation Kit (Norgen Biotek Corporation) was used. For each mPCR reaction, it were used 17.5µL of miliQ water; 2.5µL of buffer (10mM Tris HCl pH 8.0, 50mM KCl); 0.75µL of $MgCl_2$ (1.5mM); 0.5µL of dNTP (0.2mM); 2.0µL of each primer (10ρM); 0.5µL (0,2 units) of *Taq Platinum* DNA polimerase (Invitrogen), and 3µL of genomic DNA (10ng). Positive controls were reference strains (ATCCs), obtained from the Research Institute Fundação Oswaldo Cruz (FIOCRUZ) [*S. aureus* (ATCC 25923); *E. coli* (ATCC 11229), *S. agalactiae* (ATCC 13813)]. MiliQ water was used as negative control. For the specificity test of primers, it were used the DNAs of reference strains of *Staphylococcus intermedius, S. epidermidis*, *Streptococcus uberis*, *S. dysgalactiae* and *Pseudomonas aeruginosa*, obtained from the Research

Institute INCQS (Rio de Janeiro). The termocycler conditions were: 96°C during 5'; 30 cycles at 96°C during 1', 55°C during 1'and 72°C during 2'; and a final cycle at 72°C during 8'. The visualization of amplified material was evaluated by electrophoresis in agarosis gel 1.5% with 1.0μL/mL of SYBR Safe DNA gel stain (Invitrogen). The electrophoresis was done in a horizontal cube containing TBE 1X (89 nM Tris-HCl, 89 mM boric acid and 20 mM EDTA) at 65V. Gel was visualized in a UV light transiluminator and the image was obtained by digital documentation system (Figure 1). It were used 8μL of amplified material added by 4 μL of 100pb ladder (Invitrogen). For all samples it were added 2 μL of buffer.

Table 1: Primers used for DNA amplification of *S. aureus, S. agalactiae* and *E. coli* by multiplex PCR in bovine milk samples obtained from bulk tanks. Botucatu-SP, Brazil, 2011

Target	Primers	Primers' sequency	mPCR product lenght (pb)
S. aureus	SAU1	5'-GGA CGA CAT TAG ACG AAT CA -3'	1.300
	SAU2	5'-CGG GCA CCT ATT TTC TAT CT -3'	
S. agalactiae	SAGA1	5'-CGT TGG TAG GAG TGG AAA AT – 3'	590
	SAGA2	5'-CTG CTC CGA AGA GAA AGC CT – 3'	
	SIP3(F)	5'-TGA AAA TGC AGG GCT CCA ACC TCA -3'	293
	SIP4(R)	5'-GAT CTG GCA TTG CAT TCC AAG TAT -3'	
E. coli	Ecoli1	5'-GCT TGA CAC TGA ACA TTG AG -3'	660
	Ecoli2	5'-GCA CTT ATC TCT TCC GCA TT -3'	

bp: base pairs
Reference: (2)

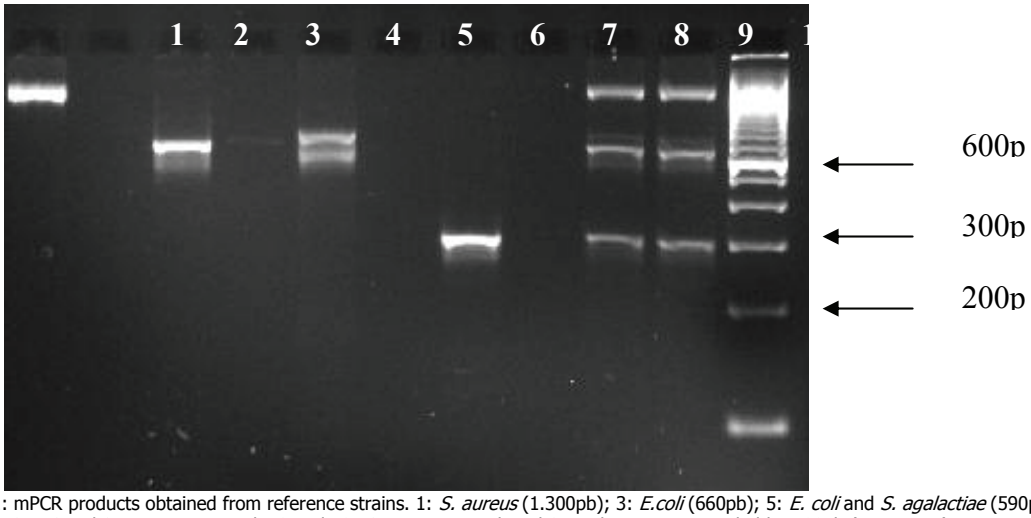

Figure 1: mPCR products obtained from reference strains. 1: *S. aureus* (1.300pb); 3: *E.coli* (660pb); 5: *E. coli* and *S. agalactiae* (590pb); 7: *S. agalactiae* (293pb); 2,4, 6 and 8: negative controls; 9 and 10: *S. aureus, E. coli and S. agalactiae*; 11: DNA ladder 100pb (Invitrogen).

RESULTS

S. aureus, *S. agalactiae* and *E.coli* were isolated in 6, 2 and 8 bulk milk samples, respectively, by bacteriological culture, and in 0, 2 and 7 samples, by mPCR (Table 2). mPCR presented 78,3% accuracy and 93,2% specitivity. Detection limit was 100pg (picograms) for each pathogen. However, sensitivity value of mPCR was 35.7%.

Table 2: Frequency of *Staphylococcus aureus*, *Streptococcus agalactiae* and *Escherichia coli* detected in 20 bulk tank milk samples, by microbiological culture and multiplex PCR (mPCR). Botucatu-SP, Brazil, 2011.

Microorganism	**Microbiological culture**		**mPCR**	
	Positive	**Frequency (%)**	**Positive**	**Frequency (%)**
S. aureus	6/20	**30**	0	**0**
S. agalactiae	2/20	**10**	2/20	**10**
E. coli	8/20	**40**	7/20	**35**

Table 3: mPCR results compared to microbiological culture results for *S. aureus, S. agalactiae* and *E. coli* detection in 20 bulk tank milk samples obtained from dairies of Sao Paulo State. Botucatu-SP, Brazil, 2011.

		Microbiological culture *S. aureus, S. agalactiae, E. coli*	
		Positive	**Negative**
mPCR *S. aureus, S. agalactiae, E. coli*	**Positive**	**6**	**3**
	Negative	**10**	**41**

P (McNemar test) = 0.0923; Kappa= 0.3564
Low replicability (P=0.0016)

Sensitivity: 37.5%; Specitivity: 93.2%; Positive Predictive Value: 66.7%; Predictive Negative Value: 80.4%; Frequency: 26.7%; Accuracy: 78.3%.

DISCUSSION

Despites 78.3% accuracy of mPCR compared to microbiological culture, the sensitivity value of molecular test was low (37.5%), despite the excellent detection limit of the target DNAs. mPCR sensitivity, in the present study, was 93.2%, considered very good, similarly to the verified in other studies, ranging from 96.3% to 100% (3; 4; 10). The mPCR high specificity and low sensitivity values for bacterial pathogens detection in samples obtained from bulk tanks were also verified in other studies (8; 6; 2; 5; 9).

CONCLUSIONS

The evaluated molecular test presented high specitivity for *S. aureus*, *S. agalactiae* and *E. coli* detection, working like the microbiological culture. However, the low sensitivity value, despites the excellent detection limit level, indicate that this mPCR protocol can be used as an additional diagnosis to the routine methods for bovine mastitis and milk quality's monitoring.

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SOMATIC CELL COUNT THRESHOLD IN DAIRY SHEEP AFFECTED BY THE PREVALENCE OF MAMMARY INFECTION

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SUMMARY

A total of 9,592 milk samples of half udders (3,908 and 5,684 samples for lactations prior and subsequent to antibiotic dry therapy, respectively) were collected aseptically in monthly samplings throughout lactation from 1,322 Churra ewe lactations belonging to 7 separate flocks. Dry therapy allowed to establish two different prevalences of intramammary infection (IMI): high (pretreatment lactations) and low (posttreatment lactations), and somatic cell count (SCC) thresholds were studied for each IMI prevalence. Only half-udders infected by major pathogen (novobiocin-sensitive coagulase-negative staphylococci included) were considered as positive in this study. For pretreatment lactations (IMI prevalence: 30.7%), the SCC threshold of 300 x 10^3 cells/ml showed values of 84.1%, 74.5%, 88.4%, 8.1%, 7.8%, 73.9% and 88.7% for half-udders correctly classified, sensitivity, specificity, false positive, false negative, and predictive values of positive and negative results, respectively. After dry therapy (IMI prevalence: 7.7%) the SCC threshold which showed a reliable discrimination of infection status was 600 x 10^3 cells/ml with values of 94.4%, 64.1%, 96.9%, 2.8%, 2.7%, 63.4% and 97.0% for half-udders correctly classified, sensitivity, specificity, false positive, false negative, and predictive values of positive and negative results, respectively. As a result, the variation in prevalence affects the accuracy of predicting infected half-udders, so it is essential to make SCC interpretation and mastitis control recommendations according to prevalence variations.

INTRODUCTION

Milk somatic cell count (SCC) is a main criterion for qualitative and hygienic assessment of raw milk because it is recognized as a reliable indicator of animal udder health in dairy cow, and several studies on the validation of this variable have been performed in dairy sheep [1] [5]. In this species, SCC is a general indicator of udder health, and high SCC reflects udder damage regardless of cause. However, effective use of the milk SCC depends greatly on a clear understanding of factors affecting them (e.g., prevalence of intramammary infection (IMI)). Thus, the SCC applicability for diagnosing subclinical mastitis in dairy sheep needs to be studied under different IMI prevalences, similarly to dairy cattle [6]. In this sense, the variation in the prevalence of herd mastitis could affect both the interpretation of SCC data and the mastitis control recommendations on an individual herd basis. The present study analyzes the SCC thresholds for discrimination between healthy and infected half-udders according to two levels (high and low) of IMI prevalence induced by antibiotic dry therapy (DT), in Churra breed.

MATERIAL AND METHODS

A total of 9,592 milk samples of half udders (3,908 and 5,684 samples for lactations prior and subsequent to complete DT, respectively) were collected aseptically in monthly samplings throughout lactation from 1,322 Churra ewe lactations belonging to 7 separate flocks enrolled in the recording scheme of the Nacional Association of Spanish Churra Breeders. Sampling and methods used to isolate and identify the different organisms were those recommended by the National Mastitis Council [4], with the modifications introduced by [1] and [2] in dairy ewes. Only half-udders infected by major pathogen (novobiocin-sensitive CNS included) were considered as positive in this study. After bacteriological plating, SCC was determined by Fossomatic method. Parameters of SCC threshold were: percentage of half-udders classified correctly, sensitivity, specificity, false negatives, false positives, and predictive values of positive and negative results. According to Renau [6] the criterion used to determine the SCC threshold in this study was selected in a range where false positives were equal than false negatives, because the goal was to express properly the true dynamics of the disease.

RESULTS

Prevalence of half-udders infected by major pathogens in preDT and postDT lactations were 30.7% and 7.7%, respectively (Table 1). The global prevalence was 17.0%. High IMI prevalences were associated to higher values of sensitivity, false positives, false negatives and predictability of positive result than low IMI prevalences. Inversely, halves correctly classified, specificity and the predictive value of negative result were higher after DT (Table 2).

Table 1. Number of isolates and relative prevalence for organisms found in pretreatment (preDT) and posttreatment (postDT) lactations.

Isolates	PreDT lactations		PostDT lactations	
	No.	Half-udders (%)	No.	Half-udders (%)
Pasteurella spp.	2	0.05	7	0.12
Streptococcus agalactiae	32	0.82	7	0.12
Sthaphylococcus aureus	74	1.89	28	0.49
Arcanobacterium pyogenes	8	0.20	9	0.16
Novobiocin-sensitive CNS	925	23.67	295	5.19
Enterobacteria	0	0.00	5	0.09
Enterococcus spp	38	0.97	11	0.19
Streptococcus spp[1]	21	0.54	31	0.55
Mixed with major pathogens	98	2.51	42	0.74
Total major pathogens	**1,198**	**30.65**	**435**	**7.65**
Corynebacterium spp	210	5.37	117	2.06
Unidentified	14	0.36	57	1.00
Micrococcus spp.	7	0.18	26	0.46
Mixed with minor pathogens	20	0.51	28	0.49
Novobiocin-resistant CNS	145	3.71	106	1.86
Total minor pathogens	**396**	**10.13**	**334**	**5.87**
Total infected halves	1,594	40.79	769	13.53
Total uninfected halves	2,314	59.21	4,915	86.47
Total half-udders	3,908	100	5,684	100

[1]Other than *Str. agalactiae*. DT: Dry therapy.

For preDT lactations, SCC threshold of 300 x 10^3 cells/ml showed values of 84.1%, 74.5%, 88.4%, 8.1%, 7.8%, 73.9% and 88.7% for half-udders correctly classified, sensitivity, specificity, false positive, false negative, and predictive values of positive and negative results, respectively. After DT, SCC threshold which showed a reliable discrimination of infection status was 600 x 10^3 cells/ml with values of 94.4%, 64.1%, 96.9%, 2.8%, 2.7%, 63.4% and 97.0% for half-udders correctly classified, sensitivity, specificity, false positive, false negative, and predictive values of positive and negative results, respectively. The global SCC threshold (all data) for a IMI prevalence of 17.02% was 400 x 10^3 cells/mL, the parameters being 90.2%, 71.8%, 94.0%, 5.0%, 4.8%, 71.0% and 94.2%, respectively.

Table 2. Summary of SCC threshold parameters (CCH: percentage of halves correctly classified, SENS: sensitivity, SPEC: specificity, FP: false positive, FN: false negative, PVPR: predictive value of positive result, and PVNR: predictive value of negative result, for high and low IMI prevalences, in pretreatment (preDT) and posttreatment (postDT) lactations.

SCC [1]	Lot	Threshold parameters						
		CCH	SENS	SPEC	FP	FN	PVPR	PVNR
100	PreDT	68.9	82.7	62.8	25.8	5.3	49.6	89.1
	PostDT	71.6	82.3	70.7	27.0	1.4	18.9	98.0
	Total	70.5	82.6	68.0	26.5	3.0	34.6	95.0
150	PreDT	77.3	79.1	76.5	16.3	6.4	59.8	89.2
	PostDT	83.1	78.9	83.4	15.3	1.6	28.3	97.9
	Total	80.7	79.0	81.1	15.7	3.6	46.1	95.0
200	PreDT	80.5	77.0	82.1	12.4	7.1	65.5	89.0
	PostDT	87.8	75.9	88.8	10.3	1.8	36.0	97.8
	Total	84.8	76.7	86.5	11.2	4.0	53.9	94.8
250	PreDT	82.7	75.5	85.9	9.8	7.5	70.3	88.8
	PostDT	90.3	73.8	91.7	7.7	2.0	42.4	97.7
	Total	87.2	75.1	89.7	8.6	4.2	59.9	94.6
300	**PreDT**	**84.1**	**74.5**	**88.4**	**8.1**	**7.8**	**73.9**	**88.7**
	PostDT	91.9	71.3	93.6	5.9	2.2	47.9	97.5
	Total	88.7	73.7	91.8	6.8	4.5	64.9	94.4
350	PreDT	85.1	73.8	90.1	6.9	8.0	76.7	88.6
	PostDT	92.7	70.1	94.5	5.1	2.3	51.5	97.5
	Total	89.6	72.8	93.0	5.8	4.6	68.1	94.3
400	PreDT	85.8	72.9	91.6	5.9	8.3	79.2	88.4
	PostDT	93.2	68.8	95.3	4.4	2.4	54.6	97.4
	Total	**90.2**	**71.8**	**94.0**	**5.0**	**4.8**	**71.0**	**94.2**
450	PreDT	86.2	71.7	92.6	5.1	8.7	81.0	88.1
	PostDT	93.8	68.1	95.9	3.8	2.5	57.9	97.3
	Total	90.7	70.7	94.8	4.3	5.0	73.5	94.0
500	PreDT	86.3	70.9	93.1	4.8	8.9	81.9	87.8
	PostDT	94.1	66.4	96.4	3.3	2.6	60.6	97.2
	Total	90.9	69.7	95.3	3.9	5.2	75.2	93.9
550	PreDT	86.4	70.3	93.5	4.5	9.1	82.7	87.7
	PostDT	94.3	65.5	96.7	3.1	2.6	62.0	97.1
	Total	91.1	69.0	95.6	3.7	5.3	76.3	93.8
600	PreDT	86.4	69.1	94.0	4.2	9.5	83.6	87.3
	PostDT	**94.4**	**64.1**	**96.9**	**2.8**	**2.7**	**63.4**	**97.0**
	Total	91.1	67.8	95.9	3.4	5.5	77.4	93.6

[1] x 10^3 cells/mL. DT: Dry therapy.

DISCUSSION

Dry therapy was effective in achieving two different IMI prevalences in the population involved in this study. Overall efficiency, that is, the percentage of half-udders classified correctly relative to infection status, was suitable for thresholds proposed and ranged between 84% and 94%. However, the accuracy of SCC thresholds was affected by IMI prevalence. Indeed, as the prevalence of mastitis increased, the accuracy of correctly predicting the presence of mastitis on the basis of SCC also increased, but the percentage of halves correctly classified and negative predictability decreased. Inversely, low IMI prevalences were associated to higher values of correct classifications, specificity and negative predictability, but lower values of sensitivity and positive predictability in comparison with high IMI prevalences. As a result, the establishment of mastitis status in the flock is of interest to design an optimal mastitis control strategy on the basis of SCC. Thus, for example the evaluation of SCC trends within flocks can be useful in assessing IMI status with a view to more accurate flock specific recommendations can be made. These results are in agreement with previously obtained in dairy cattle [3] and [6] and they are important for establishing SCC threshold differences between breeds or production systems in dairy sheep. In this sense, such differences should be only stated on comparative basis of IMI prevalence and infection criteria.

CONCLUSIONS

In dairy sheep, the reduction of IMI prevalence after dry therapy increased the half-udder SCC threshold from 300 x 10^3 to 600 x 10^3 cells/ml. This latter value improved the percentage of half-udders correctly classified, the specificity and the predictive values of negative result, but decreased the sensitivity and positive predictive value. Because of the variation in prevalence of herd mastitis, which significantly affects the accuracy of predicting infected half-udders, it is essential to make mastitis control recommendations (based on SCC) on an individual herd basis.

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CORRELATION BETWEEN MILK YIELD, SOMATIC CELL COUNT AND MILK QUALITY IN DAIRY FARMING

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SUMMARY

In this study, it has been aimed to investigate between milk yield and Somatic Cell Count (SCC) by analyzing SCC's of 252 milks, when were sampled from 3 different dairy enterprises. Some chemical and microbiological parameters of sampled milks have been determined and analyzed for the relation with milk yield. Milk samples have been collected from 10 dairy cows from each dairy farm, which there were 30 dairy cows in total, two times per month during 1 year period. Bacteriological analyzes have been employed for milks, of which SCC's were over 500.000 cell/ml. Additionally, milk yield data for each cow have been recorded in every sampling day. It was not able to establish statistically important relation between milk yield and SCC of milk, which have been obtained from related farms in research period. Depending on the logistic regretion analyses on the data collected from all enterprises, it has been detected that high total plate count (TPC) and *E.coli* counts have negative effects on milk yield, but has been found significally important ($p<0,05$) only for the data of *E.coli*. counts. According to the analyses on the data derived from the enterprise group 1, it has been detected that SCC and *E.coli* counts have negative effect on milk yield and only the data of *E.coli* has been found statistically important ($p<0,05$).

INTRODUCTION

Subclinic mastitis and its chronic forms pose a significant risk in terms of both hygiene quality and plant profitability of dairy cattle (3). Udder health and somatic cell count (SCC) are among the most important criteria in evaluating the quality of milk produced and herd management, in the countries where animal husbandry is developed (4, 10).

The European Union (EU) has banned the consumption of milk, of which bulk tank SCC (BTSCC) excesses the value of 400.000 cell/ml., since 1998. This limit is set in Turkish Food Codex as 500.000 cell/ml. It became obligatory for the milk producers in Turkey which carries out EU integration procedures to take the necessary measures regarding mastitis in order to increase operational profitability and to ensure the milk hygiene.

The aim of the present study is to determine the relationship between SCC and milk yield in three intensive plants and to explore the current situation in terms of recent quality of milk produced.

MATERIAL AND METHODS

30 cows in three different intensive plants were used as animal materials in the present study.

Milk samples were taken from each udder lobe of 10 cows in each plant two times a month during one year. The milk composition analyses, fat, fat-free dry matter, density and protein analyses were performed by using the Milkana Ultrasonic Milk Analyser. After the SCC analysis of the milks obtained from dairy cattle, microbiological analyses were employed for milks, which were over the limit of 500.000 cell / ml stated in the Turkish Food Codex (9). The raw milk samples brought to the laboratory were subjected to microbiological analyses in terms of total number of aerobic mesophylic bacteria, generic *Escherichia coli* (*E.coli*) and *Staphylococcus aureus* (*S.aureus*) counts. In order to determine the relationship between SCC, milk yield, TMC, *E.coli* and *S.aureus* counts, the "logistic regression model" was used as a method of data analysis (1, 10).

RESULTS

It was not able to establish statistically important relation between milk yield and SCC of milk, which have been obtained from related farms in research period. Depending on the logistic regretion analyses on the data collected from all enterprises, it has been detected that high total plate count (TPC) and *E.coli* counts have negative effects on milk yield, but has been found significally important ($p<0,05$) only for the data of *E.coli*. counts. According to the analyses on the data derived from the enterprise group 1, it has been detected that SCC and *E.coli* counts have negative effect on milk yield and only the data of *E.coli* has been found statistically important ($p<0,05$).

DISCUSSION

Regarding to the data obtained from 3 different enterprises and statistical analyzes during this research, it was not able to found a statistically significant relationship between milk yield and SCC. There are some studies in literature, in which the milk yield losses in cows with subclinical mastitis are determined based on the relationship between milk yield and SCC (2, 5, 6, 9). The milk yield losses were reported between 5.6 and 11.9 kg at 3 million cell/ml SCC level, which is accepted as an advanced subclinical mastitis. In another study (11), it was stated that the decrease in milk yield up to SCC rate of 500.000 cell/ml was not statistically significant. In this present study, as a result of the analyses performed for each enterprises, TMC and *E.coli* counts were found to have a negative effect on milk yield; however, only the value of *E.coli* was statistically significant ($p<0.05$). Accordingly, 1 unit logarithmic increase of *E.coli* counts in milk was caused a 0,34% decrease in the total milk yield.

It was statistically exposed that there would be a decrease in milk yield in subclinical mastitis cases which may be related to *E.coli.* Among the analysed 252 milk samples, *E.coli* was found to be higher than 10 cfu/ml. In a study by Sargeant *et al.* (7), it was reported that *E.coli* was isolated in 4.8% of the infected udder lobes in milk samples, while Jones *et al.* (5) reported that they isolated *E.coli* in 54% of the infected milk samples and SCC reached the value of 800.000 - 1.000.000 cell/ml in the milks where *E.coli* grew.

When the results of the analysis performed on the data of Enterprise 1 are analyzed, SCC was found to have a negative effect on milk yield but this effect was not statistically significant. No statistically significant effect of SCC on milk yield was found in Enterprises 2 and 3.

According to the data analyses, it was agreed that the number of animals used in the present study was not sufficient to work with statistical data; therefore, it would be appropriate to use a higher number of experimental animals in the future studies.

A correlation analysis was performed in order to determine the relationship between milk efficiency and fat, non-fat dry matter, density and protein in milk in each enterprise. A positive and statistically significant relationship was found between milk yield and the amount of dry matter and protein in milk ($p<0.05$) in Enterprise 2, while there was a statistically significant relationship between milk yield and density ($p<0.01$) in Enterprise 3.

CONCLUSIONS

The findings of the present study show that the subclinical mastitis cases, which may occur in dairy plants, may cause not only losses in milk yield but also decreases the chemical quality of milk. This strengthens the idea that the financial losses encountered especially in bacterial mastitis are related to the reduced technological quality of milk as well as reduced milk yield. Given that a high rate of microbiological load to be observed in milk pose a risk for public health as well, early diagnosis of mastitis in dairy plants and steps to prevent mastitis are of great importance.

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IN VITRO SUSCEPTIBILITY OF SPANISH BULK TANK MILK ISOLATES OF MYCOPLASMA AGALACTIAE

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SUMMARY

In order to determine the antimicrobial susceptibility of recently isolated strains of Spanish *Mycoplasma agalactiae (M.a)*, 13 isolates were subjected to a commercial sensitivity test (Sensititre, TrekDiagnostics Ltd., England) according to manufacturer´s instructions. The MIC values for 13 antimicrobial compounds for veterinary use were determined and comprised: tylosin, tilmicosin, lincomycin, clindamycin, erythromycin, chloramphenicol, florfenicol, spectinomycin, oxytetracycline, danofloxacin, enrofloxacin, marbofloxacin and tulathromycin. Results showed that the most effective compounds against *M.a in vitro* were clindamycin and quinolones which are standard treatments against contagious agalactia (CA). Tylosin and tilmicosin would also be appropriate antibiotics for CA treatment. Strains were mostly resistant to erythromycin indicating that it would not be a suitable treatment.

INTRODUCTION

Mycoplasma agalactiae (*M.a*.) is the main causative agent of contagious agalactia (CA), a syndrome that affects sheep and goats and is characterized by mastitis, absence of milk production, rapid spread and long persistence in affected areas. In most cases, infected animals recover rapidly from acute signs but develop chronic disease with excretion of the agent, mainly in milk and other secretions even for years. Due to these characteristics, antibiotics used for CA treatment should have the following characterisitics: very low MIC, long persistence, excretion in milk and easy diffusion from blood to mammary tissue [2]. Antibiotics chosen traditionally for CA treatment are tetracyclines, macrolides (tylosin and tilmicosin), fluoroquinolones, erythromycin and florfenicol [2], [3]. These drugs target *Mycoplasma mycoides*s subspecies *mycoides* Large Colony type (*Mmm*LC), now *M. mycoides* subspecies *capri* (*Mmc*). *Mmc* is an important aetiological agent in goats but it is rarely found in sheep infection. Health and economic importance of CA make it necessary to determine which treatments and antimicrobial concentrations are effective against *M.a*. in herds. This study was undertaken to evaluate *in vitro* activity of several veterinary antibiotics against thirteen *M.a*. strains isolated from bulk tank and silo sheep milk samples using commercial susceptibility plates.

MATERIALS AND METHODS

***Mycoplasma agalactiae* isolates:** thirteen *M.a*. isolates obtained from bulk tank and silo ewe milk samples in Castilla-León region (Spain) were previously identified by specific PCR [11] and PCR-DGGE [7] and cultured in Eaton´s PPLO Broth [8] until turbidity appeared. Once assured to be pure cultures, strains were kept at -20 ºC until processed.

Antimicrobial agents: thirteen antibiotics of veterinary use were tested. These agents were tylosin, tilmicosin, erythromycin, Draxxin® (tulathromycin), danofloxacin, enrofloxacin, marbofloxacin, lincomycin, clindamycin, oxytetracycline, spectinomycin, chloramphenicol and florfenicol at specified concentrations on Sensititre® microplates.

MIC (Minimun Inhibitory Concentration) tests: Eaton's broth medium without antimicrobials and phenol red was used for *M.a.* strains culture [8]. The inoculum concentration was standardised by measuring the optical density (OD) of the broth medium adjusting to an OD_{450} of 0.1 which is equivalent to approximately an amount of 1×10^8 cells per mL. Fresh culture was diluted to obtain a final concentration of 1×10^5 cells per mL. Wells were inoculated with 10 µL of inoculum added to o190 µL of sterile Eaton´s medium. Microplates were cultivated for 72 hours at 37 °C with 5% CO_2 without aeration.

Reading plates: to assess killing effect after incubation, Sensititre plates were centrifuged at 800xg to concentrate *M.a*. cells at the bottom of the wells. Positive growth was examined using an inverted mirror in a light box, considering negative (absence of growth in wells) no cell deposit and colour change clearly observed. First negative well for each antibiotic was taken as end point. Results were recorded in normalized sheets.

Expression of results: results were expressed according to Hannan [4] recommendations for veterinary mycoplasma species as: MIC range (µg/mL) as a measure of variance between strains, MIC_{50} and MIC_{90} (µg/mL) mentioning the concentrations of antibiotic to which 50% or 90% of isolates are susceptible and MIC geometric mean.

RESULTS

Table 1. MIC range, $MIC_{50,}$ MIC_{90}, and MIC geometric mean values (µg/mL) of the antimicrobial agents studied. Abbreviations: MIC, minimum inhibitory concentration; TYL, tylosin; TLM, tilmicosin; ERY, erythromycin; TUL, tulathromycin; DAN, danofloxacin; ENR, enrofloxacin; MAR, marbofloxacin; LIN, lincomycin; CLI, clindamycin; OXY, oxytetracycline; SPT, spectinomycin; CHL, chloramphenicol; and FLO, florfenicol.

	TYL	TLM	ERY	TUL	DAN	ENR	MAR	LIN	CLI	OXY	SPT	CHL	FLO
MIC range	0.5-2.0	0.5-8.0	8.0 - >32	1.0-8.0	0.25-0.5	<0.12-0.5	0.5-2.0	0.5-2.0	<0.12	1.0-8.0	2.0-8.0	8.0	2.0-8.0
MIC_{50}	1.0	1.0	>32	4.0	0.25	0.25	0.5	1.0	<0.12	8.0	2.0	8.0	8.0
MIC_{90}	2.0	8.0	>32	8.0	0.5	0.5	0.5	1.0	<0.12	8.0	8.0	8.0	8.0
Geom. Mean	1.0	1.5	28.8	3.1	0.3	0.3	0.6	1.0	<0.12	5.0	3.4	7.2	5.2

Once the plates were incubated and centrifuged, clear deposits of cells were observed in wells where antibiotic was not effective. Among the thirteen antibiotics used, clindamycin was the most effective agent inhibiting the growth of 100% of strains with MIC< 0.12 µg/mL. Quinolones, danofloxacin and enrofloxacin, inhibited most isolates at a MIC ≤ 0.25 µg/mL followed by marbofloxacin which presented a MIC = 0.5-2 µg/mL. Remarkably, all strains were resistant to erythromycin showing MIC > 32 µg/mL in all cases. Acceptable intermediate MIC values between 1-2 µg/mL were obtained for other macrolides, tylosin and tilmicosin, and for lincomycin. Nevertheless two strains showed MIC = 8 µg/mL when tested against tilmicosin. Tulathromycin was found to have a less uniform response than other macrolides (MIC = 1-8 µg/mL). Chloramphenicol, florfenicol and spectinomycin showed quite high values (MIC_{90} ≤ 8 µg/mL).

DISCUSSION

Contagious agalactia has been reported all over the world. This disease is particularly common in the Mediterranean basin of Europe, Asia and North Africa. It is very important to know which antibiotics of veterinary use are effective against *M.a*. isolated from sheep farms, especially in affected areas of Spain and neighboring countries where CA is an endemic condition. It is well known that *in vitro* sensitivity of antimicrobials does not always correspond to the effectiveness of treatment in the field, but the MIC values in all its variations are of great help, so an ineffective drug *in vitro* is not very useful on the affected animal.

The results obtained in this work demonstrated the effectiveness of quinolones *in vitro* against *M. a.*, included in standard treatments against CA, and showed agreement with data provided by Hannan [4] and Antunes [1] on the susceptibility of strains of *M. a.* isolated in Spain. Clindamycin was the most effective antimicrobial effect in all cases, but there was no previous published data to compare with this study.

Erythromycin has been considered as an option for the treatment for CA. Although other mycoplasmas have been reported to be susceptible all strains of *M. a.* in the present study were resistant which is in agreement with the work of Antunes [1]. In contrast to the results of the latter author [1], isolates in this study presented much higher oxytetracycline MIC values and slightly higher in the case of tylosin, both antibiotics of choice in treatment against the disease. Results obtained on Sicilian strains [3] were equivalent for lincomycin and enrofloxacin and notably inferior in the instance of tylosin. However, referring published MIC breakpoints for *M.a*, oxytetracycline values [4] classified most of strains as intermediate resistant and tylosin values showed sensitivity or intermediate resistance. Spectinomycin MIC values were consistent with those of these authors. Tulathromycin, belonging to a group of new-generation macrolides, obtained relatively variable response depending on the strain (MIC range = 1-8 µg/mL) and gave higher MIC_{50} and MIC_{90} values compared to other antibiotics of the same family.

CONCLUSIONS

The results of this study indicate that conventional treatments against CA should be reviewed because of an apparent development of resistance of *M a* strains in the field compared to earlier studies. In particular veterinary practitioners should avoid erythromycin because of its complete ineffectiveness *in vitro*; they should also be wary of oxytetracycline for which strains have developed intermediate resistance. Fluoroquinolones remain effective but their use is not encouraged throughout Europe because of their use for human therapies. However, because of the variable results of different studies, *in vitro* MIC determination should be conducted routinely and locally on recent isolates to help guide practitioners to choose the most appropriate treatments.

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BOVINE MASTITIS ETIOLOGICAL AGENTS AND THEIR RELEVANCE TO MILK QUALITY AND PUBLIC HEALTH

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SUMMARY

Some of the bovine mastitis pathogens were relevant either in respect to the milk quality as well as to public health. The objective of this study was to evaluate the main mastitis pathogens occurrence in Brazilian dairy herds, in order to verify the persistence of classical etiological agents and the emergent ones. A total of 1473 mammary quarter foremilk samples (MQFM) were taken from each cow in lactation of ten dairy herds for microbiological analysis and SCC determination. The isolated microorganisms were further submitted to identification. The occurrence of the most important pathogens of subclinical mastitis ranged from 0.2 to 38.3%. Of the total isolated *Staphylococcus* (38.3%), 34.6% were *S. aureus*, 49.4% were CNS and 15.9% were CPS other than S. aureus. Among the isolated *Streptococcus* spp (23.9%), 41.7% were *Streptococcus. agalactiae*, 41.1% were *Streptococcus dysgalactiae* and 17.3% were *Streptococcus uberis*. *Corynebacterium* spp were isolated from 32.8 %, of the mastitis cases. The quarters infected had a significantly higher SCC than microbiological negatives quarters. *S. aureus* presented a high occurrence and it has been and still is considered an important human pathogen, due to the production of many virulence factors and antimicrobial resistance. Among the CNS, *S. epidermidis*, *S. haemolyticus*, *S. warneri*, *S. xylosus*, *S. lugdunensis* were isolated some of these strains have been assuming relevant role in the etiology of mastitis as due to production of virulence factors similar to *S. aureus*.
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INTRODUCTION

Mastitis, the mammary gland inflammatory process, can have an infectious or noninfectious etiology, and the infectious origin is the most important. It can be caused by many pathogens, such as: bacteria, viruses, yeasts and algae These different pathogens are generally characterized as either contagious or environmental [3,5].
The major contagious pathogens comprise *S. aureus*, *S. dysgalactiae, S. agalactiae*, and *C. bovis* while the major environmental pathogens comprise the members of Enterobacteriacae Family [1]. They have been classically divided in two main groups the contagious and the environmental pathogens in consequence of their epidemiological characteristics. In essence, the contagious pathogens can be considered as organisms adapted to survive within the mammary gland, and can establish infections to trigger inflammatory response, which are typically manifest as an elevation in the somatic cell count of milk from the affected quarter [2]. In contrast, the environmental pathogens are best described as opportunistic invaders of the mammary gland, not adapted to survival within the host; typically they reach the mammary gland when the teat orifice is open, e.g. at or soon after milking or secondarily to teat damage.
The objective of this study was to evaluate the main mastitis pathogens occurrence in Brazilian dairy herds, in order to verify the persistence of classical etiological agents and the emergent ones.

MATERIALS AND METHODS

A total of 1473 milk samples were obtained from dairy cows with clinical or sub-clinical bovine mastitis in ten dairy herds in the São Paulo State, Brazil. The mammary quarter foremilk samples (MQFM) were taken from each cow in lactation for microbiological analysis and SCC determination. The initial milk was stripped from each teat on the strip cup in order to evaluated clinical mastitis occurrence. In order to detect subclinical mastitis cases it was used CMT. To perform the microbiological examination, prior to sampling, the teat ends were swabbed with 70% ethyl-alcohol and, approximately 10 ml of milk were collected in a sterile container. The milk samples were chilled to 4°C and transported to the laboratory. All milk samples requiring bacterial culture were mixed well and a standard loopful (0.01 ml) from each milk sample was inoculated on the surface of blood agar containing 5% of washed sheep red blood cells and MacConkey agar plates. All plates were incubated aerobically at 37°C and examined for growth at 24, 48 and 72 h. Bacteria were identified by using colony morphology, hemolytic pattern on blood agar media and further microscopic examination (Gram staining), standard biochemical methods (catalase, haemolysis, coagulase test with rabbit plasma) [6]. To perform SCC milk samples were taken from all mammary quarters 25 mL collected in vials with Bronopol and the samples were analyzed in Bentley Somacount 300.

RESULTS

From the 1473 examined mammary glands from ten bovine dairy herds 722 (49.0%) were infected. It was verified the predominance of contagious pathogens in the etiology of the mastitis cases studied 632 (87.5%), significantly higher than the environmental pathogens 90 (12.5%). These results were presented in Table 1.

Table 1. Environmental and Contagious etiologic agents in bovine mastitis cases in Brazilian dairy herds. São Paulo. Brazil. 2011.

Microorganisms	Nº Mammary quarters	% In relation microbiologic positives	% In relation to total of quarters
Contagious pathogens	632	87.5	42.9
Environmental pathogens	90	12.5	6.2
Total Microbiologic positives	722	100.0	49.0
Total Microbiologic negative	751		51.0
Total	1473		100.0

P < 0.0001, considered extremely significant (chi-square approximation 95% CI)

The occurrence of the most important pathogens of subclinical mastitis ranged from 0.2 to 38.3%. A total of 38.3% *Staphylococcus* spp. were isolated, from these total 34.6% were *S. aureus*, 49.4% were CNS and 15.9% were CPS, other than *S. aureus*. Among the isolated *Streptococcus* spp (23.9%), 41.7% were *S. agalactiae*, 41.1% were *S. dysgalactiae* and 17.3% were *S. uberis*. *Corynebacterium* spp were isolated from 32.8 %, of the mastitis cases (Table 2).

Among the isolated Enterobacteriaceae (6.7%) it was observed the predominance of *Klebsiella pneumonieae* (3.9%) followed by *Escherichia coli* (2.1%), it was also isolated Enterobacter (0.4%) and *Klebsiella oxytoca*(0.3 %). These results are presented in Table 2.

Table 2. Etiological agents of bovine mastitis among the Brazilian dairy herds studied. São Paulo. Brasil. 2011.

Microorganisms	Nº Mammary quarters	% Among genus	% In relation to microbiologic positives
Mammary quarters	Nº 1473		Nº 722
S. aureus	90	34.6	12.5
SCP	45	15.9	6.2
SCN	128	49.4	17.7
Staphylococcus spp. (total)	263		38.3
Streptococcus agalactiae	70	41.7	9.7
Streptococcus dysgalactiae	69	41.1	9.5
Streptococcus uberis	29	17.3	4.0
Streptococcus spp. total	168		23.9
Corynebacterium spp.	230		32.8
Klebsiella pneumonie	28	58.3	3.9
Klebsiella oxytoca	2	4.2	0.3
Escherichia coli	15	31.3	2.1
Enterobacter sp	3	6.3	0.4
Enterobacteriaceae total	48		6.7
Other microorganisms*	13		1.8

Other microorganisms were isolated: *Pasteurella* sp.(5), Nocardia sp(2), *Citrobacter* sp.(2); *Pseudomonas aeruginosa* (1) among the bacteria and yeasts Candida sp(3).

In the table 3 it was presented the SCC results of CNS infected quarters.

Table 3. Mean and Median CCS in mammary glands infected by SCN. São Paulo. Brazil. 2011.

SCN	Mean CCS x 10^3 cells/mL	Median CCS x 10^3 cells/mL	Minimum CCS x 10^3 cells/mL	Maximum CCS x 10^3 cells/mL
S.epidermidis	714.5	465.0	19.0	2,158.0
S. hyicus	1,308.1	472.0	48.0	8,633.0
S. warneri	960.1	472.0	45.0	5,904.0
S. xylosus	1,300.8	838.0	105.0	4,465.0
S. haemolyticus	803.3	348.5	200.0	2,308.0

DISCUSSION

The data revealed that the contagious pathogens were the commonest cause in mastitis cases, being implicated in 87% cases. It was observed in previous Brazilian and international researches [3,7]. *Staphylococcus* spp.(38.%) continues to be the prevalent pathogens followed by *Corynebacterium* spp.(32.8%) and *Streptococcus* spp. (23.9%). Among *Staphylococcus* spp., *S. aureus* continues to be the predominant in spite of the increase of CNS isolation. It was also verified in other studies an increase of CNS and *C. bovis* mastitis [3,5]. Among *Streptococcus* spp. *S. agalactiae* closely followed by *S. dysgalactiae.* While *S.uberis* an environmental microorganisms showed a low occurrence, as the others environmental pathogens in this study. As the transmission of contagious microorganisms occurs usually during the milking [4], it can be notice that in the studied dairy herds the milking procedure constitutes main problem. Therefore to reduce the occurrence must be adopt an adequate program of classical mastitis control.

Among the CNS, *S. epidermidis*, *S. haemolyticus*, *S. warneri*, *S. xylosus*, *S. lugdunensis* were isolated some of these strains have been assuming relevant role in the etiology of mastitis as due to production of virulence factors similar to *S. aureus*. The emergence of CNS among the Staphylococci mastitis cases registered in this paper has been also notice in many countries and, deserves to be better studied, in order to establish methods of control [5].

The mean and the median of SCC of mammary quarters without infection were 65 x 10^3 cells/mL (95% CI 44,700–85,300 cells/mL). The mean and the median of geometric means of SCC of infected quarters was 657,6 x 10^3 cells/mL (95% CI: 223,4 x 10^3–1.091,8 x 10^3 cells/mL) and 355,4 x 10^3 cells/mL[8]. In the present study the quarters infected had a significantly higher SCC than microbiological negatives quarters.

CONCLUSIONS

In spite of *S. aureus* presented a high occurrence and it has been and still is considered an important human pathogen, CNS have been assuming relevant role in the etiology of mastitis as due to production of virulence factors similar to *S. aureus*.

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PREVALENCE OF SUBCLINICAL MASTITIS IN EWE WITH SOMATIC CELL COUNT PROCEDURE IN TABRIZ AREA OF IRAN

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SUMMARY

Mastitis is one of the most serious health and economic problems in dairy sheep flocks. Although clinical cases of mastitis are a source of loss, more important economically is subclinical mastitis due its higher prevalence and associated decrease in milk production.. In addition to reduction of milk yield, lower lamb performance and higher predisposition to clinical mastitis, subclinical mastitis has also adverse effects on the hygienic quality and physicochemical properties of milk. Leukocyte infiltration in the alveoli constitutes one of the main defense mechanisms of the animal against infection; thus an increase in the somatic cell count (SCC) is a reliable index of on-going mastitis. If the milk has a high SCC, the deterioration during synthesis with a longer coagulation time and a weak coagulum leads to an increased moisture content in the cheese and a lower dry matter yield. Although subclinical mastitis occurs worldwide, its economical importance is especially significant in Iran because these are the highest sheep milk producers. In this study we tried to identify rate of subclinical mastitis of ewes in Tabriz area of Iran. For this purpose referred to sheep farms of Tabriz and selected from 20 dairy ewe flocks that was free of clinical mastitis and milk samples gained. Totally 500 milk samples gathered and using Fosomatic method, somatic cells counted. According to results the rate of subclinical mastitis was 20.2 percent. Finally it seems that by training preventive methods and considering mammary glands health, rate of subclinical mastitis decreased so that economical benefit of flock and milk products market increase.

INTRODUCTION

In dairy cattle, the detection of subclinical intramammary infections is based upon the interpretation of milk somatic cell counts (SCC) determined monthly throughout lactation. Such a dynamic approach is widely used in field conditions, since the mid-1980s (6). In dairy sheep, instantaneous physiological and pathological thresholds of SCC ranging from (0.25 1.0)×10^6 cells/ml, have been available since the early 1990s (2).This work proposes In this study we tried to identify rate of subclinical mastitis of ewes in Tabriz area of Iran.

MATERIAL AND METHODS

For this purpose referred to sheep farms of Tabriz in east Azerbaijan province of Iran and selected from 20 dairy ewe flocks that was free of clinical mastitis and milk samples gained. Containers had potassium dichromate disk. Totally 500 milk samples gathered. SCC were determined by the fluoro-optoelectronic method (Fossomatic).

RESULTS

Somatic cell counts of 498 samples to be obtain and 2 sample by Fossomatic apparatus not readied. For detect of percentage of subclinical mastitis in samples to benefit from cut –off point of 500,000 cell/ml in milk. In this way, samples of lesser than it, healthy and greater than subclinical mastitis was considered. . According to results the rate of subclinical mastitis was 20.3 percent.(Table1.)Analyze of data was obtained in Table 2.

Table 1: Results of somatic cell count of samples

Groups	Frequency	Percent	Valid Percent	Cumulative Percent
Healthy	397	79.7	79.7	79.7
Subclinical mastitis	101	20.3	20.3	100.0
Total	498	100.0	100.0	

Table 2: Mean standard error and standard deviation of samples (scc/ml)

Groups	Mean ± standard error	Standard deviation
Healthy	187,452.03 ± 8196.897	103,033
Subclinical mastitis	1,245,875.00± 166,506.322	1,053,078

DISCUSSION

The strong relationship between the number of culture-positive samples throughout lactation and the mean of log SCC confirms previous results in Latxa (3) and Assaf ewes (5). Although none of the definitions of udder halves infection status would reflect perfectly the dynamics of infections observed (1-monthly sample throughout lactation), the third one (healthy, brief and durable infections) could represent an acceptable compromise describing the diversity observed under field conditions. Thus, many halves were culture-positive only during the suckling-milking period (mainly during the first week post-partum) and never later; a strict application of the first definition could lead to consider these halves as infected. Likewise, the interpretation of the bacteriological results of samples collected at the end of lactation (close to drying-off) is difficult (7).In the literature, the instantaneous (punctual) thresholds of SCC range from (0.200 to 2.0)$\times 10^6$ cells/almost of the authors proposing values smaller than 0.500×10^6 cells/ml (7,11). Two thresholds have been proposed in order to distinguish infections by "minor" versus "major "pathogens (12); it has also been proposed to take into account the lactation stage, the iSCC of uninfected ewes increasing after the fifth month(4).

A dynamic approach of iSCC at lactation level would be supported by the observed relationship, for an udder half, between the number of bacteriological isolates and the mean of log SCC throughout lactation, it is also relevant to take into account the variable duration of infections (present study) and the fluctuations of bacterial and cellular shedding in milk (4). Even when a punctual threshold is proposed, it is recommended to evaluate a series of iSCC, instead of a single value in cows (12), as well as in ewes (9).However, in dairy sheep, few studies have proposed a dynamic approach at a lactation level (3). In Latxa ewes, the geometric mean of iSCC was 0.051×10^6 cells/ml for uninfected ewes and (0.210 and 0.543)$\times 10^6$ cells/ml for uni- and bilaterally infected ewes, respectively (5).

The strong relationship observed between the annual geometric mean of bulk SCC and the estimated prevalence of intramammary infections can be considered as an indirect validation of our decision rule and thresholds (8).From a practical point of view, iSCC are used, in the Roquefort area, for subclinical mastitis control; "doubtful" ewes are grouped either with "healthy" (when farmers decide to cull "infected" females) or "infected" ewes (in order to implement a selective drying-off therapy).

CONCLUSIONS

iSCC represent a useful tool for the detection of subclinical mastitis in dairy ewes. It is recommended to evaluate a series of iSCC, take into account the stage of lactation and use two thresholds allowing to distinguish three classes of ewes: healthy, doubtful (or briefly infected) and infected (or persistently infected).The decision rules must be pragmatically adapted to the different control strategies of bulk SCC.

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RELATIONSHIP AMONG SOME INDIRECT TESTS AND BACTERIAL AGENTS OF SUBCLINICAL MASTITIS IN IRANIAN NATIVE EWES

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SUMMARY

In a field investigation of ovine mastitis in five flocks in Garmsar region, eastern Iran, a population of 150 ewes was monitored by some screening tests and bacteriological culture during lactation. The prevalence of subclinical mastitis was 6.8% by culture, and *staph. epidermidis* was the most important agent of subclinical mastitis. The result of this study showed that SCC and CMT have acceptable sensitivity and specificity to detect subclinical mastitis in sheep, but the MAS-D-TEC which was built for measuring the electrical conductivity (EC) of the cow milk is not a suitable device to detect subclinical mastitis in sheep.

INTRODUCTION

Mastitis is the inflammatory response of the mammary tissue to physiological and metabolitc changes, traumas, allergic, and most frequently, to injuries caused by micro-organisms [1].

Most often, mastitis is of bacterial origin. *Staphylococcus aureus* is the most frequent bacterium responsible for clinical mastitis (from 20 to at least 60%). Coagulase-negative staphylococci are the principal causative agents of subclinical mastitis (30–95%), mainly in dairy ewes. Using individual somatic cell counts (iSCC) or the California Mastitis Test (CMT), and clinical examinations, ewes to be culled or treated can be identified. Immediate or delayed culling and intramammary antibiotherapy at drying-off are the main measures for the elimination of intramammary infections. In meat flocks, the prevalence of subclinical mastitis is estimated by means of bacteriological analysis of half-udder or udder milk samples, iSCC or the CMT. The reported prevalence ranges from 5 to 30% per lactation [3].

The aim of this study was to evaluate and compare the characteristics of a number of indirect tests with bacteriological status of the milk samples from native meat ewes in Garmsar, eastern Iran.

MATERIAL AND METHODS

A total of 300 milk samples from 150 randomly selected native ewes were tested by MAS-D-TEC device (an indirect Electrical Conductivity Test or ECT graded 0 to 9), comparing the same samples with CMT graded 0, T (Trace), +1, +2 and +3. Then, two samples from each udder halve were collected aseptically for evaluation of SCC and culture.

RESULTS

The results of the study showed that sensitivity, specificity, positive predictive value and negative predictive value of CMT in sheep was %83.3, %77.3, %33.3.1 and %97.1 respectively. Of total samples from the ewes sent for bacteriology, 6.8 were positive. The most important agent of subclinical mastitis in sheep in Garmsar region was *staph. epidermidis*. The SCC of samples with positive cultures has been in the range of maximum, 1.7×10^6 cell/ml and minimum, 0.7×10^6 cell/ml in sheep. All the samples measured by MAS-D-TEC device (ECT), were zero.

DISCUSSION

Iranian native ewes in Garmsar region are meat ewe breed. In meat ewes, it is reported that CNS are less frequent (12–34%) and *S. aureus* represents 1–58% of isolations [3].

Incidence rates of udder infections ranging from 28 to 45% were found in other surveys conducted in conventionally managed flocks of other sheep breeds [1].

An instantaneous relationship between the isolation of bacterial species and SCC has been reported in various studies. In the ewe, the geometric means of the SCC for udder halves range from 2.3 to 5 million cells per ml for *S. aureus,* from 1 to 1.5 million cells per ml for *S. epidermidis*, from 210 to 225,000 cells per ml for *S. xylosus* and from 130 to 150,000 cells per ml for sterile halves [3].

It was also reported that cut-off points ranging between 0.3 and 1.7×10^6 SCC/ml in sheep. SCC is influenced by age, stage of lactation, level of production, season, and ...

[9]. We found that the SCC of the positive cultures was in the range from 0.7×10^6 to 1.7×10^6 cell per ml which is in the range of above mentioned studies. A significant, positive correlation existed between SCC and CMT for sheep milk (0.77; $P<0.01$). SCC was the best predictor of infection in sheep [9].

The concordance between CMT and SCC has been studied by comparing, for the same samples, SCC values to CMT scores (using the five-category grid of interpretation used in cattle). The concordance between CMT and bacteriology is close to 80% , the sensitivity and specificity being 69.3 and 76.5%, respectively (6). The result of our study suggest that the negative predictive value of CMT is greater than the positive predictive value which is accordance with some other studies [2 ,8].

A positive relationsheep between SCC and CMT demonstrated in a trial [9]. The SE and SP in sheep has been reported as 73 and 83% [9].

The result of our study showed that, all the samples measured by MAS-D-TEC device (ECT), were zero. We previously found a good relationship between bacterial isolation and EC of cow milk with this device. In that study, isolation of pathogens was detected in ECT ≥ 5, and none of the bacteria were isolated in ECT $\leq$ 4 too [5]. Conductivity has been shown to decline with increasing milk-fat concentrations [10]. The higher absolute impedance (i.e. lower conductivity) in sheep than goats may at least be partially explained by the higher milk-fat concentrations in sheep than goats (2.9-3.5% for goats compared to 4.5-6.8% in sheep [4, 10].

With increasing fat percentage in milk, EC will decrease because of the thin nonconductive membrane that covers the fat globules. Lactose and casein have only a small direct effect on EC of milk [7].

CONCLUSIONS

It seems that knowledge of the CMT score and SCC in Iranian native sheep increases the likelihood of predicting the presence of a bacterial pathogen compare to no testing at all. As the CMT is a costless tool, it is recommended to detect subclinical mastitis in Iranian meat ewes by this screening test. The MAS-D-TEC which was built for measuring the conductivity of the cow milk is not a suitable device to detect subclinical mastitis in sheep.

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INTRAMAMMARY INFECTIONS IN PRIMIPAROUS COWS AND ANTIMICROBIAL RESIDUES EVALUATION IN MILK POSTPARTURITION

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SUMMARY

The objective of the present study was to verify the occurrence of intramammary infections during the preparturition in primigravid heifers, their evolution at postparturition and the follow up during lactation, microbiological identification of the main mastitis pathogens. It was also performed treatment using commercial dry cow therapy approximately sixty days before parturition and the search for antimicrobial residues in post-partum milk samples of the treated groups. The study was performed in two dairy herds in a total of 188 mammary quarters from 47 primiparous Holstein black and white and crossbred cows The animals were assigned randomly in four groups, being, two in Herd I and the other two in Herd II, being respectively: A- Control (untreated) and the B (treated with penicillin G potassic 500.000UI, penicillin G procaine 1.000.000 UI, neomycin 0.732 g) and, in Herd II, C- Control (untreated): and D- (treated with gentamicin sulfate 677 mg). Both treated groups received the therapy by intramammary route. For microbiological analysis individual mammary gland secretion samples were taken from all four groups before the treatment administration. Samples for microbiological examination and somatic cell count were collected immediately after parturition, at the 10th day and monthly during all the lactation. The search for antimicrobials residues was performed in 92 milk samples of the treated groups using Delvotest® SP (Gis Brocades Food Ingredients, Inc). During all the lactation period strip cup test and California Mastitis Test were performed in all animals of the four groups to detect clinical and subclinical mastitis cases and milk samples were collected from positive quarters for microbiological examination. Monthly milk samples were taken during the experimental period to SCC analysis. All the four groups showed low infection level in the pre-partum evaluation, being respectively: 21.66% group A, 13.33% group B; 23.08%group C and 28.58% group D. At the parturition examination, it was not detected any statistically difference among four groups infection level. In respect of the occurrence of antimicrobial residue, five out the 92 milk samples of the treated heifers showed residue, i.e., 5.43% of them. In dairy herd I, the mean of the lactation monthly SCC of group A, was statistically different (P= 0.0326), than SCC of B group however the same was not observed in dairy herd II, it was not detected significant difference in SCC of C and D groups. In relation to milk production it was not detected any significant difference among the four groups. The main microorganisms isolated from the mammary quarter milk samples were *Staphylococcus* spp. either CNS (coagulase negative staphylococci), as well CPS (coagulase positive staphylococci), and among those *S.aureus*.

INTRODUCTION

Mastitis is considered the major disease that affects dairy herds in the world but in heifers at first calving was little studied, due to the concept that these animals would be free of infection. [7] It is of great importance to identify the occurrence of intramammary infections in first-calf heifers, because compromise animal performance and these may represent sources of infection to the herd in milk, causing serious economic losses. [9,10,11] The occurrence of intramammary infections in primigravid heifers' late gestation and during early lactation has been demonstrated in Brazil [1,2,3,4]. In States where the problem occurs with greater frequency, has been advocated in the intramammary treatment all heifers from seven to fourteen days or the past sixty days before parturition with drugs used for dry cow [4]. In this context, the objectives of this study were to search on two dairy farms, the occurrence of intramammary infections in primiparous heifers in the pre-and postpartum follow up during lactation, isolate and identify agents in the etiology of mastitis, evaluate the intrammamary treatment in heifers 60 days before parturition with drugs used to treat mastitis in dry cows.

MATERIAL AND METHODS

We evaluated 188 mammary quarters of 47 heifers (Holstein and crossbred), 29 animals from farm I (Nova Odessa) and 18 heifers from farm II (Vale do Paraiba), located in State of São Paulo, Brazil. Samples of mammary secretion for microbiological evaluation were collected from all quarters of heifers at 60 days before expected parturition. Soon after, the animals were randomly assigned treatments: Group A (control, untreated), Group B (treated with intrammamary infusion of potassic 500,000 IU penicillin G, procaine penicillin G 1,000,000 IU, neomycin 0.732 g). in farm I, Group C (control, untreated) and Group D (treated with intrammamary infusion gentamicin sulfate 677mg). in farm II. Samples of colostrum were collected from mammary glands from 47 heifers of all groups (control and treated). After 10 days, and monthly mammary quarters were tested for diagnosis of clinical mastitis (strip cup test), electronic counting of somatic cells (SCC) and California Mastitis Test (CMT) for diagnosis of subclinical mastitis. Isolation and identification of causative agents of mastitis was performed in all mammary quarters [8]. In the analysis to detect residues of antibiotics used the commercial microbiological inhibition test (Delvotest® SP). The results were analyzed by statistical tests of Fisher, Mann-Whitney and Kruskal-Wallis using the software GRAPHPAD INSTAT [6].

RESULTS

The occurrence of intramammary infection (IMI) in heifers treated and untreated pre-partum in both farms were low, ranging from 21.66 to 23.08% in the treated group and from 13.33 to 28.58% in the untreated group.

Table 1.Prevalence of IMI in primiparous cows during peri and postparurition period before and after antibiotic treatment in farm I, State of São Paulo, Brazil

	HEIFERS TREATED - A				HEIFERS UNTREATED - B			
IMI	Pre-partum		Post partum		Pre-partum		Post partum	
	A	P	A	P	A	P	A	P
Negative	47	78,34[a]	31	52,54[b]	52	86,66[a]	26	48,14 [b]
Positive	13	21,66[a]	28	47,46 [b]	8	13,33[a]	28	51,85 [b]
TOTAL	60	100	59	100	60	100	54	100

[a,b] Different letters differ statistically (P<0.05)
A=Absolute number
P= Percentage

No significant differences (P>0.05) were found in a farm I between the two groups of heifers not treated (A) and treated (B) compared isolates in pre and post parturition (table 1) However, a significant increase in the frequency of isolations of treated heifers (A) pre-partum 21.66% to 47.46% at parturition and was also significantly increased frequency of isolates in the group of heifers untreated (B) who had a pre-parturition rate of 13.33% and 51.85% at post parturition The main microorganisms isolated in the four groups during the experimental period were: *Staphylococcus* spp. with 63.90%, *Corynebacterium* spp. with 7.20%, *Streptococcus* spp. with 0.5% and G-Rods with 3%.

There were no significant differences (P = 0.2432) between the mammary glands of animals positive microbiologically untreated and treated in a farm I (25.1% and 23.7%, respectively) and farm II (14.6% and 14.9%, respectively) and mammary glands of animals negative (P = 0.2824) in a farm 1 (74.9% and 76.4% for untreated and treated, respectively) and farm 2 (61.7% and 85.1%, respectively). No differences were detected in the mammary glands infected by microorganisms of the genus Staphylococcus among the four groups (P = 0.4667) However, the difference was significant between mammary glands infected by microorganisms of the genus *Staphylococcus* in relation to infection by other microorganisms, when analyzed untreated groups of both farms A and C. (P = 0.0062) That is, the incidence of mammary infections by microorganisms of the genus *Staphylococcus* were superior to those of other microorganisms in the untreated groups of farms I and II

The percentage of mammary quarters that were positive residue was 5.43%. Four of the five mammary quarters positive for antimicrobial residues were detected with interval greater than 70 days between treatment and sample collection postparturition

There was no significant difference between treated and untreated groups in both farms and milk production. (P> 0.05)

DISCUSSION

Intramammary infections in heifers preparturition the four experimental groups were low. These results are lower than those reported by [5] in a review article on the work of several authors on mastitis in heifers, presents prevalence rates of infected quarters ranged from 28.9 to 74.6% in pre-natal and 12.3 to 45.5% in childbirth. On properties in which the infection of mammary glands in the prepartum are low, not to justify the use of antimicrobials in the pre-delivery, thereby avoiding unnecessary spending on medicines, besides the risk of antibiotic residues in milk [2]

A large number of genera and species of microorganisms have been isolated from intramammary infections in heifers' early lactation, especially with staphylococci, streptococci environmental *Enterobacteriaceae* and *Corynebacterium bovis* [1,5,9].

Antimicrobial treatment 60 days before the expected date of birth in both farms was not a factor in the prevention of mastitis in pre and post parturition and some authors have cited this practice as effective in intramammary treatment for mastitis in dairy heifers [9,12]

The use of antibiotic treatment before calving is not advisable on properties with low incidence of mammary infections in primiparous, and one should emphasize the risk of treatment intrammamary. Since, if not performed with all the care of antisepsis and sterilization, to avoid contamination of the teat canal and subsequently the penetration and multiplication of pathogens in the mammary gland, the treatments themselves may give rise to iatrogenic infections. [2]

According to the results of this study, the presence of residues of antibiotics in the interval of more than 70 days draws attention to the possibility of antibiotic residues in milk beyond the period of 60 days, contradicting the view that the risk of residue after birth occurs particularly in animals with short dry period or due to the anticipated calving, but does not exclude the occurrence of intercurrent other factors linked to the management of these animals [2,3,4]

CONCLUSIONS

The detection of heifers infected with the etiological agents of mastitis corroborate the assertion that primiparous may act as sources of infection for the herd. The treatment did not exert an effective decrease of infection in the postpartum period and did not reduce mastitis during lactation was detected persistence of antibiotic residues in milk. We conclude that the decision to use medicines intramammary in heifers pre-calving should be carefully evaluated, based on characteristics of each farm

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IMPLEMENTATION OF HACCP IN DAIRY CATTLE FARMS TO CONTROL THE MILK QUALITY

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SUMMARY

The quality of raw and processed milk is a consequence of the activities performed during all the production process. This study describes an example of how Hazard Analysis and Critical Control Point (HACCP) system can be developed on dairy cattle farms to guarantee a safety and high quality milk with absence zoonotic pathogens. It was implemented in approximately 200 farms of Galicia (northwest Spain) to evaluate and control the influence that the milking equipment and cooling tank have on milk quality. Biological (bacterial count and /or zoonotic pathogens) hazards were analyzed, critical control points were established in water supplies and capacity, cleanliness of milking equipment and of cooling tank. The majority of the critical limits were determined as execution or not of a preventive procedure, and hence the corrective actions were in most of the cases the implementation of good practices. A monitoring and recording system was established and subsequently verification procedures were done by duplicate through an internal audit by the dairy company and by an external audit by a certification company.

INTRODUCTION

The quality of raw and processed milk is somehow a consequence of the activities performed during all the production process, from the farm up to the collection by dairy industries, for this reason, primary production in dairy farms is the first step in assuring food safety. Safety of food stuffs could be ensured by implementing good farming practices, that can be integrated and even been considered prerequisites of Hazard Analysis and Critical Control Point (HACCP) methodology. HACCP is the one quality control concept which best serves the objectives of both food safety and the farmers. It is considered to be a systematic method, preventive and science-based, whose first priority is the safety of the products through risk identification and risk management in the production process. Therefore, its main objective is to identify, in the production process, problems before they occur, establishing control measures that are critical to maximizing food safety at each stage in the production.

Microbial contamination of bulk tank milk may be due to and inadequate cleanliness and disinfection of the milking equipment and refrigeration tank. There are a number of foodborne hazards that are potentially originated during animal production, such as *Listeria* spp., *Campylobacter* spp., etc., and whose presence indicates a lack of hygiene during milking routine or milking equipments and refrigeration tanks. The importance of the presence of these bacteria is related to the consumption of raw or inadequately pasteurized milk, which has been associated with several outbreaks.

This article describes an example of how the principles of HACCP system can be developed and implemented on dairy cattle farms in order to improve the conditions of milk production and to guarantee a safety and high quality milk with absence of potential bacterial zoonotic pathogens. This example is focussed on the influence that the milking equipment and cooling tank have on the milk quality, basically on bacterial count.

MATERIALS AND METHODS

The study was carried out in approximately 200 dairy cattle farms of Galicia (northwest Spain), a region accounting for 40% of Spain's total milk production. These farms sold the milk to two of the biggest dairy companies in Spain. The 7 principles of HACCP methodology were performed on each farm using the guide elaborated by FAO [3] as a reference. Besides, each farmer was interviewed on the farm using a questionnaire about the farm characteristics and aspects of management.

The multidisciplinary team was formed by the farmer, technicians of milking equipment and cooling tank, and our research team; and then established the goals of the implementation of such a program as the raw milk describe in the European directives (Council Directive 92/46/ECC and Regulation EC 853/2004). Consequently, a flow diagram of the production process was drawn (figure 1), using data from the interviews and direct observations.

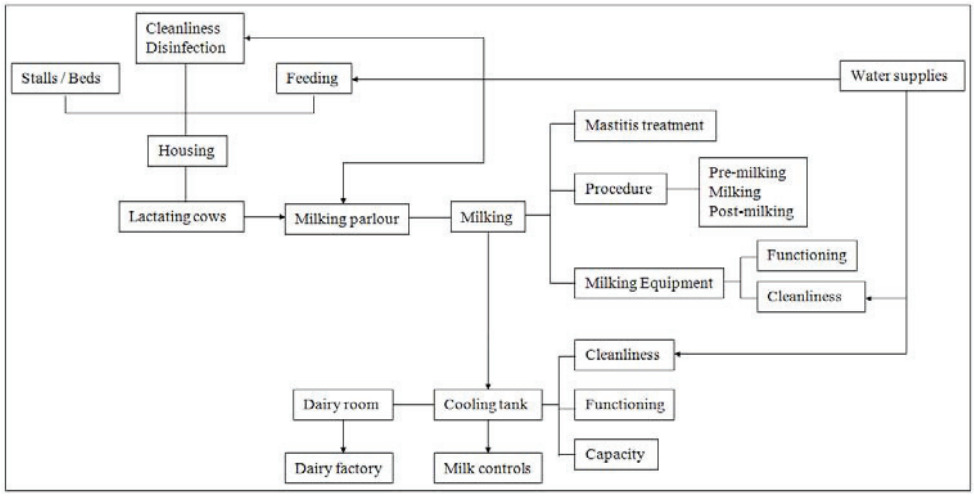

Figure 1: Diagram of production process within dairy farms

RESULTS

Biological (introduction and/or increase the number of zoonotic pathogenic bacteria such as *Listeria* spp., *Campylobacter* spp., and the increase of bacterial count in bulk tank) hazards were identified and analyzed within the steps where the milking equipment and cooling tank where involved. The different steps are presented in table 1.

The critical control points were established: 1) quality of water supplies for cleaning, as it is an important source of contamination of milking equipments; 2) cleanliness of milking equipment because whether it is not done properly will not eliminate the potential presence of bacteria (is important to keep bacteria counts as low as possible); 3) capacity and functioning of cooling tank, as bacteria could multiply rapidly when milk is not cooled quickly or collected before long; 4) cleanliness of cooling tank, as it is proved that cleaning and sanitation is one of the most important factors in obtaining high-quality raw milk.

The critical limits were determined, in most cases as the execution or not of a preventive procedure or good farming practice since objective, quick, reliable and affordable methods are not available to quantify the presence or absence of biological hazards. Therefore, the critical limits were: 1) use of dinking quality water, i.e. chlorinated; 2) use of adequate cleaning with suitable water temperature, detergent doses, etc.; 3) reach the cool temperature of 1-4ºC quickly to ensure bacteria do not multiply; 4) evaluate the washing system after every milking. Whenever possible, values from scientific literature and legal normative were used. Subsequently, the corrective actions were in most of the cases the implementation of good farming practices. A monitoring and recording system was established according to each control point: 1) revision and records of analyses of water; 2) evaluation of cleanliness through visual observations, ATP-bioluminescence measurements; 3) check temperature of cooling, annual tests of functioning; and 4) daily observations, measures of ATP-bioluminescence.

Finally, verification procedures were done by duplicate through an internal audit by personal from the quality department of the dairy company and through an external audit performed by an external and independent certification company.

Table 1: Description of the HACCP procedure

CCP[1]	Hazards	Preventive measures	Critical Limit	Monitoring	Corrective measures	Records
Water supplies	Introduction and spread of ZP[2] and BC[2]	Water analysis periodically. Chlorination. Protect wells	No chlorination. Release slurries near wells	Revision water analysis	Installation of chlorination systems	Records of analysis, dates of chlorination
Cleaning of milking equipment	Increase ZP and BC	Correct cleaning and disinfection post-milking	Not cleaning properly	Evaluation of surfaces	Check the washing system	Records of washing system tests
Functioning of cooling tank	Increase ZP and BC	Periodic maintenance	Not at 3-4ºC within 30 minutes post-milking	Check time and temperature daily	Repair the cooling tank. Review the thermometer	Annual test
Cleaning of cooling tank	Increase ZP and BC	Correct cleaning process. Drainage	Not washing with proper detergents, nor time or temperature adequate	Daily observations	Revision of washing system	Measures of ATP

[1]CCP: Critical control point
[2]ZP: zoonotic pathogens, BC: bacterial count

DISCUSSION

HACCP system has being used for years in food industry (slaughterhouses, restaurants, food facilities, etc.) to prevent, basically, microbiological hazards in public health. However, publications are still scarce at primary production level, except of some authors and for specific pathogen agents in determined animal productions. At European level, it should be noted the existence of a HACCP-like quality risk management approach programme handbook carried out by a Ducth and Portuguesse working group.

As it was described by Boersema [2], in this study most of the the critical control points were identified as managerial, in fact, the critical limits were established whether a good farming practice was carried out or not. Although this study was focused on the influence that milking equipment and cooling tank have on the milk quality, all the on-farm activities are involved in the dairy production, as it is proved in the Canadian quality milk reference manual [1]. This implies some changes in the routine of the management practices within the farm, in order to control the environmental microbiological load and its possibility to reach the bulk tank by means of an improper milking routine.

CONCLUSIONS

This study presents a first attempt to develop and implement the HACCP methodology in dairy cattle farms in Spain, as it has not yet been implemented or adapted, in order to avoid the presence of high bacterial counts and/or presence of potential bacteria zoonotic pathogens in raw milk. Therefore, this work shows the possible implementation of the HACCP, with some adaptations and modifications, at farm level to help farmers, whose actively participation is needed, to obtain safe and high quality milk, and to facilitate the certification of the production process.

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RELATIONSHIP BETWEEN ENVIRONMENTAL MICROBIAL POLLUTANTS AND MASTITIS IN EGYPTIAN BUFFALOES

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SUMMARY

The work was done on 145 Egyptian lactating buffaloes 4-5 years old. 91 milk samples were aseptically collected from mastitic animals. Also, 494 apparently normal milk samples were used for C.M.T. 28 air samples and 40 soil samples were collected from environmental of animal enclosure. All samples as well as those positive for C.M.T. were bacteriologically examined. Acutely mastitic animals showed anorexia, fever and signs of udder inflammation. Cases with chronic mastitis were recorded. The most prevalent bacterial isolates were S. agalactiae(76.5 and 92.9%) and S. aureus (52.9 and 71.4%). There is a great correlation between bacterial milk isolates and those of air and soil. Gentamycin and Kanamycin were seen to be drugs of choice for control of mastitis in buffaloes.

INTRODUCTION

Buffaloes are regarded the most important dairy animals in Egypt due to their high milk production with high fat content. The disease causes great economic losses in animal wealth [4] and zoonotic diseases to human. Air dust particles, soil and bedding materials are environmental sources of pathogenic bacteria to animals [1and8]. The objective of our work is to investigate the correlation between occurrence of mastitis in buffaloes and environmental microbial pollutants. Vitro sensitivity tests for the isolated bacteria will done.

MATERIAL AND METHODS

The work was conducted on 145 Egyptian native breed dairy buffaloes aged 4-8 years old, and belonged to some governmental and private farms in Assiut governorate. 91 milk samples were aseptically collected from mastitic animals of both farms (GF and PF). 494 milk samples were aseptically collected from normal udder quarters of both animals groups and used for CMT [3]. The milk samples positive to CMT were 52 G.F. and 43 PF.28 air samples and 40 soil samples were collected from the environment of animals enclosures. All clinical mastitis, subclinical mastitis, air and soil samples were subjected to bacteriological examination [9]. The test of antibiogram was applied on most isolated strains using discs diffusion technique of antibiotics [7].

RESULTS

The data obtained in this work were recorded in tables 1, 2, 3 and 4.

Table 1: Prevalence of bacterial mastitis among buffaloes

Animals	No. of exam. animals	No. of exam. quarters	Total no. of +ve quarters	%	Types of mastitis				Negative quarters	
					clinical		Subclinical			
					Total		Total			
					No.	%	No.	%	No.	%
Governmental Farms	80	320	95	29.7	43	13.4	52	16.3	225	70.3
Private farms	65	260	91	35	48	18.5	43	16.9	169	65

Table2: prevalence of isolated bacteria among dairy buffaloes with mastitis

Isolated bacteria	Clinical mastitis								Subclinical mastitis			
	G.F.				P.F.				G.F. (52)		P.F. (43)	
	Acute(33)		Chronic (10)		Acute (34)		Chronic (14)					
	Frequency				Frequency				Frequency			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
S. Agalactiae	20	60.6	5	50	26	76.5	13	92.9	13	25	25	58.1
S.Dysagalactiae	0	0	1	10	3	8.8	0	0	0	0	8	18.6
S.Epidermidis	8	24.2	6	60	0	0	2	14.3	6	11.5	11	25.6
S. Aureus	12	36.4	7	70	18	52.9	10	71.4	7	13.5	10	23.3
E.coli	5	15.2	5	50	5	14.7	3	21.4	5	9.6	13	30.2
K.Pneumoniae	8	24.2	4	40	2	5.9	0	0	0	0	3	7.0
P.Aeruginosa	3	9.1	3	30	1	2.9	3	21.4	0	0	4	9.3
E.aerugenes	4	12.1	5	50	11	32.4	5	35.7	1	1.9	5	11.6
Coryn. spp.	9	27.3	6	60	8	23.5	4	28.6	3	5.8	12	27.9

G. = governmental , P. = private , F. = farms

Table 3: Occurrence of bacteria in air and soil samples in houses of dairy buffaloes

Isolated bacteria	Governmental farms				Private farms			
	Air (no.14)		Soil (no.20)		Air (no.14)		Soil (no.20)	
	Frequency		Frequency		Frequency		Frequency	
	No.	%	No.	%	No.	%	No.	%
S.Aureus	3	21.4	5	25	4	28.6	6	30
S.Epidermidis	1	7.1	3	15	1	7.1	3	15
S.Faecalis	0	0	2	10	1	7.1	3	15
S.bovis	1	7.1	2	10	2	14.3	2	10
S.Faecium	1	7.1	3	15	2	14.3	4	20
S.durans	0	0	3	15	1	7.1	2	10
Coryn.spp.	3	21.4	5	25	5	35.7	7	35
E.coli	2	14.3	13	65	3	21.4	16	80
K.pneumoniae	0	0	1	5	1	7.1	3	15
P.aeroginosa	1	7.1	2	10	1	7.1	4	20
Proteus spp.	0	0	3	15	1	7.1	2	10

Table 4: Antibiogram pattern of most bacterial isolates from mastitic buffaloes

Pathogens(No.) → / Chemotherapeutic antibiotic discs ↓	S.Agalactiae(102)	S.Dysagalactiae(12)	S.Epidermidis(33)	S. Aureus(64)	E.coli(36)	k.pneumoniae(17)	P.aeruginosa(14)	E.Aerogene(31)	Coryn. Spp.(42)
Ampicillin	36	0	12	13	0	0	0	0	4
(25 ug)	35.2	0	36.4	29.7	0	0	0	0	9.5
Cefacetrile (30ug)	48	1	17	10	3	9	0	2	8
	47.1	8.3	51.5	15.6	8.3	52.9	0	6.5	19
Choramphenicol (30ug)	62	0	24	41	0	2	0	0	11
Erythromycin (5ug)	60.8	0	72.7	64.1	0	11.8	0	0	26.2
	10	3	5	7	2	3	0	12	15
Gentamycin (10ug)	9.8	25	15.2	10.9	5.6	17.6	0	38.8	35.7
	100	10	29	56	21	51	11	8	28
Kanamycin (30ug)	98	83.3	87.9	87.5	58.3	88.2	78.6	25.8	66.7
	98	10	28	58	29	16	14	26	21
Oxolinic acid(2ug)	96	83.30	84.8	90.6	80.6	94.1	100	83.9	50
	0	0	0	0	0	0	0	0	0
Penicillin (10ug)	0	5	0	0	0	0	0	0	0
	78	41.6	18	15	8	8	0	0	3
Streptomycin (10ug)	76.5	7	54.5	23.4	22.2	47.1	0	0	7.1
	76	58.3	12	45	36	7	9	7	0
	74.5		36.4	70.3	100	41.1	64.3	22.6	0

DISCUSION

Prevalence of clinical mastitis and subclinical mastitis varied among animals of both farms (table 1). The reported changes may attributed to the variations in hygienic management. The isolation of different types of bacteria from mastitic buffaloes showed various frequencies (table 2). Marked variations in the incidence of different bacterial isolates were reported between mastitic cases of both animal groups (GF and PF). Lower data of bacterial incidence in German dairy cows with clinical mastitis and subclinical mastitis were previously reported [5]. Incidences of isolated bacteria from other mastitic Egyptian buffaloes [2]. Were varied with our data. The different microbial incidences may be explained on basis of environmental conditions, endemic state of mastitis pathogens, animal management, resistance of animals, technique of milk sampling and handling of collected samples. Table3, showed various bacterial isolated with various frequencies in air and soil samples in enclosures of both farm animals. Our results were nearly similar to some investigators [6]. Soil might act as a reservoir of several pathogens as causative agents of diseases. Table 4, indicted that Gentamycin and Kanamycin were found to be the antimicrobial agents of choice to control mastitis in buffaloes.

CONCLUSION

The animal environment constitutes a dangerous vehicle for certain pathogens of veterinary and human importance. It has been proved that the air and soil of animal enclosures are the most important sources of many pathogenic and potentially pathogenic microorganisms. There was a great prominent correlation between mastitis and the environmental pathogens. Air inside animal enclosures should be improved. Soil of animal enclosure should be always kept clean with hygienic disposal of secretions and excretions as well as animal wastes. Teat disinfect must be carried out either immediately prior to milking or more commonly after milking. Gentamycin and kanamycin were proved to be highly effective antibiotics that can be used for treatment of mastitis in buffaloes. Culling all buffaloes with recurrent or chronic mastitis was among the most important preventive measures of control.

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ANTIMICROBIAL RESISTANCE EVALUATION IN *S. AUREUS* FROM BOVINE MASTITIS CASES

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SUMMARY

S. aureus from bovine mastitis is responsible for serious economic losses and public health hazard. The aim of this study was to verify mecA presence and the resistance to antimicrobials in *Staphylococcus aureus* isolates from milk of bovine mastitis cases. A total of 32 *S. aureus* isolates from mammary glands milk samples were evaluated for mecA by Polimerase Chain Reaction (PCR), using the GFX_{TM} Genomic Blood DNA Purification Kit, and primers previously described. The amplification was performed in a DNA thermal cycler (Mastercylcer® ep eppendorf, Hamburg, Germany), and the amplicons were visualized by electrophoresis in 2% agarose gel stained with ethidium bromide. In parallel, the antimicrobial resistance was detected by *in vitro* susceptibility diffusion test with the main antimicrobials used in bovine mastitis therapy as well to some of the most used in human staphylococci infection therapy. It was verified resistance to: penicillin and ampicillin 37.5%, methicillin 21.9%, neomycin 15.6%, tetracycline 12.5%, gentamicin 6.25%, vancomycin 6.25%, cotrimoxazole 3.1%. Multiple-resistance to more than two antimicrobians were presented in 40.6% strains. It was detected the presence of gene mecA in 34.4% the studied strains. A total of 15.6% were positive for mecA and simultaneously showed methicillin resistance. The detected resistance, mainly to methicillin and to vancomycin demonstrated that *Staphylococcus aureus* isolates from bovine mastitis represent a potential hazard to public health.

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INTRODUCTION

Staphylococcus aureus is the cause of a wide spectrum of infections in humans and different animal species. It has been frequently isolated from bovine mastitis [4,7]. In particular, cows with subclinical *S. aureus* mastitis can shed large numbers of *S. aureus* in their milk. *S. aureus* from bovine mastitis is responsible for serious economic losses [6], therefore, the antibiotics has been largely used in mastitis therapy. Consequently, as the expression of resistance is enhanced by the use of antibiotics, because the susceptible subpopulation is eliminated and the highly resistant subpopulation is selected out and, it was noticed an increase in *S. aureus* resistance.

The epidemiology of *S. aureus* has changed radically in particular, methicillin-resistant S. aureus (MRSA), originally restricted to hospital, has emerged as a significant pathogen in the community, and true community-acquired MRSA (CA-MRSA)

Considering the potential risk to the public health as well as to animal health, the aim of this study was to verify mecA presence and the resistance to antimicrobials in *S. aureus* isolates from milk of bovine mastitis cases.

MATERIAL AND METHODS

Milk samples were obtained from the dairy cows with clinical or sub-clinical bovine mastitis. The initial milk stripped from each teat on the strip cup in order to evaluated clinical mastitis occurrence, in order to detected subclinical mastitis cases it was used CMT (California Mastitis test).

A total of 32 *S. aureus* isolates from mammary glands milk samples. The isolates were identified by conventional methods. They were evaluated for mecA by Polimerase Chain Reaction (PCR), using the GFX^{TM} Genomic Blood DNA Purification Kit, and primers previously described [5]. The amplification was performed in a DNA thermal cycler (Mastercylcer® ep eppendorf, Hamburg, Germany), and the amplicons were visualized by electrophoresis in 2% agarose gel stained with ethidium bromide. In parallel, the antimicrobial resistance was detected by *in vitro* susceptibility diffusion test (Kirby;Bauer method) with the main antimicrobials used in bovine mastitis therapy as well to some of the most used in human staphylococci infection therapy. In vitro antimicrobial susceptibility testing was conducted by disc diffusion method. Prepared antimicrobial sensitivity discs (Oxoid®; Basingstoke, England) with 13 antimicrobial agents: ampicillin (10 µg), oxacillin (1 µg), tetracyclin (30 µg), penicillin G (10 µg), neomycin (10 µg), cephalexin (30 µg), gentamicin (120 µg), enrofloxacin (5 µg), ciprofloxacin (5 µg), vancomycin (30 µg), cotrimoxazol (25 µg), cefoxitin (30 µg) and cephalothin (30 µg). Isolates were categorized as susceptible, intermediary or resistant based upon criteria developed by National Committee for Clinical Laboratory

Standards. *S. aureus* ATCC 3565 strains were used as control.

RESULTS

It was detected the presence of gene mecA in 34.4% strains, 21.9% showed methicillin resistance, and, it was verified that 15.6% were positive for mecA and showed simultaneously methicillin resistance. These results were presented in table 1.

Table 1: Gene mecA and methicillin resistance detection on bovine mastitis *S. aureus* isolates. São Paulo.2011

Staphylococcus aureus	No.	%
mecA positive	11	34.4
mecA negative	21	65.6
methicillin resistance	7	21.9
methicillin susceptibility	25	78.2
mecA positive and methicillin resistance	5	15.6
Total of *S. aureus* isolates	32	100.0

P = 0.0318, significant statistically (Fisher's Test)

It was verified resistance to: penicillin and ampicillin 37.5%, tetracycline 12.5%, gentamicin 6.3%, vancomycin 6.3%, neomycin 15.6%, cotrimoxazole 3.1% (Table 2).

Among the methicillin-resistant group (MRSA group) the percentage of resistance was higher manly to the beta-lactam penicillins (Table 2) than among the methicillin susceptible group (MSSA). It was observed the highest resistance to penicillin and ampicillin (37.5%) and the lowest to the quinolones and to the cephalosporins, i.e. none of the *S. aureus* isolates showed resistance to those antimicrobials (Table 2).

As expected when evaluated the antimicrobial resistance among the mecA *S.aureus* isolates only to oxacillin it was verified higher percentage of resistance than the observed among the mecA negative group (Table 2).
Multiple-resistance to more than two antimicrobians were presented in 40.6% strains.

Table 2. Resistance to several antimicrobials among methicillin resistant and methicillin susceptible *S. aureus* isolates from bovine mastitis cases. São Paulo. 2011.

Resistance of *S.aureus* isolates	OXA	AMP	COT	PEN	ENR	CEF	GEN	NEO	CIP	TET	VAN	FOX	CEPH
% methicillin resistant group %	100	57.1	0	57.1	0	0	14.3	14.3	0	28.6	0	0	0
methicillin susceptible group	0	32.0	4.0	32.0	0	0	4.0	16	0	8.0	8.0	0	0
mec A positivo	45.4	36.4	0	36.4	0	0	0	9.1	0	9.1	0	0	0
mec A negativo	9.5	38.1	4.8	38.1	0	0	9.5	19.0	0	14.3	9.5	0	0
Total resistance	21.9	37.5	3.1	37.5	0	0	6.3	15.6	0	12.5	6.3	0	0

Tested antimicrobials: oxacillin, ampicillin, penicillin, cotrimoxazole, enrofloxacin, cephalexin, gentamicin, neomycin, ciprofloxacin, tetracycline, vancomycin, cefoxitin, cephalothin.

DISCUSSION

Resistance was not only the result of destruction of the antibiotic by the enzyme penicillinase produced by some microorganisms, but there is also other mechanisms termed intrinsics [9]. This fact was easily verified at table where only 15.6 % of the isolates were simultaneously mecA positive and methicillin resistant, and on the other hand, considering only the mecA positive *S.aureus* isolates, 45.5% showed methicillin resistance.

Some authors referred that since the mechanism of methicillin resistance is probably the same for all staphylococci, beta-lactam antibiotics cannot be recommended for any infection caused by these organisms [2], however, it is evident that there are other mechanisms of resistance. And on the other hand it was observed that beta-lactams penicillin (penems) and

cephalosporins are *in vitro* active against methicillin-resistant strains [3].

It was observed that in minor infections in which the bacterial load is not large (i.e., $<10^4$ to 10^6) and intact host defenses can participate in the eradication of the organisms, beta-lactam antibiotics may be effective; and there have been reports of clinical success. On the other hand, numerous clinical failures have been reported when beta-lactam antibiotics were used to treat serious infections [3].

In these cases vancomycin is the drug of choice for treatment of infections caused by methicillin-resistant staphylococci, once this antimicrobial inhibition mechanism is different from the action of beta-lactam antibiotics. Resistance to vancomycin in *S. aureus* has been seldom observed, although, an emergence of vancomycin in coagulase-negative staphylococci was referred, it has been detected in *Staphylococcus haemolyticus* [8]. In rare instances, a patient is unable to tolerate vancomycin, and another drug must be used to treat infections caused by methicillin-resistant staphylococci. Clinical experience with these drugs is very limited, and their use should be considered only when vancomycin cannot be used.

Due to the relevance of vancomycin in the treatment of infections caused by methicillin-resistant staphylococci, the 6.25% resistance to vancomycin observed in the present study it must be pointed out.

Ciprofloxacin and pefloxacin are highly active in vitro and in vivo against both methicillin-susceptible and resistant staphylococci [1]. In the present study, *S.aureus* isolates from mastitis cases showed high susceptibility to enrofloxacin and ciprofloxacin, as a matter of fact none of the isolates was resistant to them. However, resistance to quinolones has already been reported [3]. This means that as quinolone usage increases, quinolone-resistant strains may become more common.

Methicillin-resistant staphylococci are typically resistant to a variety of other antibiotics, including tobramycin, clindamycin, tetracycline, and erythromycin [3]. It was also observed in this study, however the highest percentage of resistance was verified mainly to the penicillin beta-lactams.

CONCLUSIONS

The detected resistance, mainly to methicillin and to vancomycin demonstrated that *Staphylococcus aureus* isolates from bovine mastitis represent a potential hazard to public health. Therefore, the obtained results were of great concern not only in regard of mastitis therapy but mainly to public health, due to the eventual occurrence of cross infections, as well as, to the possibility of transmission of resistance among the microorganisms by plasmids.

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THE EFFECT OF PROBIOTICS ON HUMAN SKIN STAPHYLOCOCCAL DISEASES (Abstract)

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INTRODUCTION

Species of *Staphylococcus* bacteria are identified as one of the most important causes of acute disease in humans. *Staphylococcus aureus* causes many diseases in humans and animals, particularly skin diseases (Williams & MacKie, 1993).

Researchers are attempting to find successful solutions to overcome microbial infections especially that are resistant to most antimicrobial drugs such as methicillin resistant *S. aureus* (MRSA) one potential area of investigation is the use of probiotics (Reid, Jass, Sebulsky, & McCormick, 2003).

MATERIAL

In this study, will be used several equipments, bacterial isolates (9 *Lactobacillus* isolates and 5 *Staphylococcus* isolates), MRS and Nutrient media.

METHODS

In the methods conducted agar well diffusion assay to observe effect; L*actobacillus* species (*L. casei Shirota, L rhamnosus, L. salivarius 20492, L. vaginalis 5837, L. jensenii 20557, L. reuteri 20016, L. acidophilus 20079, L. plantarum and L. fermentum*), supernatant, KOH+ supernatant and control on pathogenic human skin *Staphylococcus aureus* and *S.epidermidis*.

RESULTS

The good results with inhibition zone (mm) assay for *Lactobacillus* species were; *L. salivarius , acidophilus, L.plantarum L. casei shirota* and *L. rhamnosus* on *S. aureus* isolate respectively, and on *S. epidermidis* were*; L.plantarum L. casei shirota, L. acidophilus, L. salivarius* and *L. rhamnosus* respectively.

CONCLUSIONS

The initial results were good and agreement with previous studies, whether on human skin diseases or in the other diseases, and can be applied on human after it experimented on animal models, then observation a positive results.

PRESENCE OF *ENTEROTOXINS* GENES IN *STAPHYLOCOCCUS EPIDERMIDIS* ISOLATED FROM BOVINE MILK

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SUMMARY

Staphylococcus epidermidis is internationally considered one of the main coagulase negative staphylococci isolated from nosocomial infections causing serious problems among neonates, as well as, immune compromised patients. These microorganisms have been isolated from bovine milk causing mastitis. Therefore, the objective of this study was to verify the presence of some virulence factors among the *Staphylococcus epidermidis* isolates from bovine milk. A total of 19 isolates were evaluated. Polimerase Chain Reaction (PCR), using the GFX$_{TM}$ Genomic Blood DNA Purification Kit, and primers previously described. The amplification was performed in a DNA thermal cycler (Mastercylcer® ep eppendorf, Hamburg, Germany), and the amplicons were visualized by electrophoresis in 2% agarose gel stained with ethidium bromide. PCR assay was used to determine the presence of enterotoxin genes (sea, seb, sec and sed) in *Staphylococcus epidermidis*. The occurrence of enterotoxins genes was determined as 42.1% for *sea*, 5.3% for *seb*, 5.3% for *sed, 5.3*%) for both *sec* and *sed,* 5.1% for both *sea, sec and sed ,* respectively*.* As a conclusion*, Staphylococcus epidermidis* isolated from bovine mastitis has been found to have high enterotoxigenic potential, and therefore, represent hazard to consumers health. This might be significant for food hygiene especially in cases of subclinical mastitis.

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INTRODUCTION

In 1884, Rosenbach described the two pigmented colony types of staphylococci and proposed the appropriate nomenclature: *Staphylococcus aureus* (yellow) and *Staphylococcus albus* (white). The latter species is now named *Staphylococcus epidermidis*. *S epidermidis* is an inhabitant of the skin [2]. Nowadays *Staphylococcus epidermidis* is internationally considered one of the main coagulase negative staphylococci isolated from nosocomial infections causing serious problems among neonates, as well as, immune compromised patients.

On the other hand staphylococcal food poisoining is of major concern in public health programs worldwide, therefore research is also needed for the identification of new SEs and of new enterotoxigenic staphylococci.

The role of *Staphylococcus epidermidis* in food poisoining need to be better understood, to allow an effective prevention.

The objective of this study was to verify the presence of some virulence factors among the *Staphylococcus epidermidis* isolates from bovine milk.

MATERIAL AND METHODS

A total of 19 isolates were evaluated. Polimerase Chain Reaction (PCR), using the GFX$_{TM}$ Genomic Blood DNA Purification Kit, and primers previously described [4]. The amplification was performed in a DNA thermal cycler (Mastercylcer® ep eppendorf, Hamburg, Germany), and the amplicons were visualized by electrophoresis in 2% agarose gel stained with ethidium bromide. PCR assay was used to determine the presence of enterotoxin genes (sea, seb, sec and sed) in *Staphylococcus epidermidis*, the main coagulase negative staphylococci isolated from nosocomial infections.

RESULTS

The occurrence of enterotoxin genes was determined as 42.1% for *sea*, 5.3% for *seb*, 5.3% for *sed, 5.3*%) for both *sec* and *sed,* 5.1% for both *sea, sec and sed ,* respectively(Table 1, illustrated in Figure 1)*.*

Table 1: Enterotoxin genes (*sea, seb, sec* and *sed*) in *Staphylococcus epidermidis* isolates from mastitis bovine cases. São Paulo. Brasil. 2011.

Staphylococcus epidermidis	No.	%
sea	8	42.1
seb	1	5.3
sec	0	0
sed	1	5.3
sea + sec +sed	1	5.3
sec +sed	1	5.3
Negative	7	36.8
Total	19	

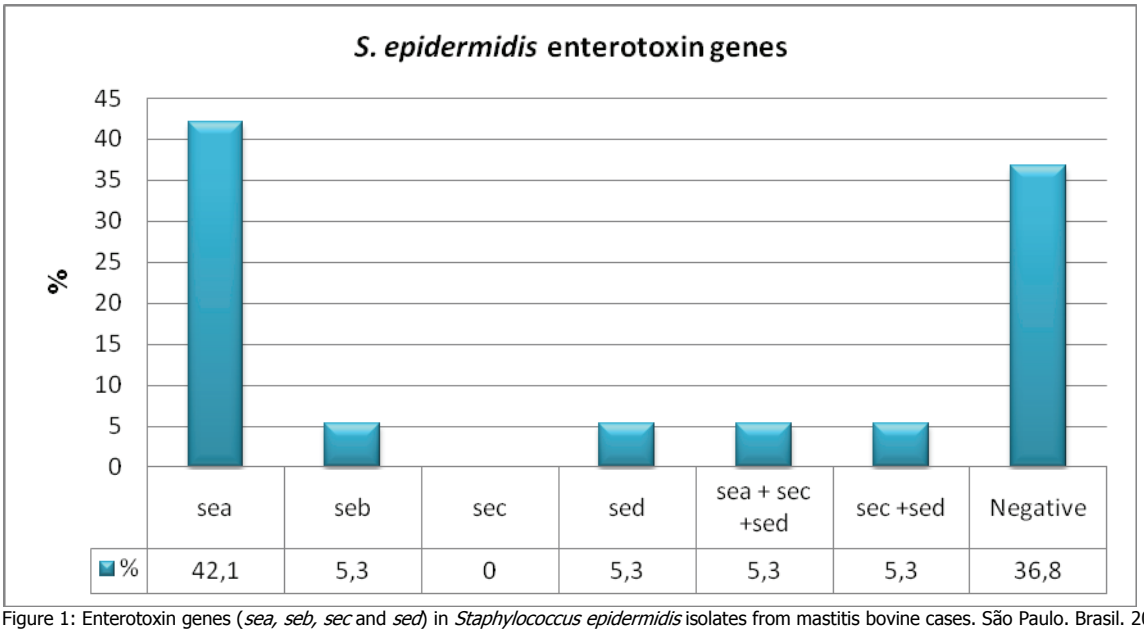

Figure 1: Enterotoxin genes (*sea, seb, sec* and *sed*) in *Staphylococcus epidermidis* isolates from mastitis bovine cases. São Paulo. Brasil. 2011.

DISCUSSION

Food-borne diseases are of major concern worldwide. To date, 250 different food-borne diseases have been described and bacteria are the causative agents of two thirds of food-borne disease outbreaks. Food poisoning is a term used to express any type of disease, illness or malaffect after consuming food. The most serious type of food poisoning is bacterial food poisoning, which may be due to bacterial infection or food intoxication. As these toxins are excreted from the organism, they are referred to as exotoxins; however, they normally exert their effects on the gastrointestinal tract and therefore are called enterotoxins. While not considered a highly lethal agent due to the low mortality associated with the illness, staphylococcal enterotoxins are considered a potential biological threat because of their stability at high temperatures (100°C for 1 h) and ability to incapacitate individuals for several days to two weeks [2].

Among the predominant bacteria involved in these diseases, *Staphylococcus aureus* is a leading cause of gastroenteritis resulting from the consumption of a food in which enterotoxigenic staphylococci have grown and produced toxins. *Staphylococcus aureus* is able to grow in a wide range of temperatures (7° to 48.5°C with an optimum of 30 to 37°C), pH (4.2 to 9.3, with an optimum of 7 to 7.5) and sodium chloride concentrations up to 15% NaCl [5].

However, nowadays it was demonstrated that other staphylococci has been associated to food borne disease.

S. epidermidis strains were able to produce staphylococcal enterotoxins (SEs) and are the causative agents of staphylococcal food poisonings. The present results showed that 63.2% of *S. epidermidis* strains isolated from milk samples of bovine mastitis case encoded enterotoxin genes, predominantly *sea* (42.%), but also *seb*, *sed*, and also genes for more than one type. Therefore, it seems to be more often than it was thought, so it must be pointed out the risk, not only represented by the consume of milk, but also by the consume of dairy products, as cheese [3].

Symptoms are of rapid onset and include nausea and violent vomiting, with or without diarrhea. The illness is usually self-limiting and only occasionally it is severe enough to warrant hospitalization. SEA is the most common cause of staphylococcal food poisoning worldwide, but the involvement of other classical SEs has been also demonstrated [1].

CONCLUSION

As a conclusion*, Staphylococcus epidermidis* isolated from bovine mastitis has been found to have high enterotoxigenic potential, and therefore, represent hazard to consumers health. This might be significant for food hygiene especially in cases of subclinical mastitis.

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SEARCH FOR ENTEROTOXINS CODIFYING GENES IN *S. AUREUS* ISOLATED FROM BOVINE MASTITIS CASES

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SUMMARY

The quality of dairy products has become an issue over the last decades due to various outbreaks of food-borne zoonoses, and therefore the consumer concern is increasing. Mastitis caused by *Staphylococcus aureus* is worldwide one of the main diseases in dairy herds. The purpose of this study was to verify the presence of some virulence factors among *Staphylococcus aureus* isolates from milk of bovine mastitis cases. A total of 32 isolates were identified biochemically based on the utilization of differents sugars, production of hemolysin, nitrate reduction, presence of urease and ornithine decarboxylase, and resistance to novobiocin. Readings of the tests were obtained after 24, 48, and 72 h of incubation and evaluated by Polimerase Chain Reaction (PCR), using the GFX$_{TM}$ Genomic Blood DNA Purification Kit, and primers previously described. The amplification was performed in a DNA thermal cycler (Mastercylcer® ep eppendorf, Hamburg, Germany), and the amplicons were visualized by electrophoresis in 2% agarose gel stained with ethidium bromide. PCR assay was used to determine the presence of enterotoxin codifying genes (sea, seb, sec and sed). The occurrence of enterotoxin genes was determined as 15,6% for *sea*, 9,4% for *seb*, 9,4% for *sec,* 3,1% for *sed, 6.3*% for both sea, *seb* and *sec; 9,4%* for *sec* and *sed* and *sed,* 3.1% for both *sea and sec,* respectively. These results demonstrated that *Staphylococcus aureus* isolated from bovine mastitis have a high enterotoxigenic potential, and therefore, represent hazard to consumer's health, particularly to children, immune compromised patients and to elders.

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INTRODUCTION

The quality of dairy products has become an issue over the last decades due to various outbreaks of food-borne zoonoses, and therefore the consumer concern is increasing. Mastitis caused by *Staphylococcus aureus* is worldwide one of the main diseases in dairy herds.

Staphylococcus aureus is an important pathogen due to a combination of toxin-mediated virulence, invasiveness, and antibiotic resistance. This bacterium is a significant cause of nosocomial infections, as well as community-acquired diseases.

Food-borne diseases are of major concern worldwide. To date, around 250 different food-borne diseases have been described, and bacteria are the causative agents of two thirds of food-borne disease outbreaks. Among the predominant bacteria involved in these diseases, *Staphylococcus aureus* is a leading cause of gastroenteritis resulting from the consumption of contaminated food. Staphylococcal food poisoning is due to the absorption of staphylococcal enterotoxins preformed in the food [11].

The purpose of this study was to verify the presence of some virulence factors among *Staphylococcus aureus* isolates from milk of bovine mastitis cases.

MATERIALS AND METHODS

A total of 32 isolates were identified biochemically based on the utilization of different sugars, production of hemolysin, nitrate reduction, presence of urease and ornithine decarboxylase, and resistance to novobiocin. Readings of the tests were obtained after 24, 48, and 72 h of incubation and evaluated by Polimerase Chain Reaction (PCR), using the GFX$_{TM}$ Genomic Blood DNA Purification Kit, and primers previously described [9]. The amplification was performed in a DNA thermal cycler (Mastercylcer® ep eppendorf, Hamburg, Germany), and the amplicons were visualized by electrophoresis in 2% agarose gel stained with ethidium bromide. PCR assay was used to determine the presence of enterotoxin codifying genes (sea, seb, sec and sed).

RESULTS

The occurrence of enterotoxin genes was determined as 15,6% for *sea*, 9,4% for *seb*, 9,4% for *sec,* 3,1% for *sed, 6.3*% for both sea, *seb* and *sec; 9,4%* for *sec* and *sed* and *sed,* 3.1% for both *sea and sec,* respectively(Table 1).

Table 1.Enterotoxin genes *Staphylococcus aureus* isolates from bovine mastitis cases. São Paulo. Brazil. 2011.

Staphylococcus aureus	No.	%
Sea	5	15.6
Seb	3	9.4
Sec	3	9,4
Sed	1	3.1
sea + seb +sec	2	6.3
sec +sed	3	9.4
sea +sec	1	3.1
Negative	13	40.6
Total	32	100.0

DISCUSSION

Genes encoding SEs have different genetic supports, most of which are mobile genetic elements. For example, *sea* is carried by a family of temperate phages [3; 5]. *Seb* is chromosomally located in some clinical isolates [13], whereas it has been found in a 750-kb plasmid in other *S. aureus* strains [14]. SEC is encoded by a gene located on a pathogenicity island [7] and *see* is carried by a defective phage [6].

In all cases of staphylococcal food poisoning, the foodstuff or one of the ingredients, was contaminated with an SE-producing *S. aureus* strain and was exposed, at least for a while, to temperatures that allow *S. aureus* growth.

In the United Kingdom, for example, 53% of the staphylococcal food poisonings reported between 1969 and 1990 were due to meat products, meat-based dishes, and 8% were due to milk products, 7% to fish and shellfish and 3.5% to eggs [15].

In France, things are different. Among the staphylococcal food poisonings reported in a two-year period (1999-2000), among the cases in which the food involved had been identified, milk products and especially cheeses were responsible for 32% of the cases, meats for 22%, sausages and pies for 15%, fish and seafood for 11%, eggs and egg products for 11% and poultry for 9.5% [8].

Various examples of staphylococcal food poisoning are described in the literature[2]. In one case, cheese was involved in an outbreak because it had been made from milk contaminated after pasteurization and before inoculation with lactic starter culture. In this particular case, the starter culture did not grow properly, resulting in a fermentation accident that allowed the *S. aureus* strain to develop and produce SE [2]. In 1985, chocolate milk was the origin of a staphylococcal food poisoning in Kentucky, USA. This chocolate milk was contaminated and stored at too high a temperature for 4 to 5 h, before pasteurization. It must be pointed out the pasteurization is able to kill the staphylococci but had no effect on the SEs that are extremely heat resistant, as they can endure boil temperature for more than 15 minutes.

In all these cases of staphylococcal food poisoning, the foodstuff were contaminated with SE-producing *S. aureus* strain and was exposed to temperatures that allow *S. aureus* growth. The foodstuff reaches this temperature due to failure in the refrigeration process or a delay in the refrigeration a very often situation in tropical countries. It can also happen when a growth-permissive temperature is required during processing, as for instance, in some cheese making process.

Many different foods can be a good growth medium for *S. aureus*, and have been implicated in staphylococcal food poisoning, including milk and cream, cream-filled pastries, butter, ham, cheeses, sausages, canned meat, salads, cooked meals and sandwich fillings.

In many cases, the main sources of contamination were humans (handlers contaminate food via manual contact or via the respiratory tract by coughing and sneezing). Nevertheless, in foods such as raw meat, sausages, raw milk, and raw milk cheese, contaminations from animal origins are more frequent and due to animal carriage or to infections, as mastitis[11].

There are many examples in literature concerning strains isolated from cows with mastitis and milk products. In France, among 61 strains isolated from raw milk cheeses, 15.9% were enterotoxigenic [12]. In Denmark, on strains isolated from cows with mastitis found that only 1 of 414 *S. aureus* isolates carried an SE gene [10]. A similar study was performed in Minas Gerais, Brazil, where 54 (43%) of 127 *S. aureus* isolates from bovine mastitis were found to be SE producers [4]. More recently in Germany, similar work on strains isolated from the milk of cows with mastitis showed that up to 72.8% of the strains were enterotoxigenic when SEA to SEJ were considered [1].

In the present study, 17 (53.1%) of 32 *S. aureus* isolates from bovine mastitis were found to be encoded enterotoxin genes and, therefore they are potentially SE producers.

In respect to antimicrobial resistance it was verified the presence of gene mecA in 34.4% strains, while 21.9% showed methicillin resistance and 15.3% were positive for mecA, as well, was expressing the methicillin resistance.

CONCLUSIONS

These results demonstrated that *Staphylococcus aureus* isolated from bovine mastitis have a high enterotoxigenic potential, and therefore, represent hazard to consumer's health, particularly to children, immune compromised patients and to elders.

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LIVESTOCK PRODUCTION SYSTEM-CHALLENGES IN MAINTAINING HEALTH AND HYGIENE IN RURAL NEPAL

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SUMMARY

Livestock farming is a major component of Nepalese farming system and it is one of the important occupations in the rural area of Nepal. Nepalese agriculture is the integration of livestock system and crops. Livestock is vital for subsistence farms as it is the source of meat, milk, drought power, manure and household income. Livestock are raised across the country but mainly represents subsistence nature of farming rather than commercial. Rural people enjoy livestock rearing as a part of their farming. Lots of potentials and challenges are existing in Nepalese rural livestock rearing. This study covers major concerns over the glimpse of livestock rearing system focusing to the rural areas and also to highlight challenges prevailing with them. Review of literatures and information are compiled to meet the objective. Rural livestock production systems have scope to further improvement but are facing basic problems regarding animal nutrition, health and hygienic condition. However, with respect to the geographical diversity and availability of various species to supplement livelihood of rural people, key issues are likely to be considered for its betterment in the days to come.

Key words: Rural area, subsistence farming, geographical diversity.

INTRODUCTION

Nepal is a small land locked mountainous country with 147,181 sq. km. in area with total household 4,253,220 (Central bureau of statistics, 2001). Of the total population 30% of Nepalese live below poverty line of us$ 12/person/month (Central bureau of statistics, 2001). Almost 70% of households have less than 1ha of land holding (Encyclopedia of nation, Nepal: country overview location and size, 2007)

Agriculture is the major sector of Nepalese economy. It provides employment opportunities to 66 percent of the total population and contributes about 39 percent in the GDP of which Nepalese livestock shares around 26% to the national agricultural GDP accounting food, fiber, power, fertilizer, fuel, transportation, income and security (MOAC 2005). Livestock provides milk, meat, draft power to cultivate lands, manure to fertilize land and cash income to the families.

Majority of the population live in rural areas of Nepal. Almost 81% of Nepalese population live in rural areas and depend on subsistence farming (Encyclopedia of nation, Nepal: country overview location and size, 2011). Most of the rural farmers do livestock farming as a secondary enterprise. Most of the people raise agriculture crops as their major source for food and income. Semi-commercialization is only in dairy farming and poultry raising mainly on urban areas. . Roughly 70% of households keep some type of livestock, including cows, buffaloes, pigs and chickens (FAO, 2005). In previous years, there used to be milk holiday which is eliminated because of dairy farming through establishment of co-operatives. The types of livestock for rearing are highly affected by the culture and religion of the society. Pigs and poultry are not selected in upper caste society, this type of cultural belief have resulted in low livestock production (Perspective *Land Use Plan, 1986-2005)*

Traditional practices of livestock farming are quite common in rural areas. Such practices are based upon the indigenous knowledge of farming. Due to ill knowledge about livestock farming system the health status and hygienic condition of animals are degrading day by day. Due to lack of knowledge, poor sanitation and poor health animal health is prevailing in the rural areas.

The overall objective is to study about the livestock production system in rural areas of Nepal and to understand the challenges in maintaining animal health and hygiene.

Table 1. Livestock Population and Growth rate of Nepal

Type/year	2004/05	2005/06	2006/07	2007/08	2008/09 A.G.R %	A G.R %
Cattle	6994463	7002916	7044279	7090714	7175198 0.33	0
Buffalo	4081463	4204886	4366813	4496507	4680486 3.12	3.12
Sheep	816727	812085	813621	809480	802993 -0.68	-0.68
Goat	7153527	7421624	7847624	8135880	8473082 3.27	3.27
Pig	947711	960827	989429	1013359	1044498 1.63	1.63
Fowl	22790224	23221439	23924630	24665820	24481286 2.59	2.59
Duck	391855	392895	394798	390748	383123 -0.85	-0.85

Table 2. Livestock Production Nepal

Type/year	2005/06	2006/07	2007/08	2008/09	A G.R %
Meat (mt.)	219205	227105	233900	241690	2.66
Milk (mt.)	1312140	1351394	1388730	1445419	3.06
Egg (core)	219205	227105	233900	241690	3.5

Source: Ministry of Agriculture and Co-operatives, Nepal

METHODS

Information were collected from available literatures, books, magazines, research proceedings, and internet and reviewed to study about the production system, status and challenges of livestock production regarding animal health and hygiene.

FINDINGS

Based on the available information, study was carried regarding the following matters:

Livestock and its contribution to house hold system in Nepal

Basically the major livestock raised by rural farmers in Nepal include cattle, buffalo, sheep, goat, pig and poultry. Cattle, buffalo, goat are raised throughout the country for milk, meat and draft power. Sheep are raised in the high and mid-hills for the purpose of meat and wool. Poultry raising is quite common in terai region (below 300 meters in elevation) semi-commercially while in case of hills and mountains poultry raising is usually done for household purpose (The Role of Large Ruminants, B.R. Joshi).). Livestock farming, especially, dairy farming alone contributes 78% in total AGDP. It is presently undergoing a transition phase from subsistence to commercial dairy farming in the various places of the Terai region due to the increase of milk marketing facilities in the area. Dairy farming has been helping the farmers to earn cash income to fulfill their basic needs, at the same time they can get manure as by-product and draft power for agricultural production (Bhatta G D; Doppler W; KC K and Shrestha G, 2009).

Livestock rearing systems

Livestock rearing in rural Nepal is quite poor and it has many challenges for further improvement. Livestock rearing in rural areas mainly include Transhumance system which consists of moving of livestock in accordance with the climate and season. During the winter, animals are moved to lowlands in search of pasture and to avoid the extreme cold, while in summer when there is no snowfall animals are moved towards highlands. The next is semi intensive system in which the animals are partly grazed and partly provided with feed. The other one is stall-feeding system.

Housing

Due to subsistence nature of farming animal sheds are built near the home. Animal housing system is quite poor, rural farmers built animal sheds with the help of straw. Use of concrete materials is only seen in commercialized farming in urban areas. In rural areas, there is no isolation of diseased animal with that of healthy ones. This helps in rapid spread of diseases. Farmers are not conscious towards the about animal sanitation and hygiene. The major problem is the lacking of animal shed disinfection and shed cleanliness. Illiteracy, lack of awareness related to animal health and hygiene, unavailability of materials are the pre-disposing factors for poor shed management (Ganesh, 1994; Kumar et al., 2009).

Feeding/ nutrition

Because livestock and crops are integrated, livestock husbandry depends on the cropping systems practiced. Feeding of animals depends upon the availability of fodder, crop residues, kitchen waste. In most poor communities animals are raised only on the basis of kitchen waste. These type of activities are deteriorating animal health in one aspect and on the other hand animals are malnourished. Mal-nutrition in rural areas is one of the major problems in livestock husbandry due to scarcity of fodder trees, grasses, and other feed concentrates. Farmers in rural areas due to lack of knowledge mix feed randomly that results to different bio-chemical reactions resulting diseases. Feed poisoning is also the another factor for poor health condition of rural livestock.

Feed Availability & Deficit in case of Nepal

- Crop residues - 60 %
- forest grazing lands - 40 %
- The feed deficit in hills (-56%)
- followed by the Terai (-42 %)
- The mountain region is at (+26%) (DLS, 2002)

This huge feed deficit has resulted in increased malnutrition resulting reduced production.

Breeding

In Nepal there are lots of indigenous breeds of cattle, buffalo, goat, sheep, pig and poultry. There are 7 indigenous breed of cattle in which Achhame (*Bos indicus*) is the smallest breed of 90 cm in height.

There are 3 indigenous breed of buffalo, 4 indigenous breed of sheep, 4 of goat and that of pig also carries 4 indigenous breed. (Agriculture and Cooperative, Government of Nepal, 2004). These indigenous breed have certain potentials to withstand adverse climate and can be adapted in any management conditions. But due to lack of genetic improvement the production of these indigenous breed could not be increased. So the country is lacking substantial production although there are huge potentials of improvement.

Health status and management

Due to poor animal husbandry, lack of knowledge about health management among rural farmers, animals are more vulnerable to diseases and parasites. There are problems related to reproductive health too. The infertility in animals is another major problem. Due to lack of knowledge about reproductive health and poor sanitation infertility problem is increasing rapidly. Lack of water sanitation is another factor in which animal are allowed to swim in ponds and same water is used in feeding animals. There is no isolation system to separate diseased and healthy animal which result in no control over diseases. More than 50% of animals are suffered by endoparasites. Mastitis is another serious problem which generally occurs due to poor sanitation and unhygienic management. Proper housing systems are not in practice which results difficulties in proper management of animals. Due to lack of knowledge about animal husbandry, people are not aware about vaccination. In some areas animals are raised without giving vaccine. Programs related to proper vaccination methods, chemical treatments insufficient so those animals are more vulnerable to different diseases. In Nepal disease reporting system is very poor so that there are chances of disease epidemics.

There is lack of biosecurity in livestock farms. There is no control during transporting, entry of outsiders. The newly bought animals are mixed with the older animals without any checking either the animal is healthy or diseased. This results in the greater spread of diseases inside the farm.

People in rural areas of Nepal consume milk without boiling. This has resulted tuberculosis spread due to drinking of unhygienic milk.

In the rural areas of Nepal, the animal diseases considered being of major importance in cattle and buffalo are rinderpest, haemorrhagic septicaemia, foot and mouth, and helminthes parasitic diseases, rabies and black quarter.

It is also reported that some diseases viz. Bovine Haematuria are limited to grazing animals. A mean rate of prevalence of 0.79% has been reported in the cattle population of the Eastern Nepalese hills (Mahato, 1986). Diseases related to plant poisoning are more frequent in grazing animals, whereas metabolic disorders are more common in stall fed animals (Mahato, 1986). Milk fever is also one of the common livestock problem.

Slaughtering places are frequently contaminated and may not be protected against dogs, rodents and insects. Meat products coming from such conditions are often deteriorated due to bacterial infection or contaminated, which may cause food poisoning or diseases in consumers. Lack of slaughter house, lack of proper infrastructure in the slaughtering places and meat shops, absence of knowledge about meat borne diseases, shortage of adequately trained personnel, improper slaughtering, handling and selling of meat and the most importantly the lack of meat inspection and examination which though is in the law have definitely bound to increase the prevalence of different pathogens and parasites, some of them being much zoonotically significant as well.

Quarantine

Nepal shares a long and open boarder with India in south and restricted boarder in north with Tibet. There is free movement of animals from India to Nepal as Nepal always been dependent on India for supply of work, meat and breeding animals as the supply of these animals and animal products are limited in Nepal. Similarly the transhumant system of animals in search of feed leads to the movement of animals in the north during the particular season. Thus free movement of animals is a pre-disposing factor for the spread of diseases along the border areas as the quarantine regulations and facilities are grossly inadequate to check the flow of sick infected animals. The gradual improvement in transport networks within Nepal tends to speed up the process of disease transmission especially during epidemics due to the absence of effective institutions and facilities for control movement of animals. Nepal has wide diversity of climate and vegetation from sub-tropical to alpine types. This predisposes the animals with major diseases and parasites of sub-tropical to temperate climates. The migratory transhumant system of movement of animals to the alpine pastures in summer and to lower altitudes in winter also predisposes animals to come in contact with disease and parasites in the alpine pastures as well as in winter grazing areas around village. Furthermore, traditional grazing practice along the roads tracks, canals, fallow lands and rice fields especially in the terai and lower hills exposes the grazing animals to internal parasites eg; liver fluke and round worms as the stall feeding practice is limited to rice growing season only

Problems associated with livestock production and management in Nepal

By studying the collected information, related to livestock production in rural areas, the major problems for improved livestock production can be concluded as below:

- Animal feed deficit – 34% TDN (Terai 42% and mid-hill 56%)
- Traditional management system
- Small scale rearing of livestock (subsistence nature)
- Inbreeding problems
- Open grazing system
- Poor extension service and research
- Integrated farming system
- High investment.
- Low production (local cattle – 450liters per lactation and local buffalo - 900 liters per lactation)
- Lack of commercialization.
- Lack of coordination between the related agencies.
- Lack of technical knowhow to the farmers.
- Lack of skillful manpower in rural areas.
- Lack of technical service delivery network (One livestock service centre and one technician covered 5 to 7 village Development Committee).
- Lack of soft loan and insurance.
- Lack of awareness on people related to animal sanitation, maintenance of animal health and hygiene
- Poor vaccination programs

Opportunities of livestock production in Nepal

- Increase household income generation and self employment generation opportunity in the rural areas.
- Import substitution and export promotion. (Import million of live animal for meat purpose and 4 lakh liters of milk)
- Shortage of milk and meat supply (14 kg meat require per person per year but only 9 kg available and 250 ml. milk per person per day but only 147 ml. available).
- Established fodder tree (especially in mid-hill areas)
- Market assist and established two powder milk plant
- Available of unproductive land for forage production.
- Development of physical infrastructure like Road, electricity.
- Develop small market in local areas.
- The potentiality of indigenous breeds can be improved through cross-breedings
- Basis of research works
- Due to greater topographic and climatic variation different livestock breeds can be raised
- Increasing rate of urbanization (5.6 percent per annum), increasing income and changing food habit have significantly increased the demands for livestock products (milk, meat and egg).

Challenges of livestock production in Nepal

- Encroachment into forest and grazing land by the people.
- High population of unproductive animal (70% indigenous breeds)
- Land fragment (majority households low landholding)
- Most of the youth are out of the village
- Low attraction of young and new generation
- Geographic areas
- Climate change
- Internal migration (Rural areas to urban areas)
- Political instability.

CONCLUSION

The livestock sub-sector has and continues to make an important contribution to the Nepalese economy and the agricultural sector in particular. As overall consumption of meat and other high protein animal products is low, and as incomes of Nepali households improve, the livestock sector should continue to see sustained growth over the coming years. Overall growth in the livestock sub-sector can be mainly achieved through increases in herd sizes. On the other hand animal health play major role in sustained production. Proper sanitation, proper care, better hygienic condition are indispensible elements in livestock production. Unless proper health and care is not given the production of animal cannot be obtained. In case of improper hygienic condition there can be huge losses in the herd because of higher spread of diseases. Thus to increase the production and to increase the national economy the unhygienic rearing of animals in the rural areas should to controlled and farmers should learn about the proper livestock production and management. The main point to be emphasized in case of animal husbandry in rural Nepal is that lack of sanitation and hygiene is producing adverse effect on animal health and productivity. Thus it is of foremost importance to all government agencies, non-governmental sectors, policy makers, planners to emphasis on raising awareness to rural farmers about animal sanitation and hygiene if the world dreams of bringing sustainable changes in the livelihood of these people and ensuring production of quantitative and qualitative animal products for fostering food security.

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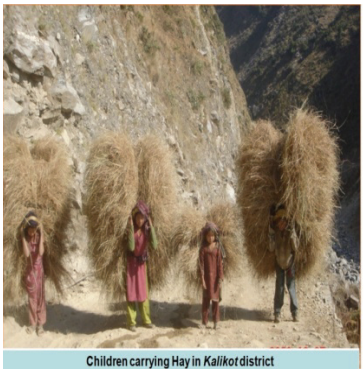
Grazing area

Pastureland

Housing of animal during winter



Children carrying Hay in *Kalikot* district

Women passing the *Tila* river with forage in *Kalikot*

Livestock rearing system in rural areas of Nepal (housing system)

Livestock rearing system in rural areas of Nepal (housing system)

MICROBIOLOGICAL PROFILE AND ANTIMICROBIAL RESIDUES IN MILK SAMPLES OF COWS FROM FARMS OF CAMPINAS – SP, BRAZIL

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SUMMARY

This study was conducted to evaluate the microbiological profile and occurrence of antimicrobial residues in milk samples from five dairy farms belonging to a line of milk collection in the metropolitan region of Campinas – SP, Brazil. We assessed the level of occurrence of clinical and subclinical mastitis by examination of the mammary glands of lactating females, using screening tests Tamis and *California Mastitis Test*, respectively. Milk samples were collected from the mammary glands for isolation and identification of the causative agents of mastitis. The somatic cell count was performed on milk samples of balloons collectors and cooling tanks. The presence of antimicrobials was evaluated in samples of milk from mammary glands, balloons meters and cooling tanks through microbiological testing (Delvotest®) The results presented by the study showed a prevalence of clinical mastitis on properties between 2.32 to 16.66%. The somatic cell counts in cooling tanks ranged from 221 to 1.124 x 1.000 cells / mL. The results of the CCS balloons collectors and CMT levels of mammary quarters were significant (P<0.05), indicating that differences in the occurrence of CMT are associated with classes of CCS. Of milk samples of isolation (41.98%), the largest genre's profile was isolated *Staphylococcus* spp (55.79%), *Corynebacterium* spp (24,60%) and *Streptococcus* spp (16.73%).The frequency of mammary quarters with CMT and levels of isolation was significant (P <0.05), indicating that differences in the occurrence of positive and negative isolates were associated with levels of CMT. Scores 2 and 3 CMT occurred in a higher percentage of mammary quarters with positive isolations. Statistical analysis for the frequencies of mammary quarters with isolates of each property was significant (P <0.05), indicating that differences in the occurrence of positive and negative isolates were associated with characteristics of the properties. The analysis of antimicrobials in balloons collectors and mammary glands showed 8.33% and 4.30% of samples in the positive level, respectively. Statistical analysis for the frequencies of mammary quarters with CMT and residue levels, adding the scores of CMT 1 and 2 was significant (P <0.05). The CMT score of 3 occurred in higher percentages when the residue was positive. Antimicrobial residues were detected in 5% of cooling tanks. The results highlight the need to implement policies focused on aention to quality of milk.

INTRODUCTION

Assessing the quality of milk has been a poin of intense concern to public health. The somatic cell coun, isolation and identification of pathogens and the presence of antimicrobials, have been important factors in evaluating the quality of the produc [6]. The objective of this study was to evaluate the microbiological profile and the occurrence of antibiotic residues in milk samples from five dairy farms, region of Campinas, São Paulo State, Brazil.

MATERIAL AND METHODS

Dairy farms were characterized according to management, milking hygiene, control and prevention of mastitis and milk production in leers (Table 1) A total of 20 samples of milk cooling tanks, 604 milk samples from milk collectors balloons and 2,363 samples of milk from mammary glands of lactating cows from five dairy farms were analyzed every 15 days during the period of 45 days The strip-cup test was performed to detect clinical mastitis cases. California Mastitis Test (CMT) and somatic cell count (SCC) using the apparatus *Somacoun*t 300 ("*Bentley Analytical Instruments for Dairy Industry*").were used to select subclinical mastitis. The analyses for the detection of antimicrobials in milk were performed with commercial microbiological test (Delvotest ® SP). Isolation and identification of causative agents of mastitis was performed in all mammary quarters as described [3] Statistical analysis was performed by Chi-square ($\chi 2$) and Kruskal-Wallis employing the "*Software Graphpad Instat 2003".*

Table 1: Characteristics of managemen, milking hygiene, prevention and control of mastitis in dairy herds from Campinas, Sao Paulo State, Brazil

Characteristics	A	B	C	D	E
Animal breeds	Holstein	Brown Swiss	Holstein	Crossbred*	Crossbred*
Mean number of milking cows in herd	110	12	69	25	43
Milk production per farm (Kg)	2.600	95	728	350	150
Type of milking	Tandem	Bucket basic	Tandem	Bucket basic	Bucket basic
Number of milking	3	1	2	2	1
Bulk milk	yes	yes	yes	yes	yes
Veterinary care	yes	No	yes	No	No
Strip cup test	yes	yes	yes	No	yes
Predipping	yes	No	No	No	No
Postdipping	yes	No	yes	No	No
Single use paper towel	yes	No	yes	No	No

*Crossbred Gir x Holstein

RESULTS

Farms (A, B and C) showed higher rates to 3.17%, and the property B presented clinical mastitis in 16.66% of lactating cows Most of the properties (A, C, D and E) showed much of their negative results to the examination of CMT, with rates ranging from 44.21% to 87.32%, while B owned only 29.79% of mammary quarters was negative to the CMT.The results of the somatic cell count (SCC) held in the cooling tanks of dairy farms showed that at certain periods of the properties had counts exceeding 750,000 cells / mL. The farm B exceeded the count of 1.000.000 cells /. mL. The chi-square test for frequencies of classes of SCC and CMT scores was significant at 5% probability ($P < 0.05$), indicating that differences in the occurrence of CMT were associated with CCS class, and the CCS in balloons collectors increased with the highest scores of CMT.The largest genera isolated were *Staphylococcus* (55.75%), *Corynebacterium* (24.60%), *Streptococcus* (16.73%), followed by yeasts and filamentous fungi with 4.74% and 2.62% respectively. Among the genus *Staphylococcus, Staphylococcus* coagulase negative with the highest frequency of isolates (45.93%), followed by coagulase-positive staphylococci (28.57%), *Staphylacoccus aureus* (17.90%) and *Staphylococcus spp*. (7.59%). There was significant differences ($P < 0.05$) between the agents of positive and negative isolates of mammary glands in dairy cows in different CMT scores, and in the CMT scores 2 and 3 showed the largest frequency of mammary quarters with positive agents.Of the total cooling tanks in the dairy farms evaluated for the presence of residual antibiotics 5% were positive for microbiological testing (Delvotest ® SP). Antimicrobial residues were detected in balloons collectors' threshold level ranging between 0.00% and 1.85%, and positive level ranging between 0.00% and 8.33%. Significant difference ($P < 0.05$), when added together the scores of CMT 1 and 2 and residue levels, indicating that differences in the occurrence of CMT scores are associated levels of waste, and CMT score of 3 occurred in higher percentages when the residue analyzed by antimicrobial Delvotest ® SP was in the positive level.

DISCUSSION

The high incidence of clinical mastitis found in the properties, corroborate those found by [1] that in a survey conducted in five dairy farms in the State of São Paulo, found in 201 animals evaluated, 14.29% of animals with clinical mastitis, and the lower frequency of clinical mastitis was around 3.45%. The occurrence of clinical mastitis is closely linked to the characteristics of production and handling of animals. The high incidence of clinical mastitis in the property B, despite having a herd of purebred animals' specialized dairy, milking management demonstrates inadequate without the use of minimal care for the prevention and control as the disinfection of the teats, using paper towels and disposable individual, hygiene facilities and limited number of milking [3].The high SCC in the tank indicates a loss of milk production, while maintaining low SCC in the tank is an indicator of good health of the gland [8, 9]. The CCS in balloons collectors has increased with the highest scores of CMT. This result highlights the importance of the CMT as an auxiliary tool in the identification of samples with high SCC. The results of microbiological isolates are consistent with studies that point to the staphylococci as the main causative agent of mastitis in Brazil. In 19.113 milk samples evaluated in dairy cows with mastitis has the highest prevalence of coagulase-positive staphylococci (30.1%), followed by *Corynebacterium bovis* (22.5%), *Staphylococcus epidermitis* (13.1%) and *Streptococcus spp*. (14.2%) [2]. Samples of milk from mammary quarters with CMT scores negative in microbiological culture were positive (25.43%). The observed data shows that despite the detection of mammary glands were not reactive to CMT, we cannot state with certainty the absence of microorganisms in the mammary gland. In contrast, mammary glands positive for CMT, but without microbiological isolation can occur due to an inflammation of noninfectious origin [8, 9].The sample of milk cooling tank positive microbiological test for detecting residual antimicrobial property is related to milk (B) that showed

high percentage of clinical mastitis. These results corroborate those found by [6] who also noted the influence of clinical mastitis in the presence of antimicrobial residues in cooling tanks at dairy farms. All properties were evaluated in samples of milk from mammary glands of antimicrobial residues in threshold levels and positive, so the balloons are being filled with contaminated milk. Property B in addition to presenting outstanding results in the occurrence of antimicrobial residues in milk also showed a higher incidence of clinical and subclinical mastitis. This result emphasizes some aspects already proposed in the literature, including the relationship of clinical and subclinical mastitis with the occurrence of residues in milk [7]. The presence of inflammation in the mammary gland, as well as its intensity, clinical or subclinical mastitis, are determining factors for the occurrence of antimicrobial residues in milk. Animals with clinical mastitis treated with antibiotics had a higher occurrence of residues (47.7%) compared to animals with subclinical mastitis (34.9%) [5].

CONCLUSION

The results show the great need to implement public policies in the production of milk in order to minimize the involvement of production, milk quality and consumer health.

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ANTIMICROBIAL RESISTANCE OF BACTERIA ISOLATED FROM MASTITIS MILK SAMPLES FROM GOATS IN BRAZIL

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SUMMARY

Mastitis is an infection of mammary glands and is considered the most important diseases of udder. This disease causes the most loss of milk production in goats, reducing the quantity and decreasing the quality of milk and its derivates. This study deals with samples of milk which were collected from goats with positive response to California mastitis test, from farms in Bahia state, Brazil. The agar disk diffusion was used for susceptibility testing of mastitis pathogens. All isolates were subjected to susceptibility testing to the following antimicrobials: amoxicillin (10μg), amoxicillin+clavulonic acid (30μg), ampicillin (10μg), cefalonium (30μg), cephalothin (30μg), gentamicin (10μg), norfloxacin (10μg), novobiocin (30μg), sulfazothrim (25μg) and tetracycline (30μg). Gram positive bacteria were also tested for sensitivity to streptomycin (10μg), oxacillin (1μg) and penicillin (10IU), whereas for Gram negative bacteria were tested for polymyxin B (300IU). The interpretation of the results followed the recommendations of the National Committee for Clinical Laboratory Standards. The higher prevalence of isolated bacteria belonged to the genus *Staphylococcus* (90.3%), being *S. aureus* (29%) the most isolated species. Bacteria from other groups like *Streptococcus* (4.9%), *Micrococcus* (1.7%) and *Enterobacter* (1.7%) were also isolated. All bacteria were submitted to antimicrobial susceptibility testing, and 54.1% of the strains studied were sensitive to all drugs tested, and 45.9% of identified strains were resistant to at least one antimicrobial agent. Of the 67 *S. aureus* and 208 coagulase negative staphylococci strains, 31.3% and 8.2% were resistant to penicillin, 29.9% and 1.0% to ampicillin, 1.5% and 10.6% to erythromycin, and 3.0% and 7.7% to tetracycline, respectively. The present study confirmed the *Staphylococcus* spp. as the main role in the etiology of mastitis in goats in Brazil, which reinforces the concern about the possibility of pathogens being served by food of animal origin like milk and dairy products to humans can cause serious damage to public health.

Keywords: Caprine mastitis; *Staphylococcus*; Antimicrobial resistance.

INTRODUCTION

World production of goat milk is estimated at 7.3 million liters per year, behind only the production of cow and buffalo milk. Greece is the country that has the largest goat herd with approximately 4 million head, followed by Spain with around 2.2 million animals. Brazil currently holds the world's ninth largest goat herd. However, it is known that many problems in the sanitary conditions or management of goats may result in loss of milk quality and cause serious harm to public health. Given that reality, mastitis in goats is considered the main disease that affects dairy goats, not only in Brazil but worldwide, causing large economic losses to producers of milk and dairy industry. Mastitis is an infection of mammary glands and is considered the most important diseases of udder. A wide variety of microorganisms may be associated with mastitis in goats. This disease causes the most loss of milk production in goats, reducing the quantity and decreasing the quality of milk and its derivates (5). According to the intensity of the inflammatory process, the mastitis is also classified in clinical or sub clinical. Clinical mastitis is characterized by inflammatory symptoms evident commitment of the mammary gland and visible changes in milk, as the presence of fibrin clots or pus (1). The sub clinical mastitis, however, shows no obvious clinical signs, and often the infection can only be detected by indirect evidence of milk as California mastitis test. Among the bacterial species involved in the etiology of infectious mastitis, some strains are resistant to several antibiotics used routinely in the treatment of mastitis. Thus, the purpose of this study was to determine the bacterial etiologic agents most involved in goats mastitis in Bahia state, Brazil, and to assess the resistance profile of these microorganisms against antimicrobial agents commonly used in treating animal diseases and humanities.

MATERIAL AND METHODS

Were evaluated 218 lactating goats of various ages and breeds from farms in Bahia state, Brazil, from August to December 2007. Goats were subjected to clinical examination and then carried out the CMT for presumptive diagnosis of sub clinical mastitis. It was considered positive samples for testing when the CMT was the formation of a firm gel, corresponding for two to three crosses for bovine milk test. Only milk samples considered positive (107 samples) were collected and sent under refrigeration to the laboratory for microbiological analysis. In the laboratory, 30μL of each sample of milk was seeded in sheep blood agar at 10%, Mc Conkey agar and BHI enrichment broth (HiMedia Laboratories Put. Ltd. - India), all incubated aerobically at 36°C, with readings at 24 and 48 hours. Colonies were stained by Gram, observed under light microscope and identified according to the following tests: production of catalase, oxidation and fermentation test, coagulase test and specific biochemical tests for identification of Gram negative rods or coccobacilli (4). Finally, to determine the pattern of antimicrobial susceptibility testing was performed by agar diffusion (2) and tested the following antibiotics: amoxicillin (10μg), amoxicillin+clavulonic acid (30μg), ampicillin (10μg), anhydrous cefalonium (30μg), cephalothin (30μg), gentamicin (10μg), norfloxacin (10μg), novobiocin (30μg), sulfazothrim (25μg) and tetracycline (30μg). Gram positive bacteria were also tested for sensitivity to streptomycin (10μg), oxacillin (1μg) and penicillin (10 Units), whereas for Gram negative bacteria we tested to polymyxin B (300 Units). The interpretation to the sensitivity profile was based on Clinical and Laboratory Standards Institute.

RESULTS

Of the total of 218 females studied, only 58 (26.6%) showed positive result to CMT, which were collected 107 samples of milk. Of this total sample, 61 (57%) showed bacterial growth, and after the bacteriological results found to correlate with the total number of females positive for CMT (58 animals), it was found that 82.7% of females (48 animals) had sub clinical mastitis. Of the total number of microorganisms isolated from mastitis studied, 98.4% were bacteria, and in only one sample (1.6%) was isolated yeasts. Almost all mastitis were caused by a single agent (93.8%), with the exception of three cases (6.2%) where there was mixed infection, and one female showed infection by *Streptococcus* spp. and S. *epidermidis* in the same gland, and the other two had infection with *Streptococcus* spp., *Micrococcus* spp., S. *epidermidis* and yeast, but only one type of pathogen in each gland. In all three cases the bacterial samples were resistant to most antimicrobials tested and all females were in the same herd. *Staphylococcus* spp. (90.3%) and *S. aureus* (29.5%) was the most frequently isolated agent, followed by *S. intermedius* (26.2%), *S. epidermidis* (21.3%) and other coagulase-negative *Staphylococcus* (14.7%). Were also isolated *Streptococcus* spp. (4.9%), *Micrococcus* spp. (1.7%) and *Enterobacter* spp. (1.7%). All isolates were subjected to antimicrobial susceptibility testing, which showed 54.1% sensitivity and 45.9% showed resistance to one or more antimicrobials. The only antimicrobials to which all bacterial samples shown to be sensitive was polymyxin B (PMX) and amoxicillin+clavulonic acid (AMX+C). Regarding other antimicrobials at least one of the samples was resistant. All species of *Staphylococcus* spp. were also sensitive to amoxicillin (AMX), anhydrous cefalonium (CFN), gentamicin (GEN), norfloxacin (NOR) and oxacillin (OXA). Still related bacterial species in the genus, the antibiotics to which most of the strains showed resistance were ampicillin (AMP) (43.5%), streptomycin (EST) (47.8%) and penicillin (PEN) (52, 2%). In contrast, although the small number of bacterial samples belonging to the genus *Streptococcus*, *Micrococcus* and *Enterobacter* found in this work is not possible to assess accurately the impact of these strains as to susceptibility to antimicrobial agents, it is noted that some of these bacterial strains were resistant to most of antimicrobials (Table 1).

Table 1: Bacteria isolated from mastitis in goats subjected to antimicrobial susceptibility testing that showed resistance to one or more antibiotics.

	Bacteria							
	S.aureus	*S.intermedius*	*S.epidermidis*	SCN*	*Streptococcus spp.*	*Micrococcus spp.*	*Enterobacter spp.*	Total of samples
Total of samples	7	6	6	4	3	1	1	28
AMX	0	0	0	0	55 58	59	0	3
AMX+C	0	0	0	0	0	0	0	0
AMP	0	34 38 49 64 65 88	28 55	84 89	55 58	59	41	14
CFN	0	0	0	0	0	0	41	1
CFL	0	0	55	0	0	0	41	2
EST	07 30 40 82 83 91	34 38	100 101	20	0	59	0	12
GEN	0	0	0	0	55 58	59	0	3
NOR	0	0	0	0	0	59	0	1
NOV	40	0	05 06	20	0	59	0	5
OXA	0	0	0	0	55 58	59	0	3
PEN	40	34 38 49 64 65 88	05 06 55	66 89	55 58	59	0	15
PMX	0	0	0	0	0	0	0	0
SUF	0	64 65	05 06 55	66	39 55 58	59	0	10
TET	92	64 65 88	55	66 89	55 58	59	41	11

**Staphylococcus* coagulase negative. AMX: amoxicillin; AMX+C: amoxicillin+clavulonic acid, AMP: ampicillin; CFN: cefalonium anhydrous CFL: cephalothin; EST: streptomycin; GEN: gentamicin; NOR: norfloxacin; NOV: novobiocin; OXA: oxaciclina; PEN: penicillin; PMX: polymyxin B; SUF: sulfazothrim; TET: tetracycline. Highlighted numbers (bold) to identify each of the resistant strains.

DISCUSSION

The present study revealed Gram positive cocci as the main agent of infectious mastitis in goats in Brazil. *Staphylococcus aureus* is considered the most important etiologic agent involved in mastitis goats, both for being the most isolated as the most pathogenic. In the United States and Europe *S. aureus* is more contagious and more prevalent in cases of mastitis (7). A study conducted in Norway (3), detected the presence of *S. aureus* in 96.2% of raw goat milk samples analyzed and this high prevalence was also reported by other authors in European countries (6). In dairy cattle there are numerous works of antimicrobials for etiological agents of mastitis, but it is not to goats. Regarding the results of antimicrobial susceptibility test, Table 1 shows two strains of *S. intermedius* (samples 64 and 65) and another strain of *Enterobacter* spp. (sample 41) resistant to four drugs, a strain of *S. epidermidis* (sample 55) resistant to five drugs, two strains of *Streptococcus* spp. (samples 55 and 58) resistant to seven drugs, and one strain of *Micrococcus* spp. (sample 59) revealing resistance to ten different antibiotics, indicating the multiple resistance of these drugs. Some types of antibiotics are widely used for treatment of intramammary infections, contributing further to the possibility of bacterial resistance against these antimicrobials and suggesting public health risks if these strains were transmitted to humans through the consumption of goat milk raw or poorly thermally processed.

CONCLUSIONS

The present study confirmed the *Staphylococcus* spp. as the main role in the etiology of mastitis in goats in Brazil, which reinforces the concern about the possibility of pathogens being served by food of animal origin like milk and dairy products to humans can cause serious damage to public health.

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DETECTION OF ANTIMICROBIAL RESIDUES IN MILK FROM COWS WITH AND WITHOUT SUBCLINICAL MASTITIS: MICROBIOLOGICAL TESTING

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SUMMARY

Mastitis is an inflammatory process of the mammary gland, usually of infectious origin, which requires frequent treatment with antibiotics. The presence of antimicrobial residues in milk cause allergic reactions and the selection of resistant microorganisms, which pose a serious public health problem. This study aimed to verify the presence or absence of inflammation influence the time of removal of antibiotics given to cows during lactation. We used 20 cows Holstein Black and White, 10 without inflammatory process (control) and 10 with subclinical mastitis assessed by the California Mastitis Test (CMT) and treated with intrammamary infusion of aminoglycoside antibiotics (gentamicin) for three days. For detection of antimicrobial residues in milk, we used the commercial test (Delvotest® SP). Samples of milk from mammary quarters before the beginning of the treatments were withdrawn for determining the microbiological profile. After the last application of the antibiotic, samples of milk were collected from mammary glands treated and balloon for six days (four days of lack of medication plus two days afterwards), for detection of antimicrobial residues. We evaluated the antimicrobial resistance of strains isolated use of antimicrobials in veterinary medicine in Brazil. The results showed that 76.88% of the mammary glands were evaluated the negative CMT and 23.12% had some scores to the test. From the positive samples to test 73.08% showed bacterial growths were isolated: *Corynebacterium* spp (38.89%), *Streptococcus* spp (38.89%), *Staphylococcus aureus* (5.56%) and coagulase negative *Staphylococcus* (16.67%). Antimicrobial residues were detected after the grace period of medication in 50.00% and 66.70% of mammary quarters and 40.00% and 20.00% of the balloons collectors of animals with and without inflammation, respectively. Strains of coagulase negative staphylococci were resistant to the antibiotic oxacillin (1µg) and penicillin G (10mg) in 18.18% and 36.36% of the samples, respectively.

INTRODUCTION

Mastitis is an inflammatory process of the mammary gland that causes severe losses in milk quality and milk production [2,15] The indiscriminate use of drugs in the treatment of mastitis encourages bacterial resistance and the presence of antimicrobial residue in milk.[16] Antibiotics residues are undesirable for public health reasons and because of their potential impact on the manufacturing process [10,12] The main causes of residues in milk are the use of drugs not recommended and not well established pharmacokinetics, drug abuse, non-compliance of the period of disposal of milk from animals in treatment, the errors in the identification of treated animals, annotation errors in data processing, errors during milking and milk mixture with and without waste [8,9,13] Some studies have found the presence of antimicrobial residues in milk after the period of disposal recommended by the pharmaceutical industry, raising concerns that the cooling tanks are being filled with residues of these drugs [9]. Given the above, the present study was to evaluate the influence of the inflammatory process in the persistence of antibiotic residues in milk samples of dairy cows and to evaluate the microbial profile and sensitivity compared to bacterial antibiotics used to treat mastitis in Brazil

MATERIAL AND METHODS

We used 20 Holstein Black and White cows, 10 animals with subclinical mastitis and 10 without inflammatory process selected through CMT reactions [14] The animals that reacted +++ (score) were classified with subclinical mastitis and those negative were classified without inflammation Intramammary aminoglycoside antibiotics infusion was applied in 18 mammary quarters that showed + + + reactions of 10 selected animals with subclinical mastitis. The animals without inflammation (control group) were treated in the same proportion as the group of animals with inflammation (18) Before the treatments, milk samples were collected from the mammary gland for isolation and identification of causative agents of mastitis. [11]. Milk samples from balloons collectors and mammary gland were removed for analysis of antibiotic residues for 6 days starting 24 hours after the last application of the drug's profile. Antimicrobial sensitivity of isolated strains of coagulase negative *Staphylococcus*, *Staphylococcus aureus* and *Streptococcus* spp. was determined by the diffusion test on Mueller-Hinton agar, using disks containing the following antibiotics: oxacillin (1µg), gentamicin (10mg), kanamycin (30µg), cefaclor (30 mg),

neomycin (10mg), penicillin G (10mg) and rifampicin (30µg). The growth inhibition zones were evaluated according to the standards of the National Committee of Clinical Laboratory Standards [17]. The evaluation of the presence of antibiotic residues in milk were carried out with the microbiological test Delvotest® SP (DSM Foods Specialties.).[1] All statistical analyses were performed using the GRAPHPAD INSTAT software [7]

RESULTS

Of the total of 173 mammary glands of cattle evaluated, 133 (76.88%) showed negative reaction, 13 (7.51%) + reaction, 9 (5.20%) + + reaction, 18 (10.41%) + + + reaction. The microbiological examination of quarter milk samples from 80 of the 10 selected animals with and without inflammation showed that the total of 54 samples of mammary quarters with a negative result to CMT, 26 (48.15%) had microbiological growth and 28 (51.85%) had negative cultures. Of the 26 positive samples from mammary glands to CMT, 7 (26.92%) were negative for microbiological growth and 19 (73.08%) had positive cultures for growth of microorganisms

Among the microorganisms isolated in animals with subclinical mastitis, *Streptococcus* spp (38.89%), *Corynebacterium* spp. (38.89%) and coagulase negative *Staphylococcus* (14.82%) were the most prevalent causative agents. Milk samples from animals without inflammatory process had the most common pathogens: *Corynebacterium* spp. (50.00%) followed by coagulase negative *Staphylococcus* (28.57%) and gram negative bacilli (14.29%).

The microbiological test (Delvotest® SP) detected antibiotic residues in milk after the grace period stipulated by the manufacturer, in 50.00% and 66.70% of mammary quarters and 40.00% and 20.00% of the balloons collectors animals with and without inflammation, respectively. According to the results obtained from analysis of antibiotic residues in milk, there were no statistically significant differences ($p > 0.05$) between groups of animals studied, and thus, no evidence of animal groups (with and without subclinical inflammation) influence the results of Delvotest® SP levels in negative and positive threshold. The sensitivity profile of the strains tested were isolated from animals with and without inflammation showed that 18.18% and 36.36% of coagulase negative staphylococcal strains were resistant to antibiotics oxacillin and penicillin G, respectively. The strains of *Staphylococcus aureus* and *Streptococcus* spp. were sensitive to all antibiotics tested.

DISCUSSION

The positive reactions to the CMT were low compared to those reported by several authors in Brazil [3,4,5] The concern with hygiene, good milking techniques, proper nutrition of the flock, may reflect low or negative reactions in CMT [3]

The results of the microbiological profile are similar to the study by [3,4,5] who observed a higher prevalence of *Staphylococcus* spp., *Streptococcus* spp. and *Corynebacterium* spp. were shown. In Brazil, *Staphylococcus* spp., followed by *Streptococcus* spp. and *Corynebacterium bovis,* are cited as the main microorganisms associated with cases of bovine mastitis [5]

The occurrence of antimicrobial residues in milk after the recommended withdrawal time period was also observed in several studies [8,9,10,13]. It was verified the persistence of waste disposal in 18.75% of samples from mammary glands of animals treated with cefacetrile, 18.10% to 11.10% with gentamicin and tetracycline, where the period of disposal of waste detected by Delvotest® SP exceeded period recommended in the instructions [13].

The results of this study concur with those reported by [5] to evaluate the influence of the occurrence and intensity of the inflammatory process in the presence of residues of antibiotics, found no statistically significant differences. However, in a study by [13] which assessed the persistence of antibiotics applied intrammamary, it was observed that the mammary glands with inflammation had higher residues of antibiotics than mamary glands without inflammatory process. In a study by [6] the authors observed that the sensitivity of strains of coagulase negative staphylococci, found that 27.60% of these strains were resistant to penicillin, and 100% were sensitive to gentamicin, confirming the results obtained in this study.

CONCLUSIONS

There were no influences of the inflammatory process in the elimination of antibiotics, but antibiotic residues were found after the grace period determined by the product manufacturer in the milk of mammary glands and balloons collectors of treated animals. *Staphylococcus* coagulase negative were resistant to the antibiotics tested against

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ANTIBACTERIAL DRUG RESIDUES IN TISSUES OF ANIMALS SLAUGHTERED IN ASSIUT CITY

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SUMMARY

Seven hundred (diaphragm and kidney) samples were collected from cattle and buffaloes slaughtered in Assiut city for detection of antibacterial residues by four plate method. Antibacterial drug residues detected in tissues of cattle, with percentages 58 and 14 in kidney and muscle, while in buffaloes with percentages 40 and 20,respectively. The different methods of prevention and control have been discussed for protection of the consumer from the hazards of antibiotic residues in meat.

INTRODUCTION

In Poland**,** a study was conducted on in 1992 and pointed that 0.2% of cattle's meat , and 0.5% of calve's meat were positive for Streptomycin residues(6).In Saudia Arabia, Youssef et al. (2002), detected antibacterial residues in muscles and livers of camels, cattle and sheep slaughtered in six different localities of Al Gassem region with 2.5-5%. Salem (2003) showed that the most used antimicrobials in food animals can be grouped into five major classes.These include the βeta-lactams (e.g.Penicillins and Cephalosporins),Tetracyclines (e.g.Oxytetracyclines, Tetracyclines, Chlorotetracyclines), Aminoglycosides (e.g.Streptomycin, Nemomycin and Gentamycin), Macrolides (e.g. Erythromycin) and Sulfonamides (e.g. Sulfamethazine).The aim of present study was planned to detect antibacterial residues in tissues of animals slaughtered in Assiut city.

MATERIAL AND METHODS

Collection of samples: Seven hundred samples were collected from carcasses of cattle (300 from diaphragm muscle, and 300 from kidneys) and buffaloes (50 from diaphragm muscle , and 50 from kidneys) slaughtered in Assiut city, during the period of March 2006 to July 2007.Each sample weighted 100 gm was collected in sterile plastic bag and transferred to the laboratory in where these samples subjected for detection of antibacterial drug residues by using of four plate method according to (4).

Test organisms:Bacillus subtitlis BGA was obtained from the department of Microbiology,
Faculty of Veterinary Medicine, Cairo University, for detection of B-lactam antibiotics
and Tetracyclines at pH 6.0, Aminoglycoside antibiotics at pH 8.0 and Sulpha drug residues
at pH 7.2.While Micrococcus luteus ATCC from the department of
Microbiology, Faculty of Veterinary Medicine, Cairo University, for detection of Macrolides
and Aminoglycosides at pH 8.0.

Medium and preparation of test plates: Standard II nutrient agar (Merck Art. Nr .7883)
+0.1 KH_2PO_4.at pH 7.2.
Test agar at pH 6.0 (Merck Art. Nr .10663) and at pH 8.0 (Merck Art. Nr .10664)
Brain Heart Infusion broth (Oxoid CM 225) pH was adjusted at 37°C to 7.4.
After incubation ,the zones of inhibition were accurately measured and the results were interpreted as follows:
Inhibition zone of 2.0 mm or more=Positive Inhibition zone of 1 below 2.0 mm=Suspicion
Inhibition zone below 1 mm=Negative.

RESULTS

Table (1):Antibacterial drug residue in muscle and kidney of cattle.

TYPES OF ANTIBIOTICS	MUSCLES		KIDNEYS	
	Incidence of positive	%	Incidence of positive	%
β- lactam and Tetracyclines	12	4%	64	21.3%
Sulpha drugs	12	4%	56	18.7%
Aminogylcosides	10	3.3%	37	12.3%
Macrolides and Aminoglycosides	8	2.7%	17	5.7%
Total	42	14%	174	58%

*Total number=600 (300 each of muscle & kidney).

Table (2): Antibacterial drug residue in muscle and kidney of buffaloes.

TYPES OF ANTIBIOTICS	MUSCLES		KIDNEYS	
	Incidence of positive	%	Incidence of positive	%
β- lactam and Tetracyclines	3	6%	6	12%
Sulpha drugs	3	6%	5	10%
Aminogylcosides	2	4%	5	10%
Macrolides and Aminoglycosides	2	4%	4	8%
Total	10	20%	20	40%

*Total number=100 (50 each of muscle & kidney).

DISCUSSION

The positive samples of kidney and muscle tissues of cattle & buffaloes for the presence of β-lactam and Tetracycline, Sulpha, Aminoglycosides and Macrolides and Aminoglycosides residues are tabulated in tables (1 &2).

The data obtained in present work proved that β-lactam and Tetracyclines residues were detected in kidneys of cattles slaughtered in Assiut city with higher percentage (21.3%), followed by Sulpha drugs residues (18.7%),and Aminoglycosides residues (12.3%), while lower findings were detected in kidneys of cattle were Macrolides and Aminoglycosides(5.7%).The present results indicated that muscle samples were contained antibiotic residues with lower percentages (14%) than kidney samples (58%).The percentage of positive samples of muscles and kidneys in cattle at different pH were 72%. Nearly similar findings were recorded by (1, 3,5, 8 & 9).

With respect to buffalo tissues, the result showed that 3(6%), and 6(12%) samples of diaphragm muscles and kidneyscontained β-lactam and Tetracycline residues, respectively. A higher finding carried out by (2 & 8),they detected β-lactam and Tetracycline residues in muscles and kidneys of buffaloes.

For Sulpha drugs residues, three samples (6%) of diaphragm muscles were positive for Sulpha drugs residues. In case of kidney, the positive samples were 5 which constitute 10% of total kidney samples.Data of Aminoglycosides residues in muscles and kidneys of baffaloes slaughtered in Assiut city showed that two samples (4%) of diaphragm muscles were positive for Aminoglycosides residues.

In case of kidney, Aminoglycosides residues were detected in five samples only (10%). Moreover, The result tabulated in table (2), indicated that only two samples (4%) of diaphragm muscles were positive for Macrolides and Aminoglycosides. In case of kidney four samples were contained Macrolides and Aminoglycoside. Nearly similar findings were registered by (2 & 8).

Filteration and clearance of the blood from abnormal constituents and drugs take place in the kidneys. So they contained the highest concentration of antibiotics among other organs and muscles..

CONCLUSION

Many public health problems may arise from antibacterial drugs residues in food .To overcome such hazards, prohibiting administration of antibacterial drugs before slaughter is necessary for withdrawal of any residues. All animals sent to emergency slaughter which might have been recently treated with antibiotics should be examined for the presence of antibacterial drug residues and if antibacterial drug residues were found, the meat should be excluded from human consumption.

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MONITORING OF ANTIMICROBIAL RESISTANCE OF ANIMAL PATHOGENS IN ESTONIA

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SUMMARY

According to the results of antimicrobial resistance monitoring programm in Estonia, indicator bacteriae isolated from pigs faeces show highest resistance to streptomycin, tetracycline and sulfamethoxazole, isolates from cattle were resistant to gentamycin and kanamycin. Resistance of *E. coli* as the representative of normal microflora of a gastrointestinal tract has been decreased during last five years. *S.aureus* isolated from clinical mastitis samples showed highest resistance to penicillin, clindamycin and tetracyclin.

INTRODUCTION

Antimicrobial resistance of most common animal pathogens is a worldwide problem. In Estonia antimicrobial resistance of bovine mastitis pathogens and indicator bacteria has been investigated regularly over 10 years. Data was collected during national antimicrobial resistance montoring programme in Estonia. In the current paper the results of investigation carried out during years 2008-2010 are presented.

MATERIAL AND METHODS

Indicator bacteria *Escherichia coli(E.coli)* (n=144) and *Enterococcus* spp. (n=86) were isolated from faecal samples from healthy pigs and dairy cows. The isolates of *Staphylococcus aureus(S.aureus)* (n=162*)* originated from routine bacteriological examinations of clinical mastitis samples at Estonian Veterinary and Food Laboratory. The *in vitro* antimicrobial susceptibility was determined by using microdilution method (VetMIC ®, Sweden). Epidemiological cut-off values issued by the EUCAST were used for interpretation of results of susceptibility testing of indicator bacteria and clinical breakpoints for *S.aureus*. The clinical breakpoints recommended for animal pathogens by CLSI (2008) were also taken into consideration. Chi-square test was used to evaluate differences between isolates collected from cattle and pig faeces. Statistical significance was assumed at $p \leq 0.005$.

RESULTS

Indicator bacteria isolated from pig faeces showed higher resistance compared to samples from cattle faeces ($p<0.005$). *E.coli* strains (n=71) isolated from pig faecal samples were resistant to streptomycin (33.8%), tetracycline (28.1%) and sulfamethoxazole (22.5%), isolates from cattle were resistant only to gentamycin (10.9%) and kanamycin (12.3). Resistance of *E. coli* as the representative of normal microflora of a gastrointestinal tract has been decreased during last three years.

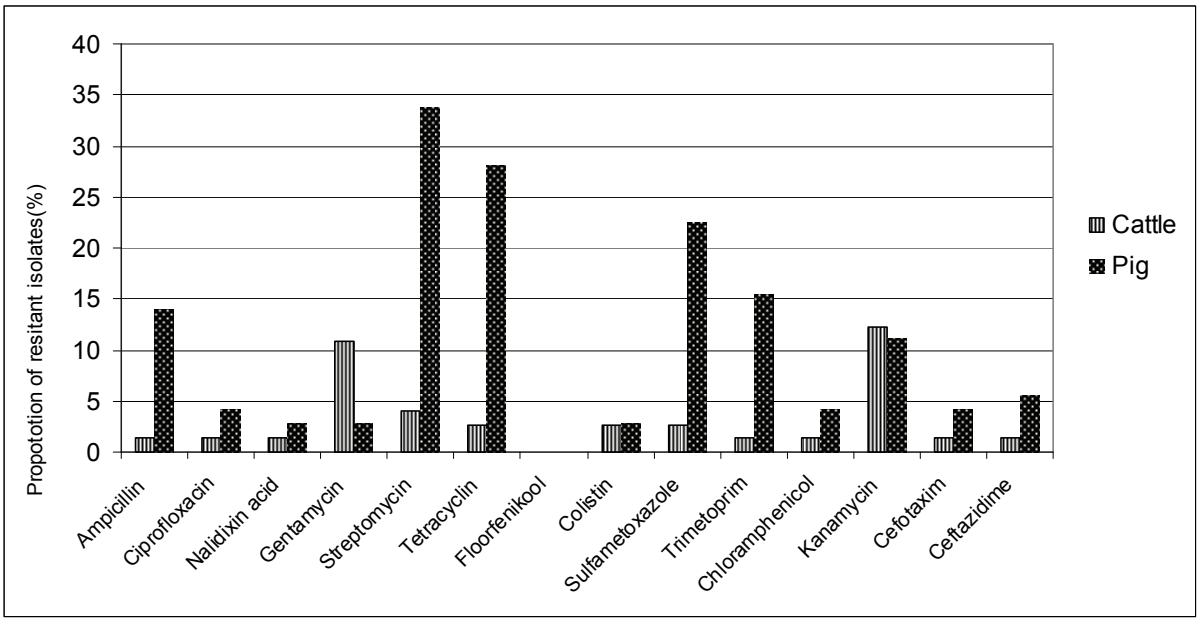

Figure1. Propotion of resistant *E.coli* isolates collected from cattle (n=73) and pig (n=71) faecal samples during 2008-2010 in Estonia.

Highest resistance during recent years has been detected against streptomycin and tetracyclin. The amount of multiresistant strains has been on the same level from year to year, being 10%. Resistance against chloramphenicol has decreased because chloramphenicol has not been used in veterinary practice in Estonia for more than 10 years.

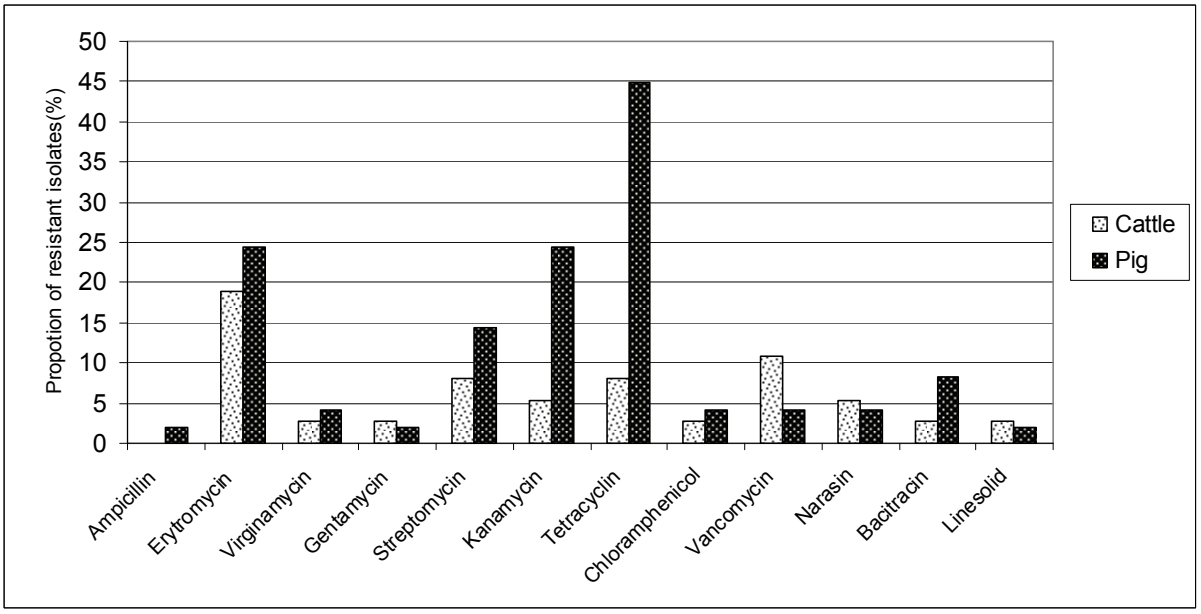

Figure 2. Propotion of resistant *Enterococcus faecalis/faecium* isolates collected from cattle (n=37) and pig (n=49) faecal samples during 2008-2010 in Estonia.

Among the *Enterococcus spp.* isolates from pig faeces, the highest percentage of isolates were resistant to tetracyclin (44.9%), erytromycin (24.5%) and kanamycin (24.5%). Isolates from dairy cow faeces (n=37) showed resistance only to erytromycin (18.9%) and vancomycin (10.4%). Amount of multiresistant bacteria has been about 30% during years 2008-2010. Multiresistance occures mainly against kanamycin, streptomycin and tetracyclin.

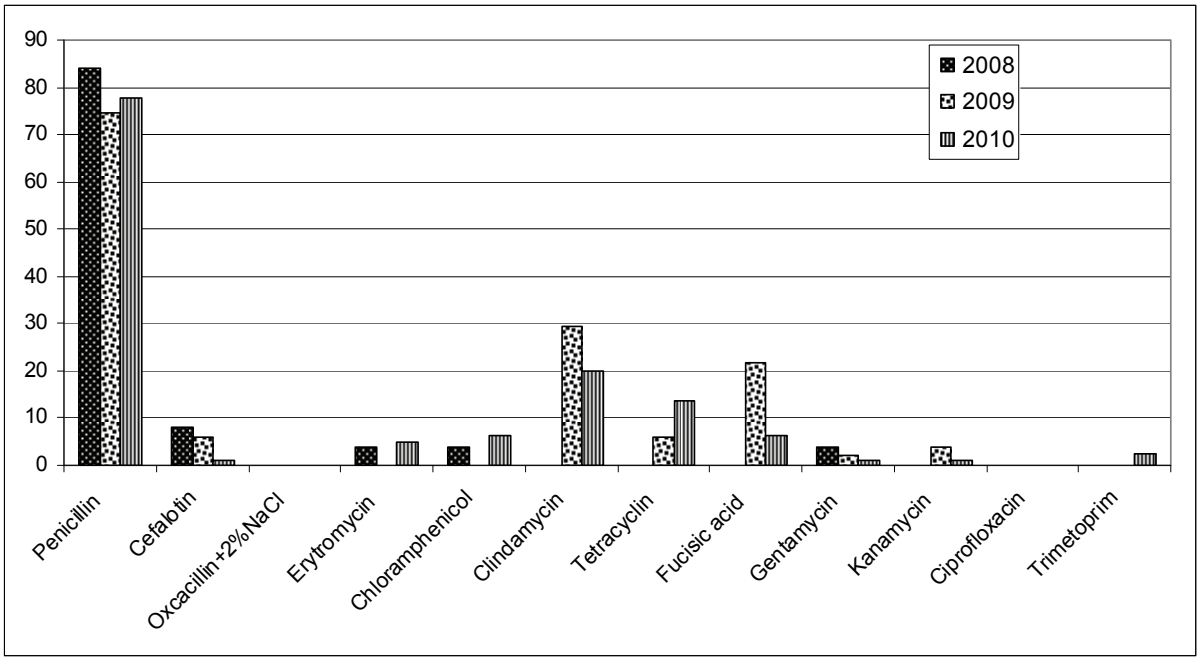

Figure 3. Propotion of resistant *S.aureus* clinical mastits isolates (n=162) in years 2008-2010 in Estonia.

Penicillin resistance in *S. aureus* isolated from bovine clinical mastitis samples was 77.7%. *S. aureus* resistance against clindamycin (19.1%) and tetracyclin (8.9%) was also common. Six (3.8%) isolates were resistant to chloramphenicol. Alltogether twenty-one isolates (25.9 %) showed multiresistance.

DISCUSSION

In comparison with *E. coli,* enterococci show high resistance to several antibiotics. Resistance has been similar over the years against erythomycin, tetracyclin, streptomycin and kanamycin. It is clearly visible that resistance is highest to antibiotics used in pig and cattle for group therapy for treatment of respiratory infections. Resistance to tetracyclin is probably high because of the use of doxycyclin and resistance to macrolide because of the use of tylosin for group therapy in animals. The percentage of vancomycin resistant enterococci is relatively high, that can be considered as a potential risk for human medicine. Vancomycin resistant enterococci are most common pathogens causing hospital infections in human medicine. In Europe the main pathogens causing hospital infection is *E. faecium* carring *vanA* gene and especially in hospitals adapted vancomycin resistant enterococci belonging to the clonal complex 17(Werner *et al* 2008). It is much needed to investigate vancomycin resistant enterococci isolated from pigs and cattle in relation of *vanA* and *vanB* gene in order to confirm possible transmission of the microbe from animals to humans.

Among *S. aureus* isolates from bovine mastitis samples there is relatively high amount of multiresistant strains. Reason for that can be relatively wide use of broad spectrum antibiotics for treatment of bovine mastitis.

CONCLUSIONS

Both, mastitis pathogens and indicator bacteriae isolated from animals showed relatively high resistance to most frequently used antimicrobials. The overall resistance of indicator bacteria has been slightly increased from year to year. According to the investigation results local rules for prudent use of antibiotics and continuous resistance monitoring program have been worked out.

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ANTI-TUMOR EFFECT OF ROXITHROMYCIN ON HEPATOCARCINOGENESIS INDUCED BY N-NITROSODIETHYLAMINE AND CARBON TETRACHLORIDE IN RATS

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SUMMARY

This paper evaluates the anti-tumor effect of Roxithromycin (**RXM**) on hepatocarcinogenesis. Histopathologically, proliferative and neoplastic changes were the main lesions induced in liver of rats by N-nitrosodiethylamine (**NDEA**) and carbon tetrachloride ($\mathbf{CCL_4}$). Computerized morphometrical analysis revealed that treatment with **RXM** reduced the number and size of the eosinophilic preneoplastic foci as well as inhibited the development of large cell dysplasia, hepatoma and hepatocellular carcinoma in nearly 100 % of rats. Moreover, serobiochemical studies showed that **RXM** modulated the level of Nitric oxide (**NO**) to nearly normal level to which the inhibitory action of tumor proliferation is attributed.

INTRODUCTION

N-nitrosodiethylamine (**NDEA**) is one of the most important environmental hepatocarcinogens [3]. Post-treatment with carbon tetrachloride ($\mathbf{CCl_4}$) on **NDEA** exerted a strong promoting effect on the development of hepatocellular carcinoma [7]. Roxithromycin (**RXM**) which is a 14-membered ring macrolide antibiotic is used worldwide. The 14-membered ring macrolides might have anti-tumor effect and might be useful agents for therapeutic application [8]. **RXM** inhibited oxidative stress, nuclear factor kappa- B (NF-κB) activation, and inducible nitric oxide synthases (**iNOS**) activity, and reduced tumor formation in liver [9]. This study was done to evaluate the anti-carcinogenic effect of **RXM** in a rat's model of hepatocellular carcinoma induced by **NDEA** and $\mathbf{CCl_4}$.

MATERIALS AND METHODS

Materials: One hundred and forty (140) male rats were divided into four groups and are subjected to different types of treatments as shown in **Fig. (1**).

NDEA was brought from Sigma- Aldrich, Steinheim, Germany. **RXM** (minimum 90% HPLC) was brought from Sigma-Aldrich Spruce Street, St. Louis, USA.

Methods: Specimens from liver were taken for histopathological examination. Number and size of preneoplastic foci were measured in 4 sections from different lobules of liver. The morphometrical analysis was done by **Research Microscope type Axiostar Plus** made by Zeiss upgradeable to professional digital image analysis system (Carl Zeiss Axiovision Product Suite DVD 30). **Nitric oxide (NO)** production was assayed in serum by Griess Reaction [4].

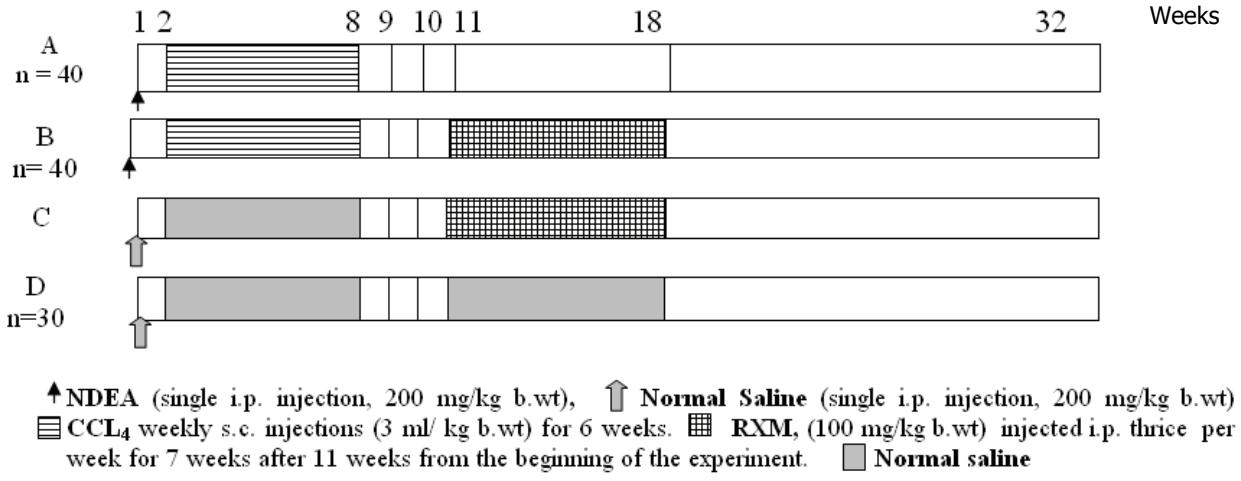

Figure 1: Schematic presentation of the experimental design

RESULTS

Early eosinophilic preneoplastic changes were found after 10 weeks in liver of rats injected with **NDEA** & **CCL_4**, while at the end of the experiment, different proliferative and neoplastic lesions were observed (**Table 1** & **Fig.2**). Treatment with **RXM** significant decrease the percentage of preneoplastic changes and inhibited the development of neoplastic changes in both hepatic parenchyma and intrahepatic bile ducts (**Table 1**).

Morphometrical analysis of the eosinophilic preneoplastic foci revealed that **RXM** decreased number and size of these foci (**Table 2**).

Biochemical results showed a significant increase in the mean ±SE of serum **NO** in **NDEA+CCL_4** group when compared with that of control group. Moreover, no significant difference was observed in the mean ±SE of serum **NO** in both **NDEA+CCL_4+RXM** group and **RXM** group when compared with that of control group (**Fig. 3**)

Table (1): Effect of RXM on different proliferative and neoplastic hepatic lesions

Groups	Group A (NDEA+CCl_4)	Group B (NDEA+ CCl_4+ RXM)
Total no. of rats at the end of the experiment	25	25
I. hepatic parenchyma: 1. Eosinophilic preneoplastic changes: a. Focal b. Diffuse 2. Hepatoma 3. Large cell dysplasia 4. HCC	 14 (56%) 10 (40%) 1 (4%) 1 (4%) 6 (24%)	 2 (8%) 4 (16%) - - -
II. Intrahepatic bile ducts: 1. Bile duct hyperplasia 2. Cholangiofibrosis 3. Cystic cholangioma 4. Cholangiocarcinoma	 10 (40%) 3 (12%) 3 (12%) 1(4%)	 4 (16%) - - -
III. Oval cell proliferation	18 (72%)	3 (12%)
IV. Miscellaneous: Spongiotic pericytoma /Spongiosis hepatic Lipoma	 6 (24%) 1 (4%)	 1 (4%) -

Table 2: The effect of RXM on number and size of preneoplastic foci exposed to NDEA + CCL_4

Groups	Total no. of the foci	Relative size (% of total no.)		
		< 0.1 mm^2	>0.1 mm^2<0.3 mm^2	>0.3 mm^2
A (**NDEA + CCl_4**)	30	7 (23.3%)	18 (60%)	5 (16.7%)
B (**NDEA + CCl_4+RXM**)	4	2	2	-

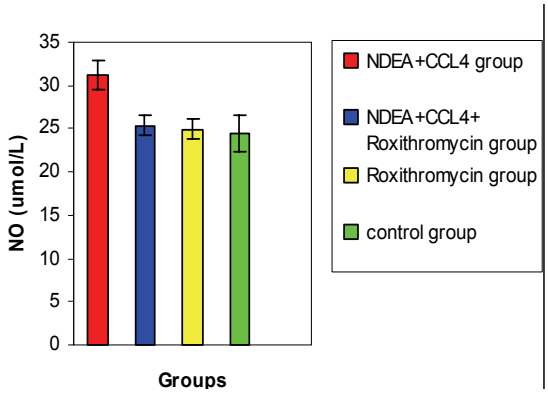

Figure 3: Average NO levels according to t-test

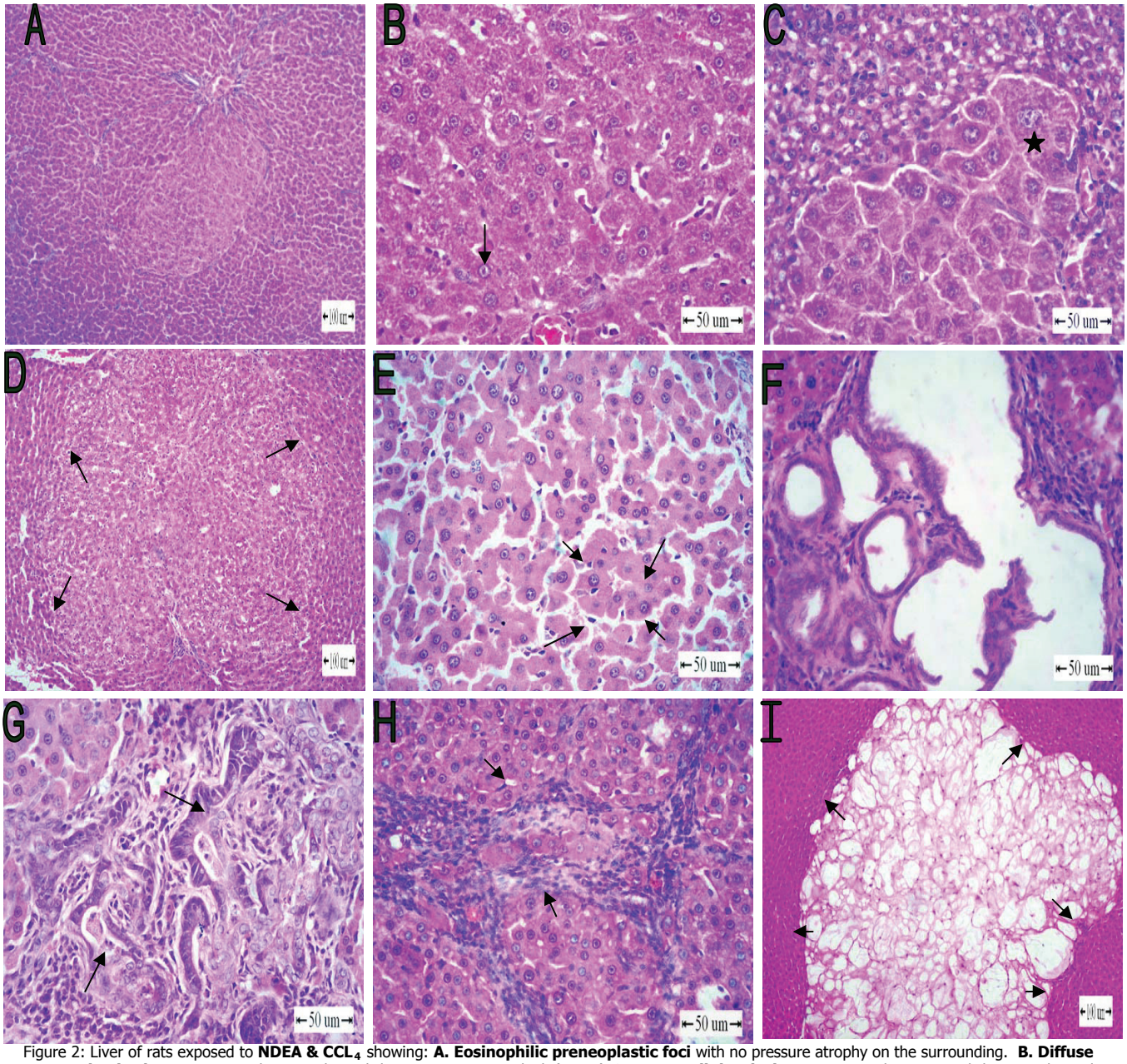

Figure 2: Liver of rats exposed to **NDEA & CCL_4** showing: **A. Eosinophilic preneoplastic foci** with no pressure atrophy on the surrounding. **B. Diffuse preneoplastic changes.** Notice large nuclei with large prominent nucleoli (arrow) **C. Large cell dysplasia.** Notice megalocytosis with large nuclei and dotted chromatin (star). **D. Hepatoma** exerting compression on the surrounding (arrows). **E. Well differentiated HCC.** Tumor cells arranged in trabecular and rosette like-acinar structures (arrows). **F. Cystic cholangioma**. **G. Cholangiocarcinoma. H. Oval cell proliferation** extended among the hepatic parenchyma in strands or rows (arrows). **I**. **Lipoma** exerting pressure atrophy on the surrounding (arrows) and consisted of mature fat cells of various size. H&E.

DISCUSSION

In the present study, effective treatment with **RXM** was obtained when it began after 11 weeks from beginning of the experiment as early preneoplastic changes were reported in one third of cases. **RXM** protected 100% of treated rats from the carcinogenic effect of **NDEA**&**CCL_4** as none of these preneoplastic changes could be developed to neoplasms. Nearly similar results were obtained by Ueno *et al.,* (2005) who found that **RXM** (100 mg/kg /day i.p.) reduced tumor formation in the liver of rats to 6.7% and reduced the average volume of hepatocellular carcinoma [9]. Different mechanisms of the antitumor effect of macrolides were postulated. These mechanisms included antioxidative activities [1], antiangiogenic [10], immunomedulatory [11].

Our results revealed a significant increase in the mean ±SE of serum **NO** in **NDEA**+**CCL_4** group when compared with that of control group. It was recently reported that serum **NO** level significantly increased in **NDEA** treated animals [6]. Inducible nitric oxide synthase (**iNOS**) may contribute to tumor promotion via **NO** radical production and subsequent action of peroxynitrite. **NO** seems to have a dual role in tumor progression as its high concentrations for long periods could result in damage to DNA, leading to mutations and cancer formation [2]. In this study, **RXM**

significantly reduced **NO** during **NDEA** induced hepatocarcinogenesis. Similar result was obtained by Ueno *et al.* (2005) [9]. Gao *et al.,* 2010 strongly suggested that the suppressive activity of **RXM** on **NO** generation was in response to lipid peroxides stimulation *in vivo* [5].

CONCLUSION

The usage of **RXM** as inhibited the development of both preneoplastic changes in nearly 76 % of rats and neoplastic changes in nearly 100% of rats during **NDEA** and **CCL_4** induced hepatocarcinogenesis. Moreover, serobiochemical studies reported that **RXM** modulated the level of nitric oxide to nearly normal level to which the inhibitory action of tumor proliferation was attributed.

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MICROBIAL POPULATIONS AND ANTIBIOTIC RESISTANCE IN *ESCHERICHIA COLI* ISOLATED FROM POULTRY SLAUGHTERHOUSE

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SUMMARY

The aim of the study was to investigate the source of environmental microorganisms and to antimicrobial resistance in *Escherichia coli* strains isolated from a poultry slaughterhouse. Micro-organisms found in slaughtered poultry, can originate from the environment of the slaughter house, also. The highest air coliforms contamination was during shackling, killing and evisceration of poultry.

INTRODUCTION

Microbial contamination of poultry meat can be affected by breeding conditions, feeding, manipulation before slaughtering, slaughter treatment hygiene, slaughterhouse hygiene and workers hygiene (Guerrero-Legarreta, 2009).

Slaughter treatment was performed in high automated line. In the slaughterhouse was not detected any disruption of hygienic request, regulation and good production practice. There were supplied every condition of manipulation with meat products, which are contained in valid laws of Slovak Republic.

Slaughterhouse was divided into clean and dirty zone, which minimize contamination of final products, ensure continuous technical processes and material flow. Dirty zone is composed of shackling are where is poultry shackled upside down by their feet and area for poultry carcass after electrical immobilization. Then are dead birds scaled with water in closed tunnel. Clean zone consist of eviscerated area, water-chilling and packaging are and cutting and de-boning area.

Micro-organisms found in slaughtered poultry, originate from two main sources: the environment of the slaughter house (live poultry, equipment, staff) and the digestive track of the animals (Tsola et al., 2008).

MATERIAL AND METHODS

Three collections of swabs (equipment, tables, walls and floor area) and bioaerosols were made in different times from various poultry breeding in slaughterhouse. Bacteria and fungi were isolated on Nutrient agar, Endo agar and Sabouraud agar.

Susceptibility (MIC - minimal inhibitory concentration) was determined by broth microdilution method according to CLSI guidelines M31-A3 (2008) using ampicillin, ampicillin and sulbactam, ceftiofur, ceftriaxon, ceftazidime, ceftazidime and clavulanic acid, gentamicin, streptomycin, neomycin, spectinomycin, nalidixic acid, enrofloxacin, ciprofloxacin, chloramphenicol, florfenicol, tetracycline and cotrimoxazol. Betalactamase genes for ESBL CTX-M and plasmid AmpC (CMY-2) were detected by PCR.

RESULTS

Air samples were multiple taken from every five parts of the plant and from these areas (equipment, tables, walls, floor) were swabbed with sterile sponge.

The highest air concentration of bacteria was in dirty zone of processing plant, but also in eviscerating area, where high humidity and temperature that support suitable condition for microbial growth. Bioaerosol originate from this section can contaminate poultry meat and cause food born diseases in consumers.

Table 1. Concentration of microorganisms in specific places of poultry slaughterhouse

The place of sampling	TCB CFU/m^3	CB CFU/m^3	Moulds CFU/m^3
I. measurement			
Portioning area	$6{,}8 \cdot 10^3$	$0{,}75 \cdot 10^3$	$1{,}3 \cdot 10^3$
Packaging area	$2{,}8 \cdot 10^3$	$0{,}9 \cdot 10^2$	0
Eviscerating area	$>10^6$	$2{,}6 \cdot 10^4$	$1{,}9 \cdot 10^3$
Killing area	$>10^6$	$2{,}5 \cdot 10^4$	$3{,}8 \cdot 10^3$
Shackling area	$>10^6$	$2{,}07 \cdot 10^4$	$>10^4$
II. measurement			
Portioning area	$4{,}2 \cdot 10^3$	$1{,}0 \cdot 10^2$	$1{,}0 \cdot 10^2$
Packaging area	$3{,}5 \cdot 10^3$	$2 \cdot 10^2$	$0{,}7 \cdot 10^3$
Eviscerating area	$>10^6$	$0{,}4 \cdot 10^3$	$0{,}8 \cdot 10^3$
Killing area	$>10^6$	$1{,}8 \cdot 10^4$	$1 \cdot 10^3$
Shackling area	$>10^6$	$5{,}9 \cdot 10^4$	$2{,}5 \cdot 10^3$
III. measurement			
Portioning area	$4{,}9 \cdot 10^3$	$0{,}5 \cdot 10^2$	$1 \cdot 10^2$
Packaging area	$7{,}6 \cdot 10^3$	$0{,}5 \cdot 10^2$	$0{,}5 \cdot 10^2$
Eviscerating area	$>10^6$	$0{,}4 \cdot 10^3$	$0{,}6 \cdot 10^3$
Killing area	$>10^6$	$1{,}0 \cdot 10^2$	$1{,}0 \cdot 10^3$
Shackling area	$>10^6$	$1{,}0 \cdot 10^2$	$0{,}5 \cdot 10^3$

The highest air bacterial contamination was during shacling, killing and evisceration (higher than 10^6 CFU in $1m^3$) were detected.

Investigation of *Escherichia coli* isolates from poultry abbatoir was found 89% resistance to ampicillin, 62% resistance to ceftiofur, 22% resistance to cefquinome, 6% resistance toampicillin with sulbactam, 14% isolates resistance to streptomycin and gentamicin, 10% resistance to chloramphenicol and florfenicol, in 35% isolates to cotrimoxazol and in 43 % isolates to enrofloxacin. We detected in ESBL positive strains CMY-2 genes and CTX-M genes.

DISCUSSION

The use of HACCP for the meat and poultry industry must begin at the farm because certain safety concerns cannot be eliminated during the slaughtering process (Sun and Ockerman, 2005).

There are various approaches aiming at reducing the presence of pathogenic micro-organisms in chickens production, which focus on various check points "from farm to fork". Given the fact that the finding of pathogenic micro-organisms in living birds is connected to their presence in carcasses, it is desirable that poultry reaching the slaughter house have a low number or absence of pathogens. It is logical, therefore, to carry out the initial control for pathogens in the environment of poultry farms (Sinell, 1995).

The use of drugs in food animals is fundamental to animal health and well-being and to the economics of the industry. However, their use also is associated with human health effects. Antibacterials and other chemicals are frequently used for controlling other infections such as coccidia, worms, fungi, ectoparasites, and several bacterial infections. But drugs used in food-animal production and residues of those drugs could enter human food and increase the risk of ill-health in persons who consume products from treated animals. Moreover, the use of antibiotics in food animals could contribute to the emergence of antibiotic-resistant microorganisms in animals that could be transmitted to humans and result in infections that could be difficult to treat (Committee on Drug Use in Food Animals, 1999).

We found resistant ESBLs *E. coli* on water-chilling system that present mechanisms for transport of bacteria from chicken to chicken (Petrak et al., 1999).

CONCLUSIONS

The results confirmed that slaughterhouse environment could be a reservoir of *E.coli* antibiotic resistance for poultry meat.

The reason of higher environmental contamination in all sections of processing plant at the end of work was probably insufficient division between some parts of processing. Partition from plastic materials between respective areas were override scores of the time, by reason of simple manipulation with boxes, that support chance to air contamination.

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INCIDENCE OF RESISTANCE OF *E. COLI* ISOLATED FROM BROILER CHCIKS TO SOME ANTIBIOTICS

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SUMMARY

Colibacillosis causes important economic losses in the poultry industry. The objective of our work was to evaluate drug resistance of *E. coli* isolates in broilers referred to veterinary diagnostic laboratory. For this, a total 1055 cases of broilers were tested between September 2005 to October 2010. *E. coli* isolation was carried from heart and liver and serotyping done using specific antisera to O1, O2 and O78. Drug sensitivity test was carried out using antibiogram technique. Our results showed that *E. coli* was isolated from 327 cases. The serotype's percentage was 16, 23 and 59 for O1, O2 and O78, respectively. The antibiogram test showed that 79% of isolates were resistant to Amoxicillin, 52% resistance to Oxytetracyclin and Nalidixic Acid, 27% Enrofloxacin and only 9% to Flumequin. As these antimicrobial agents may cause cross-resistance with enteric pathogens; their wide administration may increase their resistant in poultry industry.

Keywords: *Escherichia coli*, Antibiotic resistance, Broiler chicks

INTRODUCTION

Escherichia coli is one of the common microbial flora of gastrointestinal tract of poultry and human being including other animals but may become pathogenic to both. Although most isolates of *E. coli* are nonpathogenic but they are considered as indicator of fecal contamination in food and about 10-15 % of intestinal coliforms are opportunistic and pathogenic serotypes [1] and cause a variety of lesions in immunocompromised hosts as well as in poultry. Among the diseases some are often severe and sometimes lethal infections such as meningitis, endocarditis, urinary tract infection, septicemia, epidemic diarrhea of adults and children and yolk sac infection, omphalitis, cellulitis, swollen head syndrome, coligranuloma, and colibacillosis [4]. Antibiotics are extensively used as growth promoters in poultry production or to control infectious disease. Anti-microbial exercise and/or especially abuse are considered to be the most vital selecting force to antimicrobial resistance of bacteria [8]. Moreover, antibiotic treatment is considered the most important issue that promotes the emergence, selection and spreading of antibiotic-resistant microorganisms in both veterinary and human medicine [11]. It was stated by well established evidence that antibiotics can lead to the emergence and dissemination of resistant *E. coli* which can then be passed into people via food or direct contact with infected animals. These resistant microbes may function as a potential source in the transportation of antimicrobial resistance to human pathogens [7].

Due to enormous exploitation of antibiotics in the field of veterinary medicine, an increased number of resistant bacterial strains were developed in recent years. The transmission of plasmid mediated resistance between different bacterial species and genera are now widely occurred [3]. In different parts of the world, multi drug resistant strains of *E. coli* are ubiquitous in both human and animal isolates and multiple drug resistant, nonpathogenic *E. coli* found in the intestine is probably an important reservoir of resistance genes and momentarily drug-resistant *E. coli* of animal origin may colonize the human intestine [9]. Acquired multi drug resistance to antimicrobial agents creates an extensive trouble in case of the management of intra and extra intestinal infections caused by *E. coli*, which are a major source of illness, death, and increased healthcare costs [7]. Therefore, the present study was designed to isolate *E. coli* strains from broilers referred to veterinary diagnostic laboratory for assessing their susceptibility and resistance patterns to some selected antimicrobials.

MATERIAL AND METHODS

Sampling sites

From September 2005 to October 2010, a total of 1055 poultry carcasses referred to veterinary diagnostic laboratory and suspected to infection with *E. coli* were considered for our study [7] and samples from heart and liver were taken from each carcass. All samples were taken from poultry farms located at suburb of Ahvaz (a city in the south-western, Iran).

Bacteriological analysis

After collection, all the samples were transported for isolation, immediately. Samples were spread on the solid surface of Eosine Methylene Blue (EMB) agar medium (Hi-Media, India), then incubated for 24 h at 37°C in three triplications of EMB plates for successful isolation of typical colonies. Identification was done following a series of biochemical tests included gram staining, tests for oxidase, methyl red, Voges-Proskauer reactions, indole, citrate, catalase, urea hydrolysis, gelatin hydrolysis, lactose fermentation, nitrate reduction, casein hydrolysis and sugar fermentation. Identification of *E. coli* was further confirmed by latex agglutination tests using polyvalent antisera, moreover serotyping done using specific antisera to O1, O2 and O78 (Denka Seiken Co. Ltd, Tokyo, Japan).

Drug sensitivity test

Single disc diffusion method [2] was used to examine bacterial susceptibility to antimicrobial agents. A total of 5 antibiotic discs (Becton Dickinson, U.S.A.) with Amoxicillin 10µg, Oxytetracyclin 30µg, Nalidixic Acid 15µg, Enrofloxacin 10µg, and Flumequin 10µg were used. By the standard method of inoculation, the top of a single and well-isolated colony was touched with a sterile loop and the growth was inoculated into 2 mL of Mueller-Hinton broth. The broth culture was then allowed to incubate at 37 °C for 4 hours to obtain the young culture. The turbidity of actively growing broth cultures was then adjusted to a 0.5 McFarland standard and then a sterile cotton swab was dipped into the adjusted suspension within 15 min and excess broth was purged by pressing and rotating the swab firmly against the inside of the tube above the fluid level. The swab was then spread evenly over the entire surface of the plate of LB agar to obtain uniform inoculums. The plates were then allowed to dry for 3-5 min. Antibiotics impregnated discs were then applied to the surface of the inoculated plates with sterile forceps. Each disc was gently pressed down onto the agar to ensure complete contact with the agar surface. Even distribution of discs and minimum distance of 24 mm from center to center were ensured. Six discs (five antibiotics discs and one blank disc as control) were placed in each petri-dish. Within 15 min of the application of the discs, the plates were inverted and incubated at 37 °C. After 16-18 h of incubation, the plates were examined, and the diameters of the zones of complete inhibition to the nearest whole millimeter were measured. The zone diameter for individual antimicrobial agents was then translated into susceptible, intermediate and resistant categories according to the interpretation table of the Becton Dickinson Microbiology Company, USA.

RESULTS

Selective processing of 1055 samples yielded 327 unique *E. coli* isolates, which constituted the study population. Of the 327 isolations, 89 (27.2 %) were from heart alone, 112 (34.2%) were from liver, and 126 (38.5%) were simultaneously isolated from heart and liver. The median number of unique *E. coli* isolates per sample was 1 for heart and 2 for liver (range 1–4 for both). Antibiotic susceptibility pattern of *E. coli* isolates from samples of poultry sources has been outlined in Table 1. Moreover, the serotype's percentage of isolates O1, O2 and O78 antigens were 16, 23 and 59%, respectively.

DISCUSSION

The prevalence of *E. coli* in 31 % of carcasses in the present study was less than Rahman *et al*. records [10]. All the isolates of present study exhibited multiple resistances to more than six antibiotics. Similar findings on multiple drug resistance of *E. coli* strains has been reported from other parts of the world [6,13]. Due to indiscriminate exploitation of antimicrobial agents, such high incidence of multi drug resistance may apparently be occurred which may ultimately replace the drug sensitive microorganisms from antibiotic saturated environment [12].

CONCLUSION

Risk assessment should reflect the increasing weight of scientific evidence indicating the potential for even non-pathogens carrying and transferring genetic determinants for antibiotics resistance to human pathogens, cross-resistance development, and potential link between resistance to critical antibiotics in human medicine and use of similar drugs in poultry feeds. Appropriate use of antibiotics in humans and farm animals needs to be addressed in the world.

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Table 1. Antibiotic susceptibility pattern of 327 selected strains of Escherichia coli

	Resistant		Sensitive	
Antibiotics	%of strains positive	Inhibition zone (mm)	%of strains positive	Inhibition zone (mm)
Amoxicillin	79	<13	21	>17
Oxytetracyclin	52	<25	48	>29
Nalidixic Acid	52	<12	48	>17
Enrofloxacin	27	<30	73	>33
Flumequin	9	<14	91	>18

MOUTH MICROBIOLOGICAL AND SALIVA PH CHANGES IN DOGS WITH PERIODONTAL DISEASE

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SUMMARY

In this research, a correlation had been searched among the microflora spectrum of the dogs mouth cavity, pH level changes in the mouth cavity, and different stages of periodontitis development. Fifty dogs of different ages were included in the research in which a profound examination of the mouth cavity was performed by summarizing data on the status of the gingiva, teeth and alveolar bone as well as the pH parameters of the mouth cavity. It was ascertained that with the development of periodontitis in the dogs mouth cavity the spectrum of gram-positive and gram-negative bacteria somehow changed – *Escherichia coli* prevailed of the gram-negative microflora but *Staphylococcus* genus bacteria of the gram-negative ones. Dogs have a tendency to maintain the pH level in the mouth cavity within a certain ratio from pH 5.8 to 6.8.

INTRODUCTION

Authors consider that animals suffer from the mouth cavity pathologies throughout their lifetime [6]. An important place among them is taken by periodontitis or periodontal disease which, as it is known, is an inflammation of the periodontal tissue [9]. It was established that in 75% to 85% of cases, dogs more than three years of age were affected by periodontitis [3]. A change of bacterial spectrum in the mouth cavity is considered as one of the factors causing periodontitis in dogs [1, 7]. Literature sources indicate that the gram-positive microflora predominates at the initial stages of periodontitis while at more severe stages of its development it changes to the gram-negative one [5].

It is known that the microbiological spectrum is dependent, to some extent, on the animal feeding and pH level in the mouth cavity. When eating, dog intakes together with food different nonpathogenic and potentially pathogenic microorganisms [8]. It was shown that exactly pH level in the mouth cavity was one of the factors influencing most significantly the microbial spectrum that was present and consequently the possible development of periodontitis [4]. Some of the authors are of the opinion that in dogs there is a stable typical alkaline reaction [4]. And the alkaline pH level in the mouth cavity might "work" as a natural protective barrier that inhibits the development and growth of *E.coli, Streptococcus* and other bacteria [11].

Objectives of the research: 1.To investigates the microbial spectrum of the mouth cavity in dogs and association of its changes with the development of different stages of periodontitis. 2. To ascertain the background pH level of the mouth cavity in dogs at which periodontitis develops.

MATERIAL AND METHODS

Basically, investigations were carried out at the Clinic of the Faculty of Veterinary Medicine of the Latvia University of Agriculture. Fifty dogs were selected for a profound examination of the mouth cavity: the status of the gingiva, teeth and alveolar bone as well as the pH level. To measure pH in dogs, "Oaktan" pH-meter was used the electrodes of which were placed under the tongue on the right side. In order to define more precisely the stage of periodontitis, we used criteria of the development of periodontitis worked out by Rawlinson [10]. Depending on the obtained examination results of the mouth cavity, animals were conditionally divided into five groups – dogs with the first, second, third and fourth stage of periodontitis, and dogs in which periodontitis was not found. To establish the bacteriological spectrum of the mouth cavity in practically healthy dogs and in animals with periodontitis of different stages of development, samples were taken from the mouth cavity by using sterile swabs. In each animal, samples were taken from the area of the gingival sulcus of the lower and upper jaw lateral surface, and within 12 hours delivered to the Zemgale Regional Laboratory of the Food and Veterinary Service of the Republic of Latvia. Colonies of bacteria grown from the delivered samples were removed, and repeatedly plated on the blood agar culture medium, then differentiated by using API test.

RESULTS AND DISCUSSION

First of all we will analyze the microbial spectrum of the mouth cavity in dogs in which periodontitis was not found and animals with periodontitis of different stages of development.

Results showed evidence that in dogs without signs of periodontitis the bacteriological spectrum contained gram-positive and gram-negative bacteria in a conditional balance of 52.6% and 47.4%, respectively. These results generally agree with the data of other authors [8].

Analyzing bacterial species in the mouth cavity in dogs which were not diagnosed periodontitis, *Escherichia coli* and *Actinobacter lwolfi* were prevalent among gram-negative bacteria. Of gram-positive bacteria species in dogs *Staphylococcus intermedius* was found most often.

Table 1: Gram-positive and Gram-negative bacteria percentage in the mouth of dogs without periodontitis and with different degrees development of periodontitis (n = 10 in each stage of periodontitis)

Cast of bacteria	Without of periodontitis		With 1. stage of periodontitis		With 2. stage of periodontitis		With 3. stage of periodontitis		With 4. stage of periodontitis	
	Quantity of species	%	Quantity of species	%	Quantity of species	%	Quantity of species	%	Quantity of species	%
Gram „+"	10	52.6	2	18.2	9	40.9	10	43.5	10	52.6
Gram „-"	9	47.4	9	81.8	13	59.1	13	56.5	9	47.4

Although the literature sources show that with advancing of periodontitis gram-positive bacteria are changed by gram-negative ones [2], in this study such a tendency was observed only in the first two stages of periodontal development.

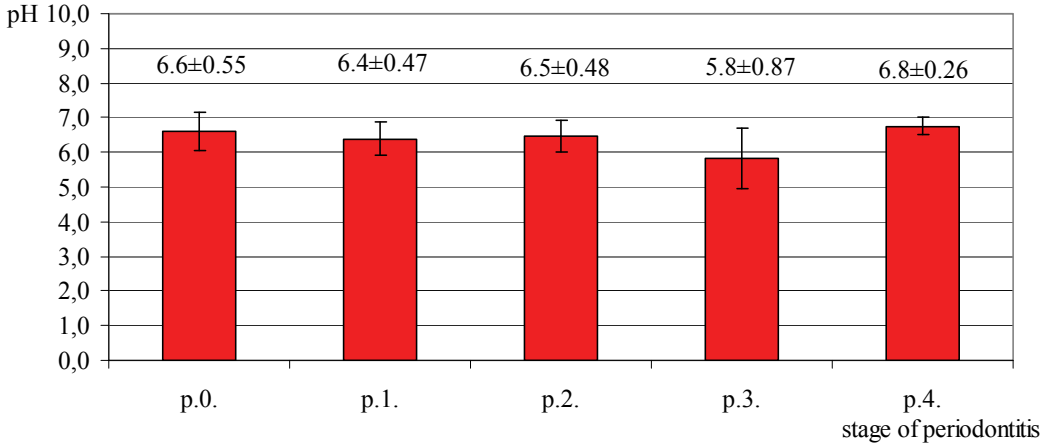
In dogs with the first stage of development of periodontitis, there were significant changes in the microbial spectrum of the mouth cavity. The number of gram-positive bacteria decreased significantly ($p<0.05$) from 52.6% to 18.2% (see Table) with prevalence of *Staphylococcus* genus bacteria (*Staphylococcus intermedius* and *Staphylococcus aureus*).

To some extent the tendency of gram-negative bacteria to prevail over gram-positive bacteria was also apparent in dogs with the second and third stage of development of periodontitis (see Table). In the mouth cavity of these animals, gram-negative bacteria were 59.1% and 56.5%, respectively, but gram-positive bacteria were 40.9% and 43.5%, respectively. Of gram-negative bacteria in dogs with the second and third stage of development of periodontitis in the mouth cavity *Escherichia coli* predominated, but of gram-positive ones – bacteria of *Staphylococcus* genus were prevailing.

Thus this study showed that with advancing of periodontitis, especially in the early period, the ratio of gram-positive and gram-negative bacteria changed radically "in benefit" of gram-negative bacteria.

As regards the microbial spectrum in the mouth cavity in dogs with periodontitis of the fourth stage of development, in these animals the percentage of gram-positive and gram-negative bacteria was the same as in animals which were not diagnosed periodontitis (see Table). Possibly, it is because the mouth cavity in the dog has already adapted to the changes caused by periodontitis.

Next objective was to determine the background pH level in the mouth cavity in dogs at which periodontitis developed.



pH level in practically healthy animals without periodontitis and in dogs with different stages of periodontal development

p.0. – without periodontitis
p.1. – with 1. stage of periodontitis
p.2. - with 2. stage of periodontitis
p.3. - with 3. stage of periodontitis
p.4. - with 4. stage of periodontitis

The determined pH level in practically healthy animals without periodontitis and in dogs with different stages of periodontal development is reflected in Figure.

Results showed that in practically healthy dogs which were not diagnosed periodontitis, the mouth cavity pH was on average at 6.6 ± 0.55 level. With developing of periodontitis, especially in the transfer period from the second to the third developmental stage, the mouth cavity had a tendency become conditionally sourer – pH lowered from 6.5 ± 0.48 to 5.8 ± 0.87 (see Figure). In dogs with the fourth developmental stage, as if the pH level in the mouth cavity returned back to its starting "positions", i.e. pH reached 6.8 ± 0.26 that differed little from pH parameters in animals without periodontitis.

We can assume that the changes of pH level in the mouth cavity in dogs determined in this study are associated to some extent with the changes in its microbial spectrum.

CONCLUSIONS

1. In practically healthy dogs without periodontitis, gram-positive and gram-negative bacteria in the mouth cavity are in a conditioned balance.

2. With developing of periodontitis, especially in the early process, the microbial spectrum in the mouth cavity in dogs radically changes to the side of gram-negative bacteria.

3. In animals with the fourth stage of periodontal development, percentage of gram-negative and gram-positive ratio in the mouth cavity becomes similar to that in the dogs without periodontitis.

4. With developing of periodontitis, pH level in the mouth cavity has a tendency to decrease from 6.6 ± 0.55 to 5.8 ± 0.87 that is typical in animals with the third developmental stage of periodontitis, but in dogs with the fourth stage of development of periodontitis pH parameters again increase

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s. Special Session ISAH-Alltech
Lecture 3


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