FOLIA VETERINARIA

The scientific journal of the
UNIVERSITY OF VETERINARY MEDICINE AND
PHARMACY IN KOŠICE — The Slovak Republic

ISSN 0015-5748

Supplementum II

LVI • 2012

EDITORIAL BOARD

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The journal is published quarterly in English (numbers 1–4) and distributed worldwide.

Subscription rate for 1 year is 120 €. Orders are accepted by *The Department of The Scientific Information – The Library of The University of Veterinary Medicine and Pharmacy in Košice* (UVIK), E-mail: palencarova@uvm.sk; the subscription is accepted by the State treasure.

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FOLIA VETERINARIA, vydáva *Univerzita veterinárskeho lekárstva a farmácie v Košiciach (UVLF),* Komenského 73, 041 81 Košice, Slovenská republika (tel.: 055/632 52 93, fax: 055/632 52 93, E-mail: vargova@uvm.sk).

Časopis vychádza kvartálne (č. 1–4) a je distribuovaný celosvetove.

Ročné predplatné 120 €. Objednávky prijíma *Ústav vedeckých informácií a knižnice Univerzity veterinárskeho lekárstva a farmácie v Košiciach* (UVIK), E-mail: palencarova@uvm.sk; predplatné Štátna pokladnica (na nižšie uvedené číslo účtu).

Bankové spojenie: Štátna pokladnica, Radlinského 32, Bratislava 15; **číslo účtu:** 7000072225/8180.

Tlač: **Univerzita veterinárskeho lekárstva a farmácie** Komenského 73, 041 81 Košice

Sadzba: **Sapfo publishers**, Szakkayho 1, 040 01 Košice

EV 3485/09

For basic information about the journal see Internet home pages: www.uvm.sk; www.uvlf.sk

Indexed and abstracted in AGRIS, CAB, EBSCO

FOLIA VETERINARIA

Folia veterinaria
Vol. 56, Supplementum II, 2012

VYDALA UNIVERZITA VETERINÁRSKEHO LEKÁRSTVA A FARMÁCIE
KOŠICE 2012

Institute of Animal Physiology
Slovak Academy of Sciences
Šoltésovej 4–6, 04001, Košice, The Slovak Republic

25th DAYS OF ANIMAL PHYSIOLOGY
INTERNATIONAL SCIENTIFIC CONFERENCE

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F O R E W O R D

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Assoc. Prof. Štefan Faix, DVM, PhD.
Director of the Institute of Animal Physiology, SAS

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THE CHANGES IN NUTRIENT UTILIZATION IN MONOGASTRIC ANIMALS AFTER PRECEDENT DIETARY RESTRICTION

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ABSTRACT

In this study we investigated the effect of previous dietary nitrogen (N) restriction on utilization of N and average daily weight gains (ADG) in pigs. After the 5-day adaptation period followed a 14-day restriction period during which experimental group (EG) was fed with LP diet with 4.9 % content of crude protein (CP) and control group (CG) was fed with SD diet (16.7 % CP). After this period the LP diet was replaced by SD diet (compensatory period) which was fed to both groups for 14 days until the end of the experiment. From day 13 of the restriction period we collected urine *via* bladder catheters. In samples of diets and urine we analyzed content of CP. Pigs were weighed periodically. During the compensatory period ADG was greater (P < 0.05) in EG compared to CG. We observed no difference in daily urinary N excretion. Nitrogen retention per kg of ADG during the compensatory period was by 21 % lower (P < 0.05) in experimental group compared with the control suggesting that the ADG in the experimental group was mostly made up of fat. Throughout the experiment (restriction + compensatory period) we observed by 21 % lower (P < 0.05) ADG and by 13 % lower (P < 0.05) daily urinary N excretion in the experimental group as a result of low N intake during the restriction period and not as a result of metabolic changes in the body.

Key words: compensatory growth; dietary restriction; nitrogen; pig

INTRODUCTION

Compensatory growth has been observed in animals fed for certain period either restricted feed intake or nutrients (1, 5). During the compensatory growth animals exposed to previous dietary restriction generally reach the same body weight as the animals which were not dietary restricted (1). The reports indicating what is causing compensatory growth are different. Some authors (8) reported that compensatory growth is due to higher feed intake which is responsible to body weight of animals. In contrary these results other authors (5) reported that compensatory growth relate to better feed utilization during compensatory period and thus the previous dietary amino acid restriction can reduce the N excretion in urine and faeces that may have positive effect on environment (3).

The aim of this study was to investigate how the previous dietary N restriction affects N excretion in urine and average daily weight gains in pigs.

MATERIAL AND METHODS

All experimental procedures were reviewed and approved by the Animal Care Committee of the Animal Production Research Centre. Altogether 12 gilts (initial body weight 44.6 kg ± 1.05 kg) were housed in balanced cages and divided into experimental group (EG; n = 6) and control group (CG; n = 6). After the 5-day adaptation period followed a 14-day restriction period during which the EG was fed low protein diet (CP = 49 $g.kg^{-1}$; ME = 14.4 $MJ.kg^{-1}$) and CG was fed standard diet (CP = 167 $g.kg^{-1}$; ME = 13.3 $MJ.kg^{-1}$). After this period the LP diet was replaced by SD diet for both groups for the next 14 days until the end of the experiment. From day 13 of restriction period to the end of experiment we collected urine *via* bladder catheters. In samples of urine and samples of tested diets we analyzed the content of CP. Using the coefficient digestibility of CP of the diets and N excreted in urine we calculated N retention during the compensatory phase. Animals were weighed periodically.

The animals were fed twice daily *ad libitum* and feed intake was recorded daily. Water was offered *ad libitum*.

The experimental data were subjected to ANOVA using Statgraphic Plus 3.1. software. When significant differences ($P < 0.05$) were detected regarding the treatment, the differences between means were separated using Fisher's LSD procedure.

RESULTS AND DISCUSSION

During the compensatory period,we observed an insignificantly higher ($P = 0.06$) daily N intake of EG (57.8 $g.day^{-1}$) in comparison with CG (54.6 $g.day^{-1}$) as a result of insignificantly higher feed intake. Also other studies (3, 7) reported unchanged feed intake during the compensatory period after previous dietary amino acid restriction. Contrary to these observations, restriction of *ad libitum* feeding increased feed intake during subsequent re-feeding period in comparison to the previously unlimited feed intake (4, 9).

During the compensatory phase the previously dietary restricted animals showed compensatory growth reflected in mean daily weight gains. We observed higher ($P = 0.02$) ADG in EG (1216 $g.d^{-1}$) in comparison with CG (827 $g.d^{-1}$). Higher ADG in pigs previously restricted from *ad libitum* feeding were reported also in other studies (1, 4) but dietary amino acids restriction did not affect ADG during the subsequent re-feeding period (2, 7).

Despite the fact that during the compensatory period we observed higher ADG in EG, N retention expressed per kg of ADG was lower ($P = 0.002$) in EG (26.7 g.kg ADG^{-1}) in comparison with CG (33.9 g.kg ADG^{-1}). Therefore we presume that ADG during the compensatory period was mostly made up of fat. Animals after dietary restriction retained more energy as fat than in the form of protein (6) and lipid deposition was increased with the feeding level (7).

We observed no difference ($P = 0.33$) in daily urinary N excretion between EG (20.6 $g.day^{-1}$) and CG (21.5 $g.day^{-1}$) but N excretion expressed per g of N intake was lower ($P = 0.02$) in EG (EG: 0.36 g.g N $intake^{-1}$; CG: 0.41 g.g N $intake^{-1}$). F a b i a n *et al.* (3) reported lower daily N excretion in urine of pigs after previous restriction of dietary lysine level.

Severe dietary N restriction resulted in lower ADG in EG during the combined (restriction and compensatory) period (EG: 643 $g.day^{-1}$; CG: 817 $g.day^{-1}$; $P = 0.01$). This agree with other studies dealing with previous restriction of feed intake (1, 9) but not to dietary amino acid restriction (7). During the combined period we observed also lower ($P = 0.001$) daily urinary N excretion in the EG (18.5 $g.day^{-1}$) compared with CG (21.2 $g.day^{-1}$) due to the lower daily N intake during the restriction period (EG: 15.9 $g.day^{-1}$; CG: 52.2 $g.day^{-1}$; $P < 0.001$).

In conclusion, accelerated growth of pigs during the compensatory period was due to higher fat deposition in the body but the compensatory effect was not sufficient to the extent that was shown during the combined, restriction and compensatory period in term of growth parameters.

ACKNOWLEDGEMENTS

This paper was supported by the project "BELNUZ 26220120052" of the Operational Programme Research and Development, funded from the European Regional Development Fund.

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FOLIA VETERINARIA, 56, Supplementum II: 11–12, 2012

CELL RECEPTORS POTENTIALLY MEDIATING INFLUENCES OF IMPAIRED MATERNAL HEALTH ON PREIMPLANTATION DEVELOPMENT

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ABSTRACT

The preimplantation embryo is highly influenced by factors of external environment. In this study, we have summarized results of experiments examining cell receptors which could mediate influences of impaired maternal health on preimplantation development. Several types of receptors that can bind molecules released in stress were detected in oocytes and preimplantation embryos which suggests that glucocorticoids and catecholamines could mediate effects of maternal stress on early embryo. In addition, receptors for other biogenic monoamines can play an important role in physiological conditions, or can influence the early embryo under unfavorable or pathological conditions. Expression of leptin and adiponectin receptors in oocytes and preimplantation embryos suggests that leptin and adiponectin could be among factors mediating effects of maternal obesity on fertilization and early embryo development.

Key words: early embryo; maternal health

INTRODUCTION

Oocytes and preimplantation embryos are equipped with variety of receptors which suggest that they are able to respond to ligands present in their environment (5). Moreover, there are data indicating that biologically active agents of maternal origin can influence oocyte maturation, fertilization and early embryo development. In this study, we have summarized results of experiments examining cell receptors which could mediate influences of impaired maternal health on preimplantation development. We have focused on maternal stress, obesity and other lifestyle disorders.

MATERIAL AND METHODS

Unfertilized oocytes or embryos at various developmental stages were isolated from the oviduct or uterus of female mice. Total RNA was extracted, reverse transcribed, and amplified by PCR. Oligonucleotide primers specific for examined receptors were used for PCR amplification. The immunostaining of oocytes/embryos with antibodies raised against examined receptors was carried out to examine protein expression. To test functionality of expressed receptors, mouse preimplantation embryos were cultured in the presence/absence of appropriate ligands, and their developmental stages were evaluated (for details see in 2, 3, 4).

RESULTS AND DISCUSSION

Animal experiments (using rodent model and farm animals) as well as observations in humans have demonstrated that prenatal maternal stress can significantly affect the pregnancy outcome. However, molecular mechanisms mediating effects of maternal stress on early embryo are practically unknown. Messenger RNA for glucocorticoid receptor was found in bovine preimplantation embryos *in vitro* (8), which indicates that glucocorticoids could directly influence preimplantation embryos. We found mRNA and protein for several subtypes of adrenergic receptors in mouse ovulated oocytes and preimplantation embryos (Table 1), and demonstrated that the exposure of mouse preimplatation embryos to adrenergic agonists can change embryo cell number (2, 3). These results suggest that catecholamines released under stress conditions from maternal sympathetic nerve terminals and

Table 1. Cell receptors which could mediate influences of stress, obesity, and other maternal life style disorders on early embryo development

Receptor	Species	Reference
Alpha 2C-AR	Mouse	3
Beta 2-AR	Mouse	2
Beta 3-AR a,b	Mouse	2
GR	Cow	8
H2R	Mouse	10
5-HT_{1D}	Mouse	6.9
5-HT_{7}	Mouse	1
AdipoR1, R2	Mouse, rat,	4.7
Ob-R	rabbit, pig, cow, mouse, human, pig	7

Receptors expressed in preimplantation embryos and/or oocytes: Alpha 2C-AR, adrenergic receptor subtype alpha 2C; Beta 2-AR, adrenergic receptor subtype beta 2; Beta 3-AR a, b, adrenergic receptor subtype beta 3, isoforms a and b; GR, glucocorticoid receptor; Adipo R1, R2, adiponectin receptor type 1 and 2; Ob-R, leptin receptors; H2R, histamine receptor subtype H2R; 5-$HT_{1D,}$ serotonin receptor subtype 5-HT_{1D},5-$HT_{7,}$ serotonin receptor subtype 5-HT_{7}

the adrenal medulla could directly influence preimplantation embryo. Moreover, receptors for other biogenic monoamines were detected in mouse oocytes and preimplantation embryos (Table 1). These receptors can play an important role in physiological conditions, contributing to embryo-maternal interactions, or can influence the early embryo under unfavorable or pathological conditions.

Accumulating evidence indicates the negative impact of maternal obesity on fertilization and early embryo development, and some effects of obesity on reproductive health can be mediated by adipokines, such as leptin and adiponectin (hormones/cytokines secreted from adipose tissue). Expression of leptin receptors in oocytes and preimplantation embryos has been demonstrated in several species (Table 1). However, conflicting results were obtained for the role of leptin in oocyte maturation (dependence on oocyte cellular context has been suggested) and preimplantation development (7). We found expression of two adiponectin receptors (adipoR1, adipoR2) in mouse ovulated oocytes and preimplantation embryos (Table 1), and demonstrated that adiponectin can directly influence development of the preimplantation embryo (4). These results suggest that leptin and adiponectin could be among the factors mediating effects of maternal obesity on fertilization and early embryo development.

ACKNOWLEDGEMENTS

The paper was supported by the project No. ITMS 26220120066 of the Research & Development Operational Programme funded by the ERDF.

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FOLIA VETERINARIA, 56, Supplementum II: 13–14, 2012

SURGICAL PROCEDURES PERFORMED IN GENERAL INHALANT ANAESTHESIA WITH CONTROLLED MECHANICAL VENTILATION AT THE EQUINE CLINIC OF HORSES OF THE UNIVERSITY OF VETERINARY MEDICINE AND PHARMACY IN KOŠICE

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ABSTRACT

Since the beginning of this year 2012, seven patients with different diagnosis and trauma cases which required a surgery in a long-term general anaesthesia were hospitalised at the Equine Clinic of the University of Veterinary Medicine and Pharmacy in Košice. The list of the surgical procedures we performed in these patients includes: colic surgery, cryptorchid castration (abdominal and inguinal), enucleation, controlled annular ligament transaction using endoscopic visual guidance, lag screw fixation of the proximal pastern fracture, stainless steel wires fixation of the depression fractures of the frontal and maxillary sinuses. In all these cases anaesthesia was maintained *via* inhalation of anaesthetic gas delivered thorough an endotracheal tube to provide safe, highly controlled anaesthesia.

Keywords: controlled mechanical ventilation; general inhalation anaesthesia; horse; surgical procedures

INTRODUCTION

Smooth and successful equine anaesthesia remains a significant challenge for all those who have to anaesthetise a horse (2).

To properly perform surgery and general anaesthesia of duration longer than 15 to 30 minutes, a room dedicated to that purpose should be included in hospital design. This operating room has to be large enough to permit easy navigation of 500–600 kg horse in lateral recumbency, permit space for surgical equipment, anaesthesia machine and monitor (1).

Clipping and the initial surgical scrub can be done before the horse is anaesthetised.

Surgical table and its accessories must provide the ability to position the horse in either lateral or dorsal recumbency without creating unnecessary pressure or tension on any muscle group or nerve (1).

The basic large animal anaesthesia machine is just an enlarged version of small-animal circle system. A machine that can be used with or without a fan will allow safer anaesthesia, in that ventilation can be assisted manually with rebreathing bag if needed (1).

Monitoring means continuous surveillance of the anaesthetised horse. In particular, the respiratory and cardiovascular systems must be monitored. A record should be kept of measurements made during anaesthesia (2).

There is a need for padded walls recovery room. Squared or rounded corners are a matter of opinion. Oxygen should be available for recovery (1).

In addition to the risk to the horse, equine anaesthesia also puts the handlers at risk of injury or even death. Owners and inexperienced onlookers must always be kept out (2). It is important to have one staff member taking primary responsibility for the equipment, supplies and training other members of the hospital team in anaesthesia (1).

MATERIAL AND METHODS

Anaesthesia was induced with intravenous (IV) xylazine (1.1 $mg.kg^{-1}$) and butorphanol (0.02 $mg.kg^{-1}$). Intravenous (IV) diazepam (0.03 $mg.kg^{-1}$) with ketamine (2.2 $mg.kg^{-1}$) was injected 3–5 minutes after apparent xylazine induced sedation and maintained using isoflurane in oxygen. Isoflurane (Forane®) was the first which really appeared to be as good if not better than halo-

thane. Minimal alveolar concentration (MAC) was about 1.3 %, so surgical anaesthesia was produced with end tidal concentrations of about 1.5–1.7 % in oxygen (2–2.4 % at vaporizer). This was reduced with premedicants and induction agents. Horses were positioned in dorsal or lateral recumbency. The lungs of horses were ventilated mechanically using intermittent positive pressure ventilation (IPPV) (Smith Respirator LA 2100, model 2002). During anaesthesia, heart rate (HR), respiratory rate (RR), temperature (T), mean arterial blood pressure (MAP) *via* a direct arterial line, capillary refill time (CRT), ECG, blood oxygen-haemoglobin saturation and end-tidal carbon dioxide measurement (capnography) were measured and recorded (Datex Ohmeda s/s™Anesthesia Monitor (GE) Healthcare).

RESULTS AND DISCUSSION

The list of the surgical procedures we performed in patients undergoing a long-term general anaesthesia includes the following cases.

Patient No. 1 was admitted with the chronic lameness for over 6 months and was diagnosed with a desmitis of annular ligament of the left hind limb. It was a 16 years old mare with a recurrent airway obstruction (RAO). We performed a controlled annular ligament transaction using endoscopic visual guidance. In this time the mare is 8 months after surgery without period of lameness.

Patient No. 2 was a trauma case. It was 8 years old mare with the depression fractures of the frontal and maxillary sinuses. She has undergone a stainless steel wires fixation of bone fractures in general inhalant anaesthesia without any complications.

Patients No. 3 and 4. Cryptorchid castrations (abdominal and inguinal) were performed in these patients. They were 2-year old healthy stallions with a good indication for general inhalant anaesthesia. No complication occurred.

Patient No. 5. Lag screw fixation of the proximal pastern fracture was performed in this patient, a 10-year old stallion without any contraindications for general inhalant anaesthesia. Five months after this surgical procedure the lag screw should be removed.

Patient No. 6 was a 3-year old stallion admitted with abdominal discomfort. After surgical exploration peritonitis was discovered. The patient had to be euthanised.

Patient No. 7. In this patient enucleation was required as a result of panophtalmitis when they could not be preserved.

Thanks to our team and the new technical equipment of the Equine Clinic we were able to admit patients that required specific surgical procedures and a long-term general and ensure complete monitoring of the patients.

ACKNOWLEDGEMENTS

The paper was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic under the contract Vega No. 1/0498/12.

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FOLIA VETERINARIA, 56, Supplementum II: 15–16, 2012

EFFECT OF DIETARY PROBIOTIC AND PLANT EXTRACT SUPPLEMENTATION ON MUCIN DYNAMICS IN THE CHICKEN INTESTINE AND ON PERFORMANCE OF CHICKENS

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ABSTRACT

The experiment was conducted to evaluate the effect of *Enterococcus faecium* EF 55 and sage dietary supplementation, challenged with *Salmonella Enteritidis* PT 4, on mucin dynamics in the intestine of chickens and on their performance. Chickens of strain ISA BROWN, one day old, were randomly divided into four groups (n = 20). The chicks of group 1 (Control – C) were fed commercial diet. Group 2 (EFS) was fed diet supplemented with probiotic strain *E. faecium* 55 at 10^9 CFU.ml^{-1} and 3 g of dry sage (*Salvia officinalis* L.). Group 3 (SE) was orally given 10^8 CFU.ml^{-1} *S. Enteritidis* PT4 in a single dose on day 4. Group 4 (ESS) was fed *E. faecium* 55 and sage and orally given *S. Enteritidis*. Duration of experiment was 21 days. Two samplings were conducted on days 3 and 17 post-challenge (PC). Our results showed that *S. Enteritidis* infection increased mucin adherent layer in the intestine and affected performance of chickens. Combination of probiotic and sage supplementation significantly reduced mucin layer in the caecum. It was concluded that feeding diet supplemented with probiotic and sage could be effective in protecting SE-infected chickens.

Key words: chicken; intestine; mucin; *S. Enteritidis*; sage

INTRODUCTION

The epithelium of the intestinal tract is covered by a layer of mucus composed predominantly of mucin glycoproteins that are synthesized and secreted by goblet cells (1). Mucin glycoproteins are responsible for the viscous properties of the mucus gel. In addition to forming a gel, which acts as a lubricant, a physical barrier and a trap for microbes, mucus provides a matrix for a rich array of antimicrobial molecules. The thickness of the mucus adherent layer is affected by the rate of mucin secretion and by the rate of mucin layer degradation. Mucin secretion occurs *via* both constitutive and regulated pathways. Constitutive pathway continuously secretes sufficient mucin to maintain the mucus layer, whereas regulated pathway affords a massive discharge as a response to environmental and/or (patho) physiological stimuli. The mucin layer at the mucosal surfaces is a nutrient source for many intestinal organisms. Degradation of mucin also occurs by the mechanical shear forces of peristalsis. Mucosal barrier could potentially be rapidly adjusted to changes in the environment, for example, in response to microbial infection (5).

The purpose of this study was to determine the effect of probiotic and sage dietary supplementation challenged with *S. Enteritidis* PT4 on mucin dynamics in the chick intestine and on performance of chickens.

MATERIAL AND METHODS

Eighty-five 1-day-old chickens of a commercial strain ISA BROWN (Párovské Háje, Slovakia) were used in a 21-day experiment. The chicks were kept in a floor-pen on wood shavings. All birds had free access to feed (commercial diet HYD O4/a, Tajba comp., Čaňa, Slovakia) and water. On the day of hatching the chicks were randomly assigned to 4 groups. The chicks of group 1 (Control C) were fed a commercial diet. Group 2 (EFS) was fed diet supplemented with probiotic and bacteriocin-producing freeze-dried strain *E. faecium* 55 at 10^9 CFU.ml^{-1} and 3 g of dry sage (*Salvia officinalis* L.) mixed in 1 kg of feed and fed for the 21 days of the experiment.

Table 1. Influence of *E. faecium* and sage extract supplementation and *S. Enteritidis* infection on mucin dynamics in the intestine of chicks (μg AB.cm^{-2})

	Day post challenge	Control (C)	*Salmonella Enteritidis* (SE)	S. *Enteritidis* + sage extract + *E. faecium* (ESS)	*E. faecium* + sage extract (EFS)
Duodenum	3	28.68 ± 1.66^{ab}	68.30 ± 4.63^{bc}	58.90 ± 4.105^{ad}	19.10 ± 1.69^{cd}
Duodenum	17	40.80 ± 3.17^{a}	62.70 ± 4.85^{ab}	55.50 ± 3.82^{c}	29.20 ± 2.34^{bc}
Jejunum	3	52.0 ± 3.61^{ab}	71.30 ± 2.73^{ac}	52.50 ± 4.16^{d}	16.50 ± 1.43^{bcd}
Jejunum	17	28.60 ± 1.60^{ab}	49.10 ± 1.79^{ace}	67.40 ± 4.42^{bde}	29.80 ± 1.92^{cd}
Ileum	3	41.20 ± 3.74	48.80 ± 1.96^{a}	40.10 ± 3.59	24.30 ± 1.53^{a}
Ileum	17	33.80 ± 3.14^{ab}	70.60 ± 2.68^{ac}	70.30 ± 5.28^{bd}	37.80 ± 2.63^{cd}
Caecum	3	81.40 ± 2.53^{abd}	161.5 ± 3.81^{bce}	39.50 ± 4.82^{ac}	21.10 ±1.23^{de}
Caecum	17	51.90 ± 5.54	42.50 ± 1.61	55.90 ± 4.02	32.70 ± 2.17

Results are presented as mean ± SEM; n = 10;
Significant differences (P < 0.001) within a row are indicated by the same superscript letter

Group 3 (SE) was orally given 10^8 CFU.ml^{-1} *Salmonella Enteritidis* PT4 in a single dose in 0.2 mL PBS on day 4. Group 4 (ESS) was fed *E. faecium* 55 and sage extract as for Group 2 and orally given *S. Enteritidis* as for Group 3. Experimental infection was carried out using *S. enterica* serovar *Enteritidis* phage type 4 (SE PT4). Dry extract of sage (*Salvia officinalis* L.) (α – thujone, 44.3 %, camphor, 21 %, eucalyptol, 12.8 % and borneol, 11 %) was added at 3 g per group and day for 21 days. At 4 and 17 days post-challenge ten chickens from each group were randomly chosen, sacrificed by overdose of anaesthetics and intestinal sections were collected and processed for mucin determination using a method by Corne *et al.* (1), modified by Smirnov *et al.* (6) and Thompson and Applegate (7). Middle segments of duodenum, jejunum, ileum and caecum were taken. Each segment was rinsed with 0.15 mol.l^{-1} NaCl, reversed and slit into two parts each about 1 × 1 cm. The segments of gut were stained in with Alcian Blue (AB) (AppliChem GmbH, Germany), rinsed with 0.25 mol.l^{-1} saccharose, immersed in solution of sodium docusate 10 g.l^{-1} (Aldrich, Germany) and kept there until the next day. The next day the samples were centrifuged (700 × g.2 min^{-1}) and the supernatant was pipetted into a 96-well plate in triplicate, 100 μl of each sample. The absorbance of samples and standards was measured by Mixplate reader (OPSYS MR, USA) at 630 nm wavelength. The amount of adherent gut mucus stained with AB was calculated by means of a standard curve and expressed as μg AB.cm^{-2} of gut.

RESULTS AND DISCUSSION

Salmonella is a facultative, intracellular pathogen capable of infecting chicks. Following oral ingestion, the pathogen penetrates the mucosal epithelium of the intestine and attacks spleen in the first few days post infection. The caecum and crop are reported to be predominantly colonized organs in chicks (4). To colonize mucosal surfaces and invade host, microbes typically must first penetrate the secreted mucus barrier and then, either attach to the apical surface of epithelial cell with cell-surface mucins or release toxins that disrupt epithelial integrity (5). Infection by pathogens can be limited by multiple mechanisms. There are several reports about the role of microbial products to stimulate increased production of mucin by mucosal epithelial cells. Our results showed that *Salmonella Enteritidis* infection increased mucin adherent layer in the intestine (Table 1). Similarly, Fasina *et al.* (3) reported a significant increase in goblet cell density at 10 days PC in the jejunum of chickens challenged orally with *Salmonella Enteritidis* (7.4 × 10^7 CFU at 3 days of age). There is an evidence that adherence of probiotic bacteria upregulates cell-surface mucins expression *in vitro*, perhaps representing an important part of the mechanism by which probiotic bacteria limit infection by pathogens (5). Another tool to limit infection by pathogen in the intestine is supplementation of diet with plants with antimicrobial properties. Constituents of essential oils possess antimicrobial action that is expected to cause structural and functional damages to the cytoplasmic membrane in the intestine. Relative weight (RW) of pancreas was higher in SE than in the C group at 3 days PC. On day 17 PC the RW of spleen was higher in the SE group than in the ESS and EFS groups, and proventriculus and gizzard RW was higher in the SE group than in the C and ESS and the EFS groups. Increased RW of the spleen in the SE group can be explained by attacks on the organ by microbes and microbial products and severe inflammatory response (2). Our results showed that combination of probiotic and sage extract supplementation significantly reduced mucin layer in the caecum of chicks challenged with salmonella. Feeding the diet supplemented with probiotic and sage extract could be effective in preventing *Salmonella Enteritidis* infection in chickens.

ACKNOWLEDGEMENTS

The study was supported by grants VEGA No. 1/0859/11 and No. 2/0010/10.

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FOLIA VETERINARIA, 56, Supplementum II: 18–19, 2012

OXIDATIVE CHANGES IN KIDNEYS INDUCED BY HIGH FAT DIET

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ABSTRACT

The study observed the effect of high fat diet and its combination with selected active substances on kidney antioxidant status. Sixty three rats weighing 300–400 g were divided into seven equal groups; the control group received normal diet (rat chow) for whole experimental period, the HFD group received high fat diet with 20 % share of sunflower oil with a wide ratio of ω-6/ω-3 (610 : 1) polyunsaturated fatty acids and the remaining experimental groups were supplemented with linseed oil, probiotic and horse chestnut extract added to high fat diet. Kidney mitochondria were used for determination of antioxidant enzyme activities and level of reduced glutathione. Results indicate negative effect of higher intake of ω-6 PUFAs on oxidative state in kidney mitochondria.

Key words: antioxidant status; high fat diet; kidney mitochondria

INTRODUCTION

Dietary polyunsaturated fatty acids (PUFAs) are known as precursors of biological active compounds. They mediate a broad range of actions, including effects on membrane fluidity and intracellular cell-signalling cascades. However, they should be present in the nutritionally proper ratio. Imbalance in the ω-6/ω-3 PUFAs ratio might be responsible for the development of cardiovascular diseases. PUFAs are sensitive to oxidative damage and therefore their consumption should be covered by a sufficient intake of antioxidants. The natural antioxidant system consists of a series of antioxidant enzymes and numerous endogenous and dietary antioxidant compounds that react with and inactivate reactive oxygen species (ROS). Kidneys are metabolically very active in effecting the biotransformation of a variety of chemicals and drugs and, in some cases, surpasses the liver (1). Due to the abundance of long-chain polyunsaturated fatty acids in the composition of renal lipids, kidneys are highly vulnerable to damage caused by ROS (7). Our experiment was preferentially focused on monitoring the changes in antioxidant enzyme activities in kidney mitochondria after high fat diet with wide ratio of ω-6 : ω-3 PUFAs.

MATERIAL AND METHODS

This study was carried out on sixty three Sprague-Dawley rats, aged four months, with body weight ranging between 300–400 g. The animals were obtained from the Animal Facility of the University of Pavol Jozef Safarik. They were kept in cages with *ad libitum* access to water. The rats were randomly divided to seven equal groups: Group C (control group) received only normal diet (rat chow) for six months. Group HFD received high fat diet containing 20 % sunflower oil with a wide ratio of ω-6 : ω-3 PUFAs (610 : 1). HFD + LO group was supplemented with linseed oil as a source of omega-3 PUFAs in amount of 4.0 %, HFD+PRO group was fed probiotic *Lactobacillus plantarum* (10^9 CFU.ml^{-1}) administered in milk. Our study was carried out in accordance with the guidelines of the Animal Care and Use Committee of Pavol Jozef Safarik University. By the end of the 6 months experimental period, all rats were sacrificed by cervical dislocation. Kidney mitochondria were isolated according to Fernández-Vizzara (5). The activity of glutathione reductase (GR, E.C.1.6.4.2) was measured according to a modified method previously described by Carlberg and Mannervik (4); that of glutathione peroxidase (GPx, E.C. 1.11.1.9)

Table 1. The activities of enzymatic and levels of non-enzymatic rat kidney mitochondria antioxidants

Groups	SOD [μkat/g_p]	GPx [μkat/g_p]	GR [nkat/g_p]	GSH [nmol/mg_p]
C	5.93 ± 0.12	0.278 ± 0.042	36.17 ± 3.23	14.14 ± 2.13
HFD	5.27 ± 0.14[b]	0.063 ± 0.019[c]	68.98 ± 8.01[c]	2.0 ± 1.85[c]
HFD + LO	5.67 ± 0.44	0.062 ± 0.019[c]	22.82 ± 2.31	9.14 ± 2.48[c]
HFD + PRO	4.65 ± 0.11[c]	0.132 ± 0.037[c]	15.23 ± 3.65[c]	11.2 ± 0.60[b]
HFD + PRO + LO	4.61 ± 0.09[c]	0.152 ± 0.060[b]	26.76 ± 6.38	7.6 ± 3.01[c]

Letters in superscript indicate significant differences at [a] – $P < 0.05$; [b] – $P < 0.01$; [c] – $P < 0.001$

was measured as described by Flohe and Gunzler (6) and that of superoxide dismutase (SOD, E.C. 1.15.1.1) by means of the SOD-Assay Kit-WST (Fluka, Japan) following the user manual, provided and calculated per mg of mitochondrial proteins (mg P). Proteins were quantified using bicinchinonic acid. Values of the measured parameters were expressed as mean value ± SD and the difference between the two groups was determined using unpaired Student's *t*-test, and the significance was considered at P values < 0.05.

RESULTS AND DISCUSSION

Since the entire range of toxic metabolites in the body is excreted mainly from the kidney, this organ is endowed with significant antioxidant defence system next to liver. This is understandable because ROS play a key role in the pathophysiological processes for a wide variety of renal diseases. The results obtained (Table 1) indicated a decrease in SOD, GPx and GR activities as well as GSH level after the HFD treatment and its combination with linseed oil and probiotics in comparison to control group. Glutathione peroxidase is an antioxidant enzyme that is highly expressed in kidneys and reduces lipid peroxides and other organic hydroperoxides highly cytotoxic and responsible for renal damage (3). During oxidative stress, inactivation of GPx may occur and, on the other hand, superoxide anion itself can inhibit peroxidase function. It may be concluded that the decreased activities of SOD and GPx in kidney mitochondria present increased oxidative stress. HFD induced significant increase in GR activity and, on the contrary, decrease in GSH levels. High fat diet contains mainly polyunsaturated fatty acids, which may be very sensitive to oxidation. An increase in GR activity could therefore be accompanied with an increased rate of GSH oxidation in the body. Glutathione provides biotransformation and detoxification of xenobiotics and therefore changes in its levels may be assumed after administration of exogenous substances. Decrease in its levels in kidney mitochondria of rats supplemented with selected active substances plays a crucial role in the mechanism of the peroxidative action of these substances in the organ (7). On the contrary, a positive effect on kidney mitochondrial antioxidant status after the addition of linseed oil to high fat diet was observed. Linseed oil has been found to have antioxidant and anti-inflammatory effects (8). We presume that the imbalanced oxidative state in kidney as well as the reduction of GSH might result not only from its utilization in redox reactions or from disorders of its regeneration but also from its improper synthesis caused by conditions of high fat diet with a wide ratio of ω-6 : ω-3 PUFAs.

ACKNOWLEDGEMENTS

The study was supported by VEGA No. 1/0372/10 and 1/1236/12.

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FOLIA VETERINARIA, 56, Supplementum II: 20–21, 2012

THE ROLE OF GST ENZYMES IN DETOXIFICATION OF XENOBIOTICS

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ABSTRACT

A large majority of foreign compounds are metabolized by different enzyme systems through reactions known as biotransformations. Subsequently, the xenobiotic molecule becomes less lipophilic and better eliminable from the body (10, 15). They catalyze the conjugation of tripeptide glutathione into a wide range of electrophilic compounds including carcinogens, environmental pollutants, anticancer agents, antibiotics, and products of oxidative processes that could result in DNA or protein damage as well (7, 9). They have been considered to play a major role in Phase II of xenobiotic metabolism; susceptibility or response/effect is attributed to genetic polymorphisms (1). Cattle is an important source of animal-derived food products. However, the function and expression of glutathione S-transferases have so far been rarely investigated. In this paper we present the available knowledge on the role of glutathione S-transferase in cattle and its possible function in protection against oxidative stress and in appearance of disease.

Key words: cattle; glutathione S-transferase

THE ROLE OF GST ENZYMES IN DETOXIFICATION OF XENOBIOTICS

The enzymes involved in detoxification of xenobiotics have been categorised into two groups: Phase I and Phase II. Phase I enzymes are related to activation of the compound mainly by the cytochrome P450 system. Among the Phase II enzymes, the mammalian glutathione S-transferase (GST) family is considered as one of the most important detoxification enzymes groups (8).

Livestock are daily exposed to various toxicants, especially insecticides, herbicides, and other pesticides during their feeding processes on the pasture (6). There is evidence about the accumulation of residues in meat and milk of cattle after their exposure to several environmental pollutants (3, 4). When compared with the data in humans, rats or mice, little is known about the liver biotransformation of xenobiotics and regulation of enzyme expression in cattle. Based on amino acid sequence similarities, seven classes of cytosolic GST have been recognized in mammalian species, designated as Alpha, Mu, Pi, Sigma, Theta, Omega, and Zeta.

To our knowledge only non-protein analysis of phase II xenobiotic metabolizing enzymes has been performed (3, 4). Recently, the study of drug-metabolizing enzyme expression and regulation has begun in cattle (1, 3, 5).

Bovine GSTs have been studied in connection with their steroid isomerase activity. Using the immunohistochemistry technique R a b a h i *et al.* (11) characterised two isoenzyme subunits of bovine GST alpha class, namely GSTA1 and GSTA2. The initial finding of the bovine GSTA1 transcript was detected in ovary, testis and adrenals. Later the researchers revealed that alpha class GSTs are specifically produced by steroid active cells (12). It has been proposed that bovine GSTA1 plays a protective role during steroidogenesis, by counteracting the harmful effects of reactive oxygen species formed during the metabolism of steroids (11). Further researchers concluded that the glutathione S-transferase present in all parts of the bull and boar epididymis may be part of the naturally occurring enzymatic barrier protecting the spermatozoa against the toxic effects of various electrophilic compounds (2).

Other authors have estimated the genetic parameters in Holstein cattle. They assumed that heat tolerance ability was partially genetically controlled. However, the underlying functional gene and

the corresponding mechanism remained unknown (13). Further researchers evaluated the effect of heat stress on GSTP1 mRNA expression in the Holstein breed using the semi-quantitative RT-PCR method (16). The researchers believed that GSTP1 would play the central role in detoxification of ROS and other toxic metabolites induced by carcinogens and thus reduce the destructive process of heat stress in Holstein cattle. Although the statistically significant association of genotypes GSTP1 with heat tolerance ability was not demonstrated, the positive role of GSTP1 was assumed to consist in resisting the destructive effect of heat stress in Holstein breed cattle.

In conclusion, there is no doubt that GSTs play an important role in the detoxification of xenobiotics and in the protection of organisms against their toxic effects. Further studies on GST expression should contribute to animal health protection; improving survival and maintaining productivity, preventing oxidative stress, promoting fertility and curtailing disease. Comprehensive data are also important for the protection of human health.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Education and Science of the Slovak Republic through project VEGA No. 1/0125/11.

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FOLIA VETERINARIA, 56, Supplementum II: 22–24, 2012

ATHEROGENIC INDEX OF PLASMA IN A GROUP OF PAVOL JOZEF ŠAFÁRIK UNIVERSITY STUDENTS

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ABSTRACT

Slovakia is one of European countries with the highest cardiovascular mortality. One of the major risk factors of the development of cardiovascular diseases is dyslipidaemia, either primary or associated with hypertension, diabetes mellitus and obesity (1). Generally, atherogenic index of plasma (AIP) calculated as $\log_{10}$ (triglycerides/high density lipoproteins cholesterol) is as a useful predictor of cardiovascular risk, particularly atherosclerosis. For the current study, we have randomly selected 200 subjects out of university students. The average age was 23.19 ± 4.36 (all population). The AIP risk categories were created according to the published epidemiological data: low risk < 0.11, intermediate 0.11–0.21, and high > 0.21. The average value of AIP was –0.10 ± 0.24 in all population, –0.06 ± 0.25 in men and –0.14 ± 0.23 in women. Correlation of AIP between sexes was very strong (P < 0.001). Clinical studies showed that AIP is a predictor of cardiovascular risk. It is an easily available cardiovascular risk marker and a useful measure of response to treatment.

Key words: atherogenic index of plasma; cardiovascular diseases; cholesterol; health risk; lipoproteins

INTRODUCTION

Slovakia is one of European countries with the highest cardiovascular mortality (1, 7). One of the major risk factors for the development of cardiovascular diseases (CVD) is dyslipidaemia which may be primary or associated with hypertension, diabetes mellitus (DM) and obesity (5, 7). Dyslipidaemia usually involves elevated plasma levels of triglycerides (TAG), total cholesterol (TC), low density lipoprotein (LDL), and a very low density lipoprotein (VLDL) cholesterol and a low level of high density lipoprotein (HDL) cholesterol (3, 4, 5). Atherogenic index of plasma (AIP) is a logarithmically transformed ratio of molar concentrations of triglycerides to HDL-cholesterol ($\log_{10}$ TAG/HDL). The strong correlation of AIP with the lipoprotein particle size may explain its high predictive value (2).

The purpose of our study was to find the prevalence of higher AIP and to determine an association of AIP with parameters of CVD, lipid metabolism, and DM which was investigated in a group of Pavol Jozef Šafárik University students and compared between sexes.

MATERIAL AND METHODS

The study was carried out on 200 volunteers, 100 males and 100 females, 2319 ± 436 old (all population) who did not have CVD. We collected 10 ml of blood from each of them. The blood was allowed to clot and serum was obtained after centrifugation at 3500 rpm for 10 minutes. The sera were stored at –80 °C and analysed within one month of collection. TC, TAG, HDL and glucose levels were determined by enzymatic analyser methods COBAS MIRA. LDL, VLDL, non HDL were calculated. Cardiovascular risk ratio was calculated using AIP, which was defined as $\log_{10}$ (TG/HDL) with TG and HDL expressed in molar concentration (1, 6, 7). A two-sample *t*-test was used to determine the statistical significance of the means between different groups using $P < 0.05$ as the level of significance. Pearson correlation coefficient was used to show the level of risk of AIP and selected clinical lipid, metabolic and atherosclerotic parameters in men and women.

Table 1. Mean values, standard deviations (SD) and statistical significance of selected clinical parameters and AIP in men

Risk of AIP	Low		Intermediate		High	
n	**78**		**8**		**14**	
Age	22.90 ± 3.44		26.88 ± 6.85		23.86 ± 1.70	
AIP	−0.16 ± 0.17		0.15 ± 0.03		0.38 ± 0.11	
	Mean ± SD		**Mean ± SD**		**Mean ± SD**	
TC[1]	4.24 ± 0.84	***	4.57 ± 0.99	***	4.83 ± 0.80	***
HDL[1]	1.12 ± 0.21	***	0.90 ± 0.11	***	0.78 ± 0.11	***
non-HDL[1]	3.12 ± 0.79	***	3.67 ± 0.93	***	4.05 ± 0.73	***
LDL[1]	2.76 ± 0.74	***	3.09 ± 0.88	***	3.19 ± 0.74	***
VLDL[1]	0.36 ± 0.12	***	0.58 ± 0.06	**	0.86 ± 0.18	***
TAG[1]	0.80 ± 0.25	***	1.28 ± 0.14	*	1.88 ± 0.39	***
% HDL/TC	27.03 ± 5.56	***	20.24 ± 3.52	***	16.40 ± 2.07	***
LDL/HDL	2.53 ± 0.74	***	3.42 ± 0.83	***	4.07 ± 0.82	***

[1] – mmol.l^{-1}; Correlation with AIP: significance at the level *** – P < 0.001, ** – P < 0.01, * –P < 0.05

Table 2. Mean values, standard deviations (SD) and statistical significance of selected clinical parameters and AIP in women

Risk of AIP	Low		Intermediate		High	
n	**86**		**7**		**7**	
Age	23.05 ± 4.80		26.88 ± 6.85		23.86 ± 1.70	
AIP	−0.20 ± 0.17		0.13 ± 0.02		0.37 ± 0.16	
	Mean ± SD		**Mean ± SD**		**Mean ± SD**	
TC[1]	4.94 ± 0.95	N. S.	4.39 ± 0.86	***	5.40 ± 1.59	***
HDL[1]	1.41 ± 0.42	***	1.05 ± 0.27	***	1.06 ± 0.38	***
non-HDL[1]	3.53 ± 0.77	**	3.34 ± 0.76	***	4.34 ± 1.43	***
LDL[1]	3.11 ± 0.75	N. S.	2.68 ± 0.73	***	3.18 ± 1.15	***
VLDL[1]	0.42 ± 0.19	***	0.65 ± 0.18	***	1.16 ± 0.54	***
TAG[1]	0.92 ± 0.41	***	1.44 ± 0.40	***	2.55 ± 1.18	***
% HDL/TC	28.63 ± 6.32	***	24.16 ± 5.39	***	20.55 ± 7.33	***
LDL/HDL	2.36 ± 0.77	***	2.66 ± 0.74	***	3.20 ± 1.36	***

[1] – mmol.l^{-1}; Correlation with AIP: significance at the level *** – P < 0.001, ** – P < 0.01, * – P < 0.05; N.S. – not significant

RESULTS AND DISCUSSION

AIP risk categories were created according to the published epidemiological data: low risk < 0.11, intermediate 0.11–0.21 and high > 0.21 (1). Based on these categories, 164 of university students (82 %) fell in low risk (men 78 %, women 86 %), 15 (7.5 %) in intermediate risk (men 8 %, women 7 %) and 21 (10.5 %) in high risk category of AIP (men 14 %, women 7 %). The mean value of AIP was –0.10 ± 0.24 (all population), –0.06 ± 0.25 for men and –0.14 ± 0.23 for women.

Mean values (± standard deviations) of lipid and atherosclerotic parameters and statistical significance between evaluated and reference values in each group are presented in Table 1 for men and Table 2 for women. The mean levels of HDL cholesterol, % HDL to TC, ratio LDL/HDL were significantly higher (P < 0.001) in a group of low risk of AIP men. Correlation of risk of AIP between men and women was very high (P < 0.001). Men and women in the group of high risk of AIP showed higher values of all parameters than those in the group of low risk of AIP. Our results showed relationship between lipid metabolism and AIP and atherosclerosis. Diet, health, lifestyle and physical activity affected positively lipid metabolism and reduced risk of atherosclerosis and cardiovascular diseases. Clinical studies showed that AIP predicts cardiovascular risk. It is an easily available cardiovascular risk marker and a useful measure of response to treatment (2, 6).

ACKNOWLEDGEMENTS

This study was supported by the grants 10/GSD/2011, 1/GSŠ/2011, 3/GSŠ/2011, 4/GSŠ/2011 and VEGA 1/1236 /2012.

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FOLIA VETERINARIA, 56, Supplementum II: 25–27, 2012

MONITORING OF CADMIUM AND LEAD IN WILD BOARS IN THE SOUTH PART OF CENTRAL SLOVAKIA

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ABSTRACT

The aim of the study was to determine cadmium and lead concentrations in parenchymatous organs and muscle tissue of wild boars (20 animals) that were hunted down in Rimavska Sobota district in the south part of central Slovakia which appears to be a typical agricultural region. Concentrations of cadmium in the kidney ranged from 0.011 to 1.807 mg.kg^{-1}. Hepatic cadmium concentrations ranged from 0.012 to 0.296 mg.kg^{-1} and muscle cadmium concentrations varied from 0.01 to 0.164 mg.kg^{-1}. Renal lead concentrations varied from 0.0019 to 0.066 mg.kg^{-1}, liver lead concentrations from 0.002 to 0.023 mg.kg^{1} and muscle lead concentrations from 0.0007 to 0.051 mg.kg^{-1}. Some determined values were above the maximum allowed concentration, such as renal cadmium concentrations in 5 samples (25 %) and muscle cadmium levels in 2 samples (10 %). Cd and Pb levels in the liver and Pb in kidneys and muscles did not exceed the maximum allowed concentration. Based on the given results the Rimavska Sobota district has not been so heavily polluted as some other regions. However, the presented results documented that toxic metals in the tissues of game have remained a pressing problem.

Key words: kidney; liver; muscle; toxic metal; wild boar

INTRODUCTION

During the past two centuries industrial and agricultural development have caused a high level of environmental pollution and resulted in accumulation of environmental contaminants in all types of ecosystems. Toxic metals are a large group of anthropogenic pollutants which were distributed to the environment during industrial revolution. They do not have any physiological function as they are not essential to living organisms. Cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As) and some radioactive metals are included in the group of toxic metals. Small particles of cadmium and lead occur in different compounds and contaminated dust in the atmosphere and transfer to long distances. This results in subsequent contamination of soil and vegetation not only in industrial regions but also in regions with undeveloped industry (3, 6, 10). Both sources of toxic metals contamination (atmosphere and soil origin) cause contamination of vegetation (3, 6). Animals and humans have been contaminated primarily by inhalation and ingestion of small particles which in most cases give rise to chronic intoxication (2). Resorption of cadmium and lead is relatively low and is influenced by a number of factors, such as animal species, dose, age, sex and pregnancy (5, 11).

Higher environmental levels of these contaminants result in their bioaccumulation in plants and animals. Due to affinity of Cd and Pb to animal organs, these serve as a bioindicator of pollution and contamination of their biotopes, which was the main goal of our study.

MATERIAL AND METHODS

Twenty wild boars of different age categories from several hunting grounds in the Rimavska Sobota district in the south part of central Slovakia showing no clinical signs of any illness were included in the study. Samples of parenchymatous organs (liver and kidney) and muscle tissue were collected from hunted animals immediately after disbowelling and were stored at –20 °C until fur-

Table 1. Cadmium concentrations (mg.kg^{-1}) in wild boars hunted in district Rimavska Sobota

	Cadmium	Kidney	Liver	Muscle tissue
Central Gemer	x ± SD	0.42 ± 0.115$^{Aa, Bb}$	± 0.026^{Aa}	0.069 ± 0.032^{Bb}
n = 20	maximum	1.807	0.296	0.164
	minimum	0.011	0.012	0.01

$^{Aa, Bb}$ – in superscript indicate significance at P < 0.01
Limit values according to the Slovak Codex Alimentarius: Cd – 0.1 mg.kg^{-1} in muscle tissue, 0.5 mg.kg^{-1} in parenchymatous organs

Table 2. Lead concentrations (mg.kg^{-1}) in red deer in district Rimavska Sobota

	Lead	Kidney	Liver	Muscle tissue
Central Gemer	x ± SD	0.013 ± 0.004	0.007 ± 0.001	0.012 ± 0.003
n= 20	maximum	0.066	0.023	0.051
	minimum	0.0019	0.002	0.0007

Limit values according to the Slovak Codex Alimentarius:
Pb – 2 mg.kg^{-1} in muscle tissue, 1 mg.kg^{-1} in parenchymatous organs

ther processing. After adding 5 ml of H_2O_2 and 2 ml of HNO_3, the samples were subjected to wet-mineralization using a microwave laboratory system Microwave 3000 (Perkin Elmer, USA). Lead and cadmium levels were determined by an inductively coupled plasma mass spectrometer Agilent 7500 ICP-MS (Agilent Technologies, USA) in an accredited laboratory of the State Veterinary and Food Department in Kosice. The respective standard solution was used at the beginning, during and after finishing the analysis. Statistical analysis was carried out using Microsoft Excel (MicroSoft®, Inc., USA) statistical software. Data obtained by the analysis were divided into the groups according to animal organs. Concentrations were noted down as a mean ± standard deviation (SD), minimal and maximal values. To compare the differences between different organs one-way ANOVA and Tukey's test were used. The P < 0.01 indicated significant difference.

RESULTS AND DISCCUSION

By comparing our results with the criteria stated in the Slovak Codex Alimentarius we have noted that some values exceeded the maximum allowed concentration, such as renal cadmium concentrations in 5 samples (25 %) and muscle cadmium levels in 2 samples (10 %). Cd levels in the liver and Pb levels in all organs were below the maximum limit.

With regard to Cd the maximum mean concentration was detected in kidney 0.42 mg.kg^{-1}, lower in liver 0.1 mg.kg^{-1} and the lowest one in muscle tissue 0.069 mg.kg^{-1}, where we also detected significant differences (Table 1). Compared to other Slovak regions kidney and liver cadmium concentrations determined in our study were lower. In 2009 the mean Cd level in kidneys reached 2.38 mg.kg^{-1} in central Gemer (Revuca district). In 2003 Cd levels in kidney in central Zemplin reached 0.56 mg.kg^{-1}. Mean liver concentrations in Slovak regions reported by other authors varied from 0.28 mg.kg^{-1} to 0.72 mg.kg^{-1} (4, 7). In four regions of Croatia mean cadmium kidney concentrations varied from 3.47 mg.kg^{-1} to 5.98 mg.kg^{-1} and liver concentrations ranged from 0.3 mg.kg^{-1} to 0.49 mg.kg^{-1}; they were significantly higher than our results (1). Cadmium levels in muscle tissues were approximately at the same level as our results (1, 4, 7, 8).

Mean lead levels in selected tissues of wild boars hunted in Rimavska Sobota determined in our study were approximately at the same level (0.007–0.013 mg.kg^{-1}), and did not differ significantly (Tab. 2). However, they were significantly lower compared to lead levels in wild boars in another Slovak region and regions outside Slovakia, where lead levels varied from 0.056 mg.kg^{-1} to 2.29 mg.kg^{-1}; the highest concentrations were found in muscle tissue and kidneys (1, 4, 7, 8). Some authors reported higher levels in kidneys than in the liver; this was also observed in our study (9, 12). Similarly, our results showed that the environmental contamination by lead emissions in Rimavska Sobota district was low due to mostly agricultural character of this part of Slovakia.

Our results proved relatively low contamination of the environment; this fact was supported by relatively low number of samples that exceeded maximum allowed concentrations stipulated by the Slovak Codex Alimentarius.

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FOLIA VETERINARIA, 56, Supplementum II: 28–30, 2012

THE INFLUENCE OF DIET WITH GENETICALLY MODIFIED MAIZE ON GROWTH, NUTRIENT DIGESTIBILITY AND HEALTH STATUS OF BROILER RABBITS

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ABSTRACT

Live weight growth, feed conversion and health of rabbits after feeding the complete feed mixtures with 12 % proportion of Bt (MON 88017), isogenic maize and reference maize (PR 36D 79) was observed in 72 broiler rabbits (Hycole) between 5 to 11 weeks of age. Bt maize deteriorated neither the health in animals nor the production of animal proteins valuable for human nutrition when compared with the conventional maize.

Key words: blood parameters; Bt maize; microflora; nutrient digestibility; rabbit

INTRODUCTION

Genetically modified (GM) crops have been developed which offer a wide variety of benefits to producers including resistance to insects, disease and herbicides. Bt maize contains bacterial gene from the bacteria Bacillus thuringiensis. This gene encodes the protein which is toxic to some insects. In the European context, Slovakia ranks among 7 European Union countries (Spain, France, Romania, Portugal, Germany, the Czech Republic, Poland) which have practical experience in Bt maize cultivation (6). In this study substantial equivalence in nutrient contents between isogenic and Bt maize by means of chemical analyses was tested when the genetically modified maize (Bt maize MON 88017), isogenic maize (DKC 5143) and reference maize (PR 36D 79) were used in the experiment on rabbits.

MATERIAL AND METHODS

A total of 72 weaned rabbits (35 days old, males, Hycole hybrid) were divided to 3 experimental groups. The rabbits in the Group 1 (1-EG) were fed granulated mixture with 12 % transgenic maize (MON 88017), in the Group 2 (2-EG) granulated mixture with 12 % isogenic maize (DKC 5143) and those in the Group 3 (3-EG) granulated mixture with 12 % reference maize (PR 36D 79). The experiment lasted 42 days. The rabbits were kept in standard cages (0.61 m × 0.34 m × 0.33 m), 2 animals per cage. Body weight and feed consumption were registered weekly. Between 65 and 70 days of age, 5 rabbits from each group were selected for digestibility tests using the balance method. The digestibility test was performed in accordance with the recommended methodology (5). The samples of individual feeds were analyzed for the content of nutrients (Table 1) according to AOAC procedures (1), and starch according to the alpha-amyloglucosidase method. The rabbits were fed *ad libitum* (Table 2) and had free access to drinking water from nipple drinkers throughout the experiment. Blood samples were obtained from the marginal ear vein (vena auricularis). Blood serum was analysed for total proteins and lipids ($g.l^{-1}$), cholesterol, glucose and calcium ($mmol.l^{-1}$) (Randox, the United Kingdom). The activity of blood glutathione-peroxidase (GPx;$U.g^{-1}$ Hb) was determined by RANSEL standard set (Randox, UK). The phagocytic activity (PA) was assessed by direct counting procedure using microspheric hydrophilic particles (MSHP). Faeces as well as caecal samples (three animals from each group were slaughtered on 42nd day) were collected for microbiological analysis according to ISO, expressed in colony forming units (log 10, CFU) per g. The results were pre-

Table 1. Ingredients and chemical analysis of the experimental diets for rabbits

Ingredients	%	Parameter [g. kg^{-1}]	1- EG	2- EG	3- EG
Lucerne meal	41.0	Dry matter	901.8	895.8	895.6
Dried beet pulp	10.0	Crude protein	172.8	168.1	171.4
Rape extr. meal	20.0	Crude fibre	179.2	183.0	189.2
Wheat	3.0	Fat	38.4	35.6	32.6
Apple pomace	9.0	N-free extract	432.2	438.8	428.3
Maize	12.0	Organic matter	822.6	821.6	821.6
Carob meal	0.4	Starch	154.2	157.6	160.1
Mineral & Vitamins*	3.2	Calcium	9.3	6.7	9.58
Rape oil	1.0	Phospho-rus	6.9	4.1	3.68
Limestone, pulverized	0.4	ME [MJ. kg^{-1}]	9.42	9.16	8.99

* – Provided per kg diet: vit. A 12000 IU; vit. D_2 2500 IU; vit. E 20 mg; vit. B_1 1.5 mg; vit. B_2 7.5 mg; vit. B_6 4.5 mg; vit. B_{12} 30 µg; vit. K 3 mg; nicotinic acid 45 mg; folic acid 0.8 mg; biotin 0.08 mg; choline chloride 450 mg. Premix minerals: Ca 9.25 g; P 6.2 g; Na 1.6 g; Mg 1.0 g; K 10.8 g; Fe 327.5 mg; Mn 80 mg; Zn 0.7 mg

sented as mean ± standard deviation (SD); statistical evaluation of the results was performed by the one-way ANOVA and post-hoc Tukey test.

RESULTS AND DISCUSSION

No siginificant differences were found among experimental groups in feed intake, body weight and carcass value in the fattening experiment. The resulting digestibility coefficients for protein fell within the narrow range of 63.97 to 65.72 % and fat digestibility were in the interval from 72.50 to 78.22 %, similar to the data of Chrenková *et al.* (4). Feed mixtures differed regarding digestible energy content, i. e. crude protein, crude fibre and fat. The values of blood parameters varied within the physiological limits. On day 42 the phagocytic activity ranged from 43.7 to 45.5 %. Feeding of genetically modified Bt maize to rabbits did not influence the biochemical and mineral parameters in blood, as well as it had no negative effect on growth performance of rabbits. Concentration of the low activity of GPx in experimental groups indicated that no oxidative stress was evoked. Bacterial counts were well balanced in the caecum and faeces of rabbits after the application of the experimental diets. Low incidence of *Enterococcus* sp./*E. coli* indicates good digestibility of the feed (Table 3). The health status of animals was good.

Table 2. Results of experiments

Parameter	1- EG	2- EG	3- EG
Relation between feed consumption (n = 24)			
Daily weight gain [g.day^{-1}]	36.88	36.95	39.0
Feed conversion ratio [g.g^{-1}]	3.00	3.08	3.21
Carcass yield [%]	57.27	57.78	57.85
Coefficient of nutrients digestibility in % (n = 5)			
Crude protein	65.72	63.97	66.39
Fat	72.50	78.22Ac	76.30
Crude fibre	25.25	25.29	24.90
Nitrogen-free extract	75.57	75.30	75.88
Organic matter	61.34	62.16	62.06
Biochemical parameters in the blood of rabbits ($\overline{X}$ ± SD)			
Total proteins TP [g.l^{-1}]	61.44 ± 3.87^{b}	54.77 ± 3.80	62.28 ± 1.07^{b}
Cholesterol CHOL [mmol.l^{-1}]	2.08 ± 0.24^{bc}	1.71 ± 0.29	1.56 ± 0.2
Triglycerides TRIGS [mmol.l^{-1}]	1.10 ± 0.45	0.98 ± 0.35	0.84 ± 0.19
Glucose GLU [mmol.l^{1}]	8.10 ± 0.37	7.96 ± 0.36	7.93 ± 0.52
ALT [U.l^{-1}]	10.03 ± 2.78^{b}	8.61 ± 1.47	10.13 ± 2.35^{b}
Calcium Ca [mmol.l-1]	3.24 ± 0.14	3.07 ± 0.15	3.28 ± 0.06
Phagocytic activity PA [%]	43.7 ± 0.5	44.8 ± 0.8	45.5 ± 0.6
GSH-Px [U.ml $^{-1}$]	125.34 ± 31.58	121.54 ± 24.89	107.83 ± 34.44

Different letter in superscript (a, b, c) indicate significant differences between values in line at $P < 0.05$; (ABC) significance at $P < 0.01$; ALT – alanine amino transferase; GSH-Px – activity of blood enzyme glutathione peroxidase

Table 3. Bacteria (log 10 CFU.g^{-1}) in caecum contents and faeces of rabbits fed experimental diets

Bacterial strains	Caecum			Faeces		
	1-EG	2-EG	3-EG	1-EG	2-EG	3-EG
***Enterococcus* sp.**	3.54	3.92	3.19	< 10^2	< 10^2	< 10^2
Lactic acid bacteria	3.42	4.04	2.54	2.65	2.40	1.44
(CoNS)	4.35	4.07	3.67	2.85	2.81	2.30
(CoPS)	3.52	4.11	2.79	1.54	1.60	1.10
Clostridim-like sp.	2.38	3.37	2.10	1.89	2.39	2.45
Pseudomonas-like sp.	4.95	4.43	4.42	2.88	3.54	2.23
Enterococcus* sp./*E. coli	3.34	3.20	2.52	<10^1	2.02	<10^2

Coagulase- negative staphylococci (CoNS); Coagulase- positivestaphylococci (CoPS)hemical analysis of the experimental diets for rabbits

ACKNOWLEDGEMENTS

This study was supported by the project "BELNUZ No. 262201 20052" supported by the Operational Programme Research and Development, funded from the European Regional Development Fund. The authors would also like to thank to Dr. Rastislav Jurčik for excellent technical assistance and for co-operation in the experiments.

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FOLIA VETERINARIA, 56, Supplementum II: 31–32, 2012

STABILITY OF ENTEROCIN-PRODUCING STRAIN *ENTEROCOCCUS FAECIUM* EF 55 IN RABBITS AND INHIBITORY ACTIVITY OF ENTEROCIN 55

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ABSTRACT

In the present study we tested inhibitory activity and competitiveness of *enterocin* 55 produced by EF 55 strain (isolate from chicken crops) against different indicator strains. Using *in vivo* experiment, the colonization, stability and antimicrobial activity of EF 55 strain were monitored in rabbits. *In vitro* inhibitory activity was noted; *Ent* 55 inhibited the growth of 60 strains tested, 59 Gram-positive and 1 Gram-negative. On the basis of previous *in vitro* results the EF 55 strain was tested under *in vivo* conditions in rabbits. Colonization of the digestive tract of rabbits with EF 55 strain was sufficient. In the experimental group, a slight decrease in *Clostridium*-like and *Pseudomonas*-like bacteria was recorded after EF 55 strain action compared with the control group. As the non-rabbit-derived EF 55 sufficiently survived in the digestive tract of rabbits and exhibited antimicrobial activity, it could be considered a promising candidate supporting the health of rabbits.

Key words: enterocin; *Enterococcus faecium;* probiotic; rabbits

INTRODUCTION

Enterococci are known to produce antimicrobial substances of proteinaceous character – bacteriocins, enterocins. Enterocins that are produced by *Enterococcus faecium* species were studied the best (2). *Enterococcus faecium* EF 55 (isolate from chicken crop) is a probiotic strain which produces *Enterocin* 55 (*Ent* 55) (4). Our interest was to find if this strain with beneficial effect in chicken (3) can also colonize digestive tract of rabbits with beneficial influence. The breeders have problems with rabbits after the weaning period because of their sensitivity to different spoilage agents. Therefore, they searched for possibilities to protect the health of these animals.

The aim of our study was to investigate the antimicrobial activity due to EF 55 or its bacteriocin *Ent* 55 *in vivo* and study *in vitro* the effect of *Ent* 55 itself. *In vivo* experiment has also included a study of stability of EF 55 strain in rabbits.

MATERIAL AND METHODS

In vitro inhibitory activity of *Ent* 55 (semi-purified) was tested by the quantitative agar spot test (1) using 1.5 % Brian Heart agar (BHI), MRS agar (Oxoid, the United Kingdom; Merck, Germany) and 0.7 % BHI and Tryptone Soya Broth (TSY; Becton & Dickinson, USA) agars. Inhibitory activity was expressed in Arbitrary units per milliliter ($AU.ml^{-1}$), i. e. the highest dilution of enterocin causing the inhibition of the growth of an indicator strain. *Enterococcus avium* EA5 (isolate from piglets; 25 000 $AU.ml^{-1}$) was used as a positive control. Altogether 126 indicator strains were tested: 118 Gram-positive and 8 Gram-negative bacterial strains. The indicator strains were from different sources and of various species: *Enterococcus* (20 strains), *Staphylococcus* (85 strains), *Listeria* (10 strains), *Micrococcus* (3 strains), *Acinetobacter* (4 strains), *Escherichia* (2 strains) and *Enterobacteriae* (2 strains). Competitive studies were conducted against *Listeria* sp. and *Staphylococcus* sp. We compared growth or inhibition in EG (experimental group with *Ent* 55) and CG (control group without *Ent* 55). Cultivation was carried out in BHI and TSY broths (appropriate for the strain) for 24 hours at 32 °C or 37 °C. The *in vivo* experiment was carried out on 48 rabbits (5 weeks old, males and females, Hycole). The animals were divided to two groups (EG – experimental and CG – control, 24 animals in each).

They were fed mixed feed Tekro (Nitra, Slovakia). Moreover, the EG rabbits received EF 55 strain (10^9 CFU per animal and day) in water. They had access to water *ad libitum*. The experiment lasted 42 days; the EF 55 strain was administered for 21 days. Rabbit faeces were sampled at the beginning of the experiment and on day 21 and 42 (3 weeks after cessation of administration and at the end). The counts of EF 55 strain were determined on M-*Enterococcus* agar with rifampicin (Difco USA, 100 $\mu g.ml^{-1}$; Biomark, India). Caecum and appendixes were sampled on days 21 and 42 (3 animals were killed). The samples for microbiological analysis were processed by standard microbiological methods according to ISO, using appropriate media: MRS agar (Merck, Germany) for detection lactic acid bacteria (LAB); Mannitol salt agar (Merck, Nemecko) for coagulase-negative staphylococci (CoNS); Baird-Parker agar (B&D, USA) with supplement for coagulase-positive staphylococci (CoPS); *Clostridium difficile* agar with supplement and 7 % defibrinated horse blood (Oxoid Ltd., UK) for detection Clostridium-like bacteria, CLED agar (B&D, USA) for Pseudomonas-like sp., Mac Conkey agar (B&D, USA) for *Escherichia coli* and Enterobacteriaceae.

RESULTS AND DISCUSSION

From among 126 tested strains the growth of 60 (47.6 %) was inhibited by *Ent* 55; 59 Gram-positive (98.3 %) and 1 Gram-negative (1.7 %). Predominantly enterococci and listeriae were inhibited (100–25 600 $AU.ml^{-1}$). Competitive studies were conducted against *Listeria monocytogenes* CCM 4699, *L. innocua* LMG 13568 and *Staphylococcus aureus* Nip1. In the first two cases, we observed a bacteriostatic effect of *Ent* 55 against the tested strains. Predominant antilisterial effect of *Ent* 55 was also confirmed by Strompfová *et al.* (4). *In vitro* testing of *Ent* 55 demonstrated its broad-spectrum inhibitory activity. As the strain EF 55 was successfully applied in chicken (3), we decided to test it in rabbits. The counts of EF 55 in faeces reached up to 2.0 $\log_{10}$ $CFU.g^{-1}$. The counts of enterococci in EG (faeces) were increased on day 21 (3.45 ± 0.7 $\log_{10}$ $CFU.g^{-1}$) compared to the first day (2.29 ± 1.16) of the experiment. This indicated sufficient colonisation of digestive tract of rabbits with EF 55 strain. The counts in caecum and appendixes reached less than 1.0 $\log_{10}$ $CFU.g^{-1}$. EF 55 was present in the gut also 3 weeks after its administration. The inhibitory effect of this strain in EG was observed on day 21 (3 weeks of EF 55 application); slight reduction in Pseudomonas-like sp. (difference of 0.32 $\log_{10}$ $CFU.g^{-1}$) as well as Clostridium-like sp.(difference of 0.54 $\log_{10}$ $CFU.g^{-1}$) was found in comparison with CG. The beneficial effect of EF 55 strain was detected in chickens also by Levkut *et al.* (3). In our experiment the counts of other bacteria were not changed neither in the faeces nor in caecum. The strain EF 55 could be considered a promising candidate in terms of supporting the health of rabbits because it sufficiently colonized digestive tract of rabbits despite not being rabbit-derived.

ACKNOWLEDGEMENTS

Te study was partially supported by the Slovak Scientific Agency VEGA (Project 2/0002/11). The authors would like to thank to colleagues from Animal Production Research Centre in Nitra and to Mrs. M. Bodnárová for her excellent technical assistance in the experiments.

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FOLIA VETERINARIA, 56, Supplementum II: 33–34, 2012

DETECTION OF *ENTEROCOCCUS HIRAE* IN COMMON OSTRICHES AND PHEASANTS AND THEIR SENSITIVITY TO ENTEROCINS

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ABSTRACT

Enterococci are widespread bacteria normally found also in the intestines of humans and animals. These Gram-positive lactic acid producing bacteria belong to the phylum *Firmicutes,* family *Enterococcaceae*, genus *Enterococcus*. According to the latest validation, they are grouped into 7 groups based on 16S rRNA gene similarity. The species *Enterococcus hirae* belongs to *E. faecium* group. It was found to cause disorders, e. g. in poultry. The aim of this study was to test sensitivity to enterocins of *Enterococcus hirae* strains identified in faeces of ostriches and common pheasants. Enterocins are antimicrobial substances produced mostly by enterococci. In this study the enterocins used were produced by *E. faecium* strains (isolates of our Laboratory of Animal Microbiology) of different origin. Faecal samples were collected from 140 common ostriches (*Struthio camelus*) and 60 common pheasants (*Phasianus colchicus*). The pure colonies were identified by the novel MALDI BioTyper TM system (Bruker Daltonics, USA) based on analysis of bacterial proteins. From among the 71 strains from ostriches, 41 were taxonomically allotted to the species *E. hirae;* in pheasants 10 strains from 50 were *E. hirae*. All *E. hirae* strains from pheasants and ostriches were sensitive to the investigated enterocins with inhibitory activity up to 25 600 AU.ml^{-1}, tested by the quantitative agar spot test. These results are contribution to basic microbiology. The sensitivity of antibiotic-resistant strains of *E. hirae* to enterocins is important to health of animals.

Key words: enterocins; *E. hirae;* ostriches; pheasants; sensitivity

INTRODUCTION

Recently, husbandry of new animal species, for example ostriches or pheasants, has been wide-spread in Europe. Ostriches have good quality meat (taste, structure, well digestible, composition of necessary amino acids, low fat and cholesterol content) but are bred also for eggs or skins (7). Pheasants are bred for meat and sport hunting. It is very important for farmers to keep the animals healthy. We have only limited information on microbiota of ostriches or common pheasants. Therefore it is important to study their microflora, especially the species which can threaten their health.

Enterocins are antimicrobial substances mostly produced by enterococci (5). Microflora of ostriches and common pheasants has not been studied from this point of view. Enterococci are widespread bacteria normally found also in the intestines of humans and animals. These Gram-positive lactic acid producing bacteria belong to the kingdom Bacteria, phylum *Firmicutes,* class *Cocci*, order *Lactobacillales*, family *Enterococcaceae*, genus *Enterococcus* (1). According to the newest validation they are grouped into 7 groups based on 16S rRNA gene similarity (6). The species *Enterococcus hirae* belongs to *E. faecium* group. *E. hirae* is a frequently occurring component of the intestinal flora of several domestic animal species (2) and may cause disorders, e.g. in poultry (septicaemia and focal necrosis of the brain in chicks) (3).

The study was focused on detection of *Enterococcus hirae* in ostriches and common pheasants and on testing of their sensitivity to enterocins.

MATERIAL AND METHODS

Strains of *E. hirae* were foundd in faecal samples collected from 140 common ostriches (*Struthio camelus*) of 3 age categories and on 60 common pheasants (*Phasianus colchicus*) located in aviaries with free movement. The samples were processed by the standard microbiological method (International Organization for Standardization – ISO) using appropriate dilutions in Ringer solution. The appropriate dilutions were plated onto M-*Enterococcus* agar (Difco, Maryland, USA, ISO 15214) and incubated at 37 °C for 48 hours. The bacterial colonies obtained were checked for their purity and identified by the novel MALDI BioTyper TM system (Bruker Daltonics, USA), based on analysis of bacterial proteins. The sensitivity of strains to enterocins was tested by the quantitative agar spot test according to De Vuyst *et al.* (4) and expressed in Arbitrary units per millilitre ($AU.ml^{-1}$). In this study we used enterocins produced by *E. faecium* strains (isolates of our Laboratory of Animal Microbiology) of different origin.

RESULTS AND DISCUSSION

From among 71 strains from ostriches 41 were allotted to the species *E. hirae* and in pheasants 10 strains from 50 were identified as *E. hirae*. The study involved 8 semi-purified enterocins (*Ent* EM 41 – activity 25 600 $AU.ml^{-1}$; EM 42 – 25 600 $AU.ml^{-1}$; *Ent* A (P) – 25 600 $AU.ml^{-1}$; *Ent* 55 – 51 200 $AU.ml^{-1}$; *Ent* 2019 – 6400 $AU.ml^{-1}$; *Ent* M3a – 51 200 $AU.ml^{-1}$; *Ent* 4231 – 3200 $AU.ml^{-1}$; *Ent* M – 6400 $AU.ml^{-1}$). *Ents* EM 41 and EM 42 were produced by *E. faecium* strains EM41 and EM 42, originating from ostriches. All strains of *E. hirae* from both pheasants and ostriches were sensitive to the tested enterocins with inhibitory activity up to 25 600 $AU.ml^{-1}$. All strains of *E. hirae* from pheasants were the most sensitive to *Ent* EM 41 (ostrich), *Ent* 55 (chicken crop), *Ent* 2019 (rabbit); the least sensitive was *E. hirae* 41b to *Ent* EM 42 with inhibitory activity 200 $AU.ml^{-1}$. Four strains of *E. hirae* from ostriches (EH 152; 161/Cl; 212; 221) were the most sensitive to all tested enterocins (25 600 $AU.ml^{-1}$). *E. hirae* 2142 was sensitive to at least 4 enterocins (*Ents* 55; EM 41; EM 42; 4231) with the activity of 200 $AU.ml^{-1}$. Testing of sensitivity of *E. hirae* to enterocins is interesting from at least two aspects: to test enterocins inhibitory spectrum and to find how the antibiotic resistant *E. hirae* strains can be treated, e.g. by enterocins. These results contribute some basic knowledge in the field of microbiology. Sensitivity of antibiotic resistant strains of *E. hirae* to enterocins is important from the point of health of farmed animals.

ACKNOWLEDGEMENT

The results were achieved within the work on the project No. 2/0002/11 of the Slovak Scientific Agency VEGA. We are grateful for skillful assisstance of Mrs. Margita Bodnárová, and Mr. Szabo (ostrich farm representative), Mr. Slamečka and Mrs. Chrastinová from Nitra for their support in taking the samples.

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FOLIA VETERINARIA, 56, Supplementum II: 35–36, 2012

DECREASE IN BOVINE CHOLINESTERASE ACTIVITIES AFTER THE EXPOSURE TO TRIAZOLE PESTICIDES

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ABSTRACT

Determination of cholinesterase (ChE) activity is an accurate method for detecting exposure of animals to anticholinesterase compounds. In this paper we investigated the influence of technical triazole fungicides on bovine cholinesterase activity. Changes in cholinesterase activity were assessed spectrophotometrically after *in vitro* exposure to Orius 25EW and Prosaro 250EC® at concentrations ranging from 50 to 300 μg.ml^{-1} for acetylcholinesterase (AChE) and from 25 to 300 μg.ml^{-1} for butyrylcholinesterase (BChE). Our results confirmed a significant inhibition of AChE and BChE activity ($P < 0.001$) after exposure to both fungicides tested.

Key words: acetylcholinesterase; butyrylcholinesterase; tebuconazole; triazoles

INTRODUCTION

Triazole fungicides belong to currently used pesticides (CUPs). Their antifungal properties rely on the inhibition of enzyme lanosterol 14-α-demethylase (CYP 51) which regulates the ergosterol synthesis essential for formation of the fungal cell membrane (10). Tebuconazole fungicides are used against mildews and rust of cereal grains (wheat, barley, rice, cole seeds, etc.), fruits and vegetables. Several studies have shown that tebuconazole exhibits low acute toxicity, but may cause tumours in mice, ocular lesions in dogs and developmental anomalies in mice, rats and rabbits (8).

An inhibiting effect on cholinesterase activity has been widely studied in humans (9) and animals (6) after the exposure to organophosphate (OP) and carbamate pesticides that are typical cholinesterase inhibitors. ChE inhibition results in accumulation of acetylcholine in the nervous tissue with subsequent overstimulation of specific cholinergic receptors and disruption of the function of the nervous system (1, 5). In contrast with numerous studies investigating with changes in ChE activity induced by OP and carbamate pesticides there are rare reports dealing with AChE activity after the exposure to triazole fungicides.

The aim of our study was to assess changes in AChE and BChE activity in bovine plasma and erythrocytes after *in vitro* exposure to various concentrations of technical triazole fungicides (Orius 25EW and Prosaro 250EC®). The measurement of ChE activity in blood or tissue is a useful tool for determining the effect of exposure to anticholinesterase agents and to complement the results for genotoxicity assessment.

MATERIAL AND METHODS

Tebuconazole-based fungicides, trade names Orius 25EW (25 % of tebuconazole) and Prosaro 250EC® (12.5 % tebuconazole plus 12.5 % prothioconazole) were dissolved in 96 % ethanol and used in experiments at concentrations ranging from 50 to 300 μg.ml^{-1}. All experiments were done in 0.1 M sodium phosphate buffer, pH 7.4 at 25 °C, using acetylthiocholine (ATCh, 1mM) as a substrate. The enzyme activity was measured spectrophotometrically according to E l l m a n *et al.* (3) with thiol reagent DTNB (0.3 mM).

The experiments were performed with erythrocytes (source of AChE) and plasma (source of BChE) obtained from whole blood of 6 bulls (6 months old, Slovak Spotted cattle). The final dilution of erythrocytes was 800-fold and of plasma 25- to 100-fold. The increase in absorbance ($\Delta A.min^{-1}$) was read at 436 nm for AChE

and 412 nm for BChE within 5 min after incubation with different concentrations of fungicides in 1 ml reaction volume. All spectrophotometric measurements were performed on a CARY 300 spectrophotometer (Varian Inc., Australia). The results were evaluated statistically by linear and non-linear regression models. Differences between experimental and control groups were checked by the Tukey's multiple comparison tests (Graph Pad Prism®).

RESULTS AND DISCUSSION

A decrease in AChE and BChE activity after the exposure to both commercial fungicides tested was found. A significant inhibition in AChE activity was obtained after the treatment of bovine erythrocytes with the fungicides at concentrations ranging from 150 to 300 $\mu g.ml^{-1}$ ($P < 0.001$) for Orius and from 200 to 300 $\mu g.ml^{-1}$ ($P < 0.001$) for Prosaro. A significant inhibition in BChE activity was seen after the exposure of bovine plasma to the both fungicides, starting at the concentration of 150 $\mu g.ml^{-1}$ ($P < 0.001$).

Triazoles are not typical cholinesterase inhibitors; data about their impact on ChE activity are rare. For example, T o n i *et al.* (7) described an increase in AChE activity in the brain of *Cyprinius carpio* exposed to tebuconazole. In contrast to this, D o m i n g u e s *et al.* (2) observed decline in ChE activity in zebrafish early stages after the highest used concentration of prochloraz. Moreover, an interactive effect between ergosterol-biosynthesis-inhibiting (EBI) fungicides and OP pesticides could be contemplated (4). Presumably, EBI fungicides can induce forms of cytochrome P450 responsible for the activation of the OPs and thus might result in enhanced toxicity.

In conclusion, our results indicated a significant inhibition of bovine blood cholinesterases after the exposure to triazole fungicides. Livestock is an appropriate object for genotoxicity assessment; cattle are directly exposed to chemical agents *via* feed. Chemicals accumulated in their meat and milk could increase genetic risk to humans. The results about the ChE inhibition add to the body of knowledge about the effects of triazole fungicides.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Education and Science of the Slovak Republic VEGA1/0125/11.

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FOLIA VETERINARIA, 56, Supplementum II: 37–38, 2012

BLUETONGUE DISEASE CONTROL AS AN IMPORTANT PART OF MONITORING THE HEALTH STATUS OF SHEEP

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ABSTRACT

Monitoring of bluetongue disease (BT) including entomological survey, clinical-biochemical examinations and serological diagnostics was carried out during 2011. Entomological survey consisted of capturing and classifying midges based on characteristic marks. Clinical examination of animals was carried out monthly by general inspection of animals. Biochemical examination of blood included haematological profile, enzymatic activity (ALP, AST, GGT, CPK), concentration of total bilirubin, total protein, albumin, creatinine, total immunoglobulin, urea, beta-hydroxybutyrate and minerals (Ca, P, Fe, Cu, Zn). Monthly serological examination for the detection of anti-BTV antibodies took place at the State Veterinary Institute in Zvolen using ELISA method. Altogether 9892 midges were caught. Complex *C. obsoletus* was present in 53.21 % (5264), complex *C. pulicaris* in 4.77 % (472) and complex *C. nubeculosus* was detected in 0.06 % (6) midges. Other *Culicoides* spp. were present in 41.96 % (4151). The biggest number of *Culicoides midges* was trapped in July and August. Such findings of clinical and biochemical examinations are not typical for bluetongue disease but indicate other diseases. Serological diagnostics showed absence of anti-BTV antibodies.

Key words: bluetongue; monitoring; sheep; vector

INTRODUCTION

Bluetongue is an orbivirus disease of sheep and other domestic and wild ruminants transmitted by *Culicoides midges*. It was originally considered a disease of the African continent. First occurrence of the disease outside Africa was documented on the Island of Cypress. The main bluetongue disease outbreak occurred in Central and Western Europe in 2006. Spreading of bluetongue virus is limited to the areas with the presence of the vector, suitable climatic conditions for replication and transmission of the virus vector and the activity of adult vectors during transmission period (6). Transmission of BTV in animal population occurs almost exclusively through the bite of certain types of adult *Culicoides midges* (5). The aim of this study conducted in 2011 was to gain information about the incidence and epidemiological situation of bluetongue disease in farmed sheep.

MATERIAL AND METHODS

Bluetongue surveillance was carried out in 2011 on a sheep farm, and consisted of entomological investigation, clinically-biochemical examination of sheep and serological diagnosis. Insects were trapped in weekly intervals from April to November 2011 (insect trap JW1212). The complexes of the genus *Culicoides* are differentiated according to the wing patterning (1, 4). Clinical and laboratory examination of sheep was rcarried out in monthly intervals. The haematological parameters were determined by a hematology analyzer Vet ABC™. Total protein, albumin, creatinine, total immunoglobulin, glucose, triglyceride, β-hydroxybutyrate, activity of enzymes (AST, GGT, AF, CPK), urea and P were analysed by commercial diagnostic tests (RANDOX) using a spectrophotometric analyzer Alize (Lisabio, France). The level of total lipids was determined by spectrophotometric (SPECOL 211) commercial diagnostic test (Ecomed). The value of TBi was assayed by spectrophotometric method using SPECOL 211. Values of Ca, Fe, Cu, Zn were determined by atomic absorption spectrophotometry (AAS

AAnalyst 100-Perkin Elmer). Examination for the detection of antibodies against bluetongue disease (VP7 protein) took place at the State Veterinary Institute in Zvolen monthly, using ELISA method (ID VET ID Screen Bluetongue Competition Kit for detection of anti-VP7 antibodies by competitive ELISA).

RESULTS AND DISCUSSION

During the monitored period we caught totally 9892 *Culicoides midges*. The highest number of captured individuals was recorded in the *Culicoides obsoletus* complex (5264 units, 53.2 %). Of other BT virus-carrying *Culicoides complexes* we detected presence of 472 (4.77 %) *Culicoides pulicaris* complexes and 6 (0.06 %) belonging to *Culicoides nubeculosus*. Altogether 4151 midges (41.96 %) belonging to other *Culicoides species* were trapped. During the entomological surveillance carried out from August to December 2006 in Belgium, representatives of 16 different species of *Culicoides*, with 1959 individuals were caught (3). In contrast to our results they reported a higher percentage of *Culicoides nubeculosus* complex. Most *Culicoides midges* were captured during the summer months (July, August), which is related to climatic conditions suitable for life and development of the vector in this period. The number of trapped *Culicoides midges* were also affected by temperature and relative humidity. If the period of maximum activity is not known, the animals are tested at monthly intervals throughout the year (2). During the clinical monitoring of sheep, we have not observed clinical symptoms characteristic for bluetongue. Of other diseases that were documented in sheep during the period of observation, the most diagnosed disorders were chronic endo and ectoparasitosis, contagious footrot, soremouth, mastitis and nutritional imbalances. During BT monitoring particular attention should be paid to risk groups, i.e. newly purchased or introduced animals, especially from infected areas and animals on the pasture (2). With regard to energy metabolic profile decrease in triglyceride levels and increase in total lipids levels were recorded. During the monitored period, some parameters of hepatic profile tended to decrease (creatinine) or increase (total immunoglobulin and overal bilirubin). The mineral profile showed marginal hypozincaemia throughout the monitored period. Serological investigation of sheep failed to detect antibodies against any BT virus serotype. Virological examination of animals and the vector are necessary only in case of suspected infection (2).

ACKNOWLEDGEMENTS

The study was supported by the projects VEGA 1/0940/11 and AV4/2041/08.

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FOLIA VETERINARIA, 56, Supplementum II: 39–40, 2012

PROPERTIES OF *STREPTOCOCCUS GALLOLYTICUS* STRAINS, ISOLATED FROM BEAVERS

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ABSTRACT

Beavers as herbivores belong to order Rodentia, family Castoridae, genus Castor. *Castor fiber* is a species of beaver. In Slovakia, beavers can be seen in the west part boarding with Austria and the north-east boarding with Poland. There is only limited information about microbiota in beavers. *Streptococcus gallolyticus* is the species of *Firmicutes*, belonging to the genus *Streptococcus* which is often found in various animals. In co-operation with Polish colleagues, free-living beavers were caught in the north-east part of Poland. Faeces, colon (n = 12), caecum (n = 6) were sampled from 12 beavers, males and females, 4–5 years old, complying with all ethic rules for animal handling. The mean counts in faeces reached 6.87 ± 2.62 $\log_{10}$ CFU.g^{-1}, in caecum 3.21 ± 1.79 CFU.g^{-1} and in colon 5.00 ± 2.23 CFU.g^{-1}. Fifteen colonies (6 caecal, 4 from colon, 5 faecal) allocated to the species *Str. gallolyticus* by Maldi-TOF mass spectrometry. The strains were sensitive to antibiotics with inhibitory zones from 22 to 37 mm. They showed bacteriocin activity; the growth of indicator strain EA5 strain was inhibited by all *Str. gallolyticus* strains (inhibitory zones from 5 to 15 mm). Eight of the 15 tested strains showed inhibitory activity against SG6Hc1 strain (5 mm). Fourteen strains showed inhibitory activity against the strain SGTr1 (8–12 mm).

Up to now, nobody has studied neither bacteriocin activity nor bacteriocin activity of *Str. gallolyticus* in beavers. Therefore our results, although preliminary, are an interesting contribution to basic and general microbiology.

Key words: antibiotic; bacteriocin; beaver; *Streptococcus gallolyticus*; sensitivity

INTRODUCTION

Beavers as herbivores belong to order Rodentia, family Castoridae, genus Castor. *Castor fiber* is a species of beaver which was once widespread in Eurasia. Beaver ponds often had a beneficial effect on trout and salmon populations. In Slovakia, beavers can be seen in the west part boarding with Austria where they were reintroduced continually from the seventieth. But they can also be seen in the north-east part boarding with Poland from where they had migrated. There is only limited information about microbiota in beavers. *Streptococcus gallolyticus* is the species of *Firmicutes,* belonging to the genus *Streptococcus* which is often found as a normal member of the gut microflora in various animals (1). In co-operation with our colleagues from Poland, free-living beavers were caught in the north-east part of Poland, Województwo (Province) Podlaskie Gmina-Wizajny (GPS: 22° 52 E: 54° 22 N). We studied the properties of *Str. gallolyticus* strains isolated from beavers to extend the basic knowledge of microbiota in these animals.

MATERIAL AND METHODS

Faeces, colon (from each of 12 beavers) and caecum (n = 6) were sampled from 12 beavers of both sexes, 4–5 years)old, complying with all ethic rules for animal handling. The samples were transported to our laboratory by Delivery service. They were processed by the standard microbiological method (ISO); 1 g of sample was diluted in Ringer solution (Merck, Germany) and the appropriate dilutions were spread onto M17 agar (Oxoid, UK) enriched with maize starch and cultivated at 37 °C for 48 h. The counts of colonies were expressed as $\log_{10}$ CFU.g^{-1} ± standard deviation (SD).

Representative colonies were randomly picked up, checked for the purity prior their identification by a MALDI BioTyper TM system (Bruker Daltonics, USA) based on protein "fingerprints" measured by MALDI-TOF mass spectrometry. Lysates of bacterial cells were prepared according to producer's instructions (Bruker Daltonics-2).

Sensitivity/resistance of the identified strains to antibiotics was tested with the aim to examine it in wild-living animals. We applied the method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST-3) using Brain Heart agar (BHA, Oxoid) supplemented with defibrinated sheep blood. The following antibiotic disks were included: penicillin, vancomycine, tetracycline, chloramphenicol, cefepime, cefotaxine (30 μg) and ampicillin (10 μg, Lach-Ner, Neratovice, Czech Republic), implemented as seen on the web source (www.tgw1916.net/Streptococcus/gallolyticus.html). The inhibitory zones were reported in mm.

Bacteriocin activity of the isolated strains was tested by the qualitative method (4) using BHA; inhibotory zones were expressed in mm. Tthe isolates themselves (11) were used as indicator bacteria – cross-activity: *Streptococcus gallolyticus* SG2Tr, SG21, SG22, SG42, SG2Tr1, SG2Tr4, SG2Tr3, SG2Tr2, SG61. SG11, SG1Hc2, SG2Hc2, SG2Hc2, SG6Hc1) and *Enterococcus avium* EA5 (the principal isolate from piglets faeces).

RESULTS AND DISCUSSION

The mean colony counts in the faeces reached 6.87 ± 2.62 $\log_{10}$ CFU.g^{-1} in faeces 3.21 ± 1.79 $\log_{10}$ CFU.g^{-1} in *caecum* and 5.00 ± 2.23 $\log_{10}$ CFU.g^{-1} in colon. Fifteen representative colonies (6 caecal, 4 from colon, 5 faecal) were allocated to the species *Str. gallolyticus* by the identification in association with their phenotype (1). This species was previously identified mostly from avian samples and it was genotyped as *Str. bovis* (5). *Str. castoreus* as a species novum was isolated from beaver by L a w s o n *et al.* (6).

The strains were sensitive to antibiotics with inhibitory zones from 22 to 37 mm. Penicillin sensitivity is used as one of phenotypical parameters of *Streptococcus gallolyticus* species (1). Surprisingly, the strains were also sensitive to tetracycline and vancomycine, to which this species has been often resistant (1). However, the sensitivity of our isolates indicated their real free-living origin influenced by nothing. K i m p e *et al.* (7) reported resistance of *Str. gallolyticus* from pigeon or human to lincosamides and macrolides; the resistance genes could be required.

The *Str. gallolyticus* strains showed bacteriocin activity. The growth of EA5 strain was inhibited by bacteriocin activity of all *Str. gallolyticus* strains (the size of inhibitory zones from 5 to 15 mm). Eight from the 15 tested strains showed inhibitory activity against SG6Hc1 strain (5 mm), 14 strains showed inhibitory activity against the strain SGTr1 (8–12 mm). Up to now, nobody has studied neither bacteriocin activity nor bacteriocin activity of *Str. Gallolyticus* in isolates from beavers.

Although preliminary these results contribute to the basic and general microbiology. Additional testing is in process.

ACKNOWLEDGEMENTS

The study was supported by the project No. 2/0002/11 of the Slovak Scientific Agency VEGA and the project No. N311 510 139 of the Ministry of Science and Higher Education in Poland.

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FOLIA VETERINARIA, 56, Supplementum II: 41–42, 2012

EVALUATION OF PROTEIN AND AMINO ACID QUALITY IN PIG NUTRITION

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ABSTRACT

Amino acid composition of proteins, their digestibility and availability, determines protein quality and feeding value. At present, the ileal digestibility is used to estimate the amino acid availability in pig feed ingredients. Surgical preparation of experimental animals for collection of ileal digesta is necessary and, therefore, we gradually established and developed the following types of the digestive tract cannulation: T-cannula; re-entrant cannula; post-valve T-caecum cannula. General care and management practices for cannulated pigs were also optimized. The values for ileal digestibility were calculated by deducting the total ileal outflow of amino acids from dietary amino acids intake. The resulting values may be expressed as apparent (if digestibility is determined by the content of all amino acids present in the ileal digesta) or true – standardized ileal digestibility (it is necessary to subtract the endogenous amino acid fraction from the total amino acids in ileal digesta).

Key words: amino acid; apparent ileal digestibility; cannulated pigs; protein; standardized ileal digestibility; true ileal digestibility

INTRODUCTION

In the past, the diet formulation was based on crude protein content of the components which gave a reasonable knowledge about the amount of protein in the diet, but it lacked any information about protein quality. Amino acid (AA) composition of proteins, their digestibility and availability, determines protein quality and feeding value. Generally, the digestibility of nutrients is determined indirectly as a difference between the amount of the nutrient ingested and that excreted in the faeces. This is the faecal digestibility which could normally be found in tables of feed composition and is referred to only as "digestibility". This method is inappropriate for the determination of AA digestibility because AA are absorbed almost exclusively in the small intestine (5). Digestibility of AA is therefore detected at the end of the small intestine (the terminal ileum) and in this case we speak about ileal digestibility, the synonym being pre-caecal digestibility (2). Determination of ileal digestibility is much more difficult and laborious than faecal digestibility since it requires a surgical preparation of experimental animals.

The aim of this study was to implement and optimize method of determining ileal digestibility in our laboratory.

MATERIAL AND METHODS

During the last 6 years, a total of 95 pigs with body weight ranging from 9.5 to 60.0 kg underwent surgery in our laboratory. Gradually, we have established and developed the following types of the digestive tract cannulation, which can be used to determine ileal digestibility:

T-cannula: inserted in the terminal part of the intestine and routed through the abdominal wall to the surface of the body, usually on the left side, back of the costal arch.

Re-entrant cannula: the terminal part of ileum is interupted, the cannulas are inserted in two incurred sections of the intestine. Intestinal digesta pass outside the body of an animal through a created bridge.

Post-valve T-caecum cannula: the cannula is inserted into the caecum in the area of valve between ileum and caecum and routed through the abdominal wall to the surface of the body on the left side of the costal arch.

RESULTS AND DISCUSSION

All cannulated pigs recovered quickly after the surgery and after 10–14 days their consumption of feed returned to normal. No clinical problems related to the surgery were observed. General care and management practices for cannulated pigs were also optimized. Values for ileal digestibility are calculated by deducting the total ileal outflow of amino acids from dietary amino acids intake and may be expressed as apparent (AID), if digestibility is determined by the content of all amino acids present in the ileal digesta, or true (TID) or standardized (SID) ileal digestibility, if it is necessary to subtract the endogenous amino acid fraction from total amino acids in ileal digesta. These terms are used to specify how ileal endogenous AA loses are reflected in digestibility values (1). Ileal endogenous AA losses may be separated to basal losses, which are not influenced by feed ingredient composition, and specific losses, which are induced by feed ingredient characteristics such as levels and types of fibre and antinutritional factors (3). The AID for a given AA is calculated by subtracting the total ileal outflow of that AA from the quantity ingested by the pig. Values for SID are calculated as values for AID except that the basal endogenous ileal AA losses are subtracted from the ileal outflow. Values for TID represent the proportion of dietary AA that disappear from the digestive tract prior to the distal ileum, and they do not include ileal endogenous AA loses (4).

The ileal analysis method should be the method of choice for determining amino acid digestibility. This method is very sensitive for detecting differences in amino acid digestibility, as these result from processing conditions or from inherent differences between samples of the same feedstuff. Data on true ileal digestibility have their main application in preparing compound feed.

At present, the described method of determining ileal digestibility is routinely used in our laboratory.

ACKNOWLEDGEMENTS

This work was supported by the Agency of the Slovak Ministry of Education for the Structural Funds of the EU under the project ITMS: NFP26220220104.

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FOLIA VETERINARIA, 56, Supplementum II: 43–45, 2012

INFLUENCE OF SERVING ELECTROLYZED WATER ON SELECTED BLOOD PARAMETERS OF CALVES

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ABSTRACT

Two almost identical groups of calves, each comprising ten animals, were investigated in the on-going experiment. One group was experimental and was given a solution of electrolyzed water and the other was control. Electrolyzed water was produced in a patent-protected Envirolyte facility. Stabile electrochemically activated water solution with increased disinfectant effect was produced by an anode fraction. This solution was added to water for drinking. Blood samples were taken from the monitored calves regularly and selected blood parameters were evaluated. No significant differences were found between the experimental and control group of calves. Serving electrolytically treated water did not have any negative impact on the health of calves. The average values were in the range of reference values given by Reece (2), Sova *et al.* (3), Ulrich von Bock und Polach (4) and Vrzgula *et al.* (5) and corresponded to generally relevant physiological processes in young animals.

Key words: blood parameters; calves; electrolyzed water; Envirolyte facility

INTRODUCTION

Electrolyzed water is produced in a patent-protected Envirolyte facility (used at Cooperative Farm Krásná Hora nad Vltavou a.s., to clean dairy rooms and milking houses). Only water and kitchen salt are needed to produce it. A stable electrochemically activated water solution with increased disinfectant effect is produced by an anode fraction. Afterwards the solution is called anolyte. After decomposition and reoccurrence of active components of the anolyte, only water is the residual substance. The production of anolyte is automatic, anolyte is collected in a plastic container and its amount is regulated automatically according to the usage needs. The automatic checking of the specific production conditions the Envirolyte facility is equipped with enables to produce anolyte of neutral pH value. The neutral anolyte is very effective against bacteria, moulds, viruses and algae and it is usually used to clean and treat water in swimming pools, treat potable water or other sources of water. Contrary to the traditional disinfecting agents, the main advantage is that it is safe to people, animals and environment. The anolyte is an effective disinfectant of potable water (Test Report No. 618/2006 ZÚ Pardubice, No. 2549/2005 Chemila Hodonín) in accordance with the Amendment No. 4 to Decree No. 409/2005 Coll., Hygiene requirements for products coming into direct contact with water and for water treatment. Electrolyzed water was also applied in food industry (4).

The aim of the study was to determine the effect of electrolyzed water used for feeding the calves on their blood parameters and to verify its safety to health.

MATERIALS AND METHODS

Two almost identical groups of calves of the same age range, same breed affiliation (Red Spotted breed) were investigated in the on-going experiment on Cooperative Farm Krásná Hora nad Vltavou a.s. Each group consisted of ten calves, one group was experimental (anolyte added to water) and one served as a control (water without anolyte). Calves were housed individually in separate boxes in a large-capacity calf shed. The minimum age of calves

Table 1. Selected blood parameters (average values)

a)

Blood parameters	Hb [g.l^{-1}]	Hk [l.l^{-1}]	Ery [T.l^{-1}]	Leuko [G.l^{-1}]	Glyk [mmol.l^{-1}]	Urea [mmol.l^{-1}]	Ca [mmol.l^{-1}]	AF [mikrokat.l^{-1}]
Exp. group	127.77	0.32	6.66	6.95	3.93	2.82	2.21	3.34
Control	134.98	0.34	6.82	7.12	5.16	3.17	2.26	3.54

b)

Blood parameters	Chol. [mmol.l^{-1}]	CB [g.l^{-1}]	Zn [mg.l^{-1}]	Cu [mg.l^{-1}]	Trigl [mmol.l^{-1}]	P [mmol.l^{-1}]	Mg [mmol.l^{-1}]	GMT [mikrokat.l^{-1}]
Exp. group	2.48	71.67	1.16	0.63	0.27	2.49	0.83	0.58
Control	2.42	72.25	1.25	0.69	0.31	2.48	0.87	1.44

was 14 days at the beginning of the experiment. Water for calves was distributed manually in buckets of volume 4 litres. The experimental group of calves was supplied anolyte added to potable water from the local water main (1 litre of anolyte + 3 litres of potable water, mixed in a bucket – solution with 25 vol. % of anolyte). The calves were supplied this water once a day (twice a day in summer) from day 14 of age until they were relocated (approximately two months). Anolyte was stored in the place of water solution preparation in a closed tank of volume 50 l equipped with an outlet valve. Only fresh anolyte was used to ensure its effectiveness. Blood samples were taken from the jugular vein of calves in regular intervals using a hypodermic needle and were transferred to heparinised glass tubes. Each tube was provided with a serial number and a number of the ear tag of the respective.. In total, 140 samples were taken in 7 rounds and were analysed in a laboratory of the Department of Veterinary Disciplines and Quality Products, Faculty of Agriculture, University of South Bohemia in České Budějovice. A modern haematological and biochemical analyser by DIALAB s.r.o. Praha was used to analyse the samples for the values of haematocrit, erythrocytes, leukocytes, glucose, urea, AF GMT, cholesterol and total level of proteins and triglycerides using standard commercial kits. For other measurements the blood elements were separated in a separator. Blood plasma was examined for zinc, copper, phosphor, calcium and magnesium using absorption spectrometry.

The results of the analyses were recorded in a laboratory diary and then converted to a digital form and entered into a table using Microsoft Office Excel 2003. Sample number, number of the ear tag of an animal, date of sample taking and the farm are given in the table.

RESULTS AND DISCUSSION

Results of selected blood parameters determined in calves from both groups are presented in Table 1 a, b.

The values in the table are the average values of the monitored blood parameters of all 7 rounds. No significant differences were found between the experimental and control group of calves. Supplementation of anolyte to water had no negative impact on the health of calves. No contraindications were found throughout the experiment. The average values were in the range of reference values given by a number of authors (2, 3, 4, 5) and corresponded to generally relevant physiological processes in young animals. Haematocrit values as well as the values of erythrocytes and partially those of leucocytes in the monitored groups were at the low limit of the recommended values. This might have been caused by inferior nutrition in terms of proteins and by higher body exhaustion and stress of calves during breeding.

CONCLUSIONS

The results allowed us to conclude that the blood parameters tested were within the usual ranges in both monitored groups. There were some exceptions when the basic principles of appropriate nutrition, especially provision of sufficient water and supplying feed with lower protein content. However, such conditions were noticed in the individual groups only once. It is thus obvious that calves can successfully cope with various impacts of technologies of breeding and environmental conditions provided that relevant animal hygiene and breeding principles are observed.

ACKNOWLEDGEMENT

The study was supported by the Project NAZV No. QI111B107.

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FOLIA VETERINARIA, 56, Supplementum II: 46–47, 2012

ESSENTIAL OIL ENRICHED WITH SODIUM SELENITE IN BROILER DIET

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ABSTRACT

The study evaluated the effect of administration of 0.05 % *Thymus vulgaris* essential oil (EO), enriched with sodium selenite at a dose of 0.4 mg.kg^{-1}, on antioxidant status and gastrointestinal bacteria in fattening chickens. Activity of selenoenzymes glutathione peroxidase as well as thioredoxin reductase was significantly increased in chicken liver in groups receiving sodium selenite alone as well as together with EO. Malone dialdehyde (MDA) in duodenal mucosa as a product of lipid oxidation was significantly lower in group receiving EO enriched with sodium selenite. We observed significant decrease in bacteria (coagulase-negative staphylococci, lactic acid bacteria, *Enterobacteriacae*) in ceacum as well as in feaces (*Enterobacteriacae*) in groups supplemented with EO. This confirmed antioxidant and antimicrobial properties of EO as well as the well known strong antioxidant properties of sodium selenite. Our results showed that immunity can be supported by administration of EO as immunoglobulin A level in duodenal mucosa was significantly higher in groups with EO supplementation.

Key words: antioxidant status; broilers; essential oil; gastrointestinal microbiota

INTRODUCTION

Recent trends and development in the area of animal nutrition have been characterized by an increasing interest in the potential impact of plants, herbs and spices on the antioxidant status and immune system of animals. Approximately up to 80 % of domestic animals have been fed synthetic compounds due to their therapeutic or growth performance properties (3). Possible antibiotic residues and disease resistance are the reasons why antibiotics were banned as feed additives. This led to investigations of alternative feed additives in animal production. Essential oils (EO) are one of the alternatives. Studies about their pharmacological effects and health claims are inadequate because many of them dealt with commercial products (1). Selenium is a structural component of a several specific Se-proteins as well as an integral part of the antioxidant system.

The aim of our study was to obtain more information regarding the effects of sodium selenite enriched EO on antioxidant status and selected bacteria in caecum and faeces of broilers in the animal model experiment.

MATERIAL AND METHODS

Forty broiler chickens (Ross 308 hybrid) were randomly divided on the day of hatching to 4 groups (10 chickens in each). They were fed for 5 weeks with experimental diets. Group 1 (control, C) was given commercial diet (CD), group 2 (E1) was fed the same CD enriched with 0.05 % *Thymus vulgaris* essential oil (EO), group 3 (E2) received CD supplemented with 0.4 mg.kg^{-1} of sodium selenite and group 4 (E3) was fed CD enriched with both, EO and sodium selenite. At the end of the experiment the birds were sacrificed, samples of liver, kidney and duodenal mucosa tissues were collected and blood for analyses was sampled into heparinised tubes. We determined total antioxidant status (TAS) in plasma, activity of glutathione peroxidase (GPx) in blood and tissues, thioredoxin reductase (TRxR) activity and malone dialdehyde (MDA) concentration in the tissues. Faecal samples (mixture, n = 5 per group) and caecal samples were collected to evaluate selected bacteria.

The data obtained were analysed by one-way ANOVA with the *post hoc* Tukey multiple comparison test. The data were presented as mean values ± standard deviation.

RESULTS AND DISCUSSION

Youdim and Deans (7) reported that thyme oil supplementation of the diet acted as effective free radicals scavengers and influenced the antioxidant defence systems. The high antioxidant activity of thymol is due to the presence of phenolic OH groups which serve as hydrogen donors to the peroxy radicals produced during the first step of lipid oxidation and thus break peroxid formation. MDA is a major secondary product of lipid oxidation and a good marker of oxidative stress and such as it was investigated in our experiment. Our results confirmed findings of authors cited above because MDA was significantly reduced in duodenal mucosa of the group supplemented with EO and selenium (C = 58.38 ± 10.23; E1 = 54.64 ± 7.23; E2 = 36.75 ± 12.63; E3 = 31.98 ± 15.06 nmol.g^{-1} protein).

Free radicals can have deleterious effects and can attempt to alter the antioxidant-pro-oxidant balance. Probably, antioxidant properties of thyme oil (6) are being utilised by the cells, thus sparing the intracellular antioxidant system. The antioxidant action of selenium is determined mainly by its role as an essential component of the active centre of selenoenzymes like GPx, TrxR and many other enzymes which are the part of antioxidant system (5). Our results confirmed this theory. We observed a significant increase in activity of both GPx and TrxR in the liver in group supplemented with selenium and with EO and selenium (GPx: C = 17.54 ± 4.44; E1 = 20.94 ± 1.56; E2 = 27.70 ± 3.20; E3 = 25.32 ± 2.03 U.g^{-1} protein, respectively; TrxR: C = 42.65 ± 9.23; E1 = 47.60 ± 9.82; E2 = 102.90 ± 20.76; E3 = 141.10 ± 26.78 U.g^{-1} protein).

The predominant helper cells in surface tissues are Th2 cells that secrete a mixture of cytokines which produce mainly IgA and IgE. Nickels (4) mentioned that peppermint oil maintains the structural integrity of immune cells due to its strong antioxidant action which protects cell membrane from free radicals oxidants, thereby resulting in an improved immune response. We can deduce that plasma cells as well as helper cells are protected from attack by free oxygen radicals by antioxidative properties of *T. vulgaris* EO which can explain a significantly higher IgA concentration in duodenal tissue of broilers from groups E1 and E3, receiving thyme oil in their diet (C = 0.63 ± 0.13; E1 = 0.90 ± 0.18; E2 = 0.75 ± 0.08; E3 = 0.98 ± 0.20 mg.g^{-1}).

Phenolic structures of EO have strong antibacterial properties. Thymol, a phenolic molecule, is the main component of thyme oil which can denature proteins in cell walls of bacteria and increase cell wall permeability. Disrupted permeability of the cell wall induces the release of intracellular fluid, which consequently kills bacteria (2). This is the theory which can explain an inhibitory effect of *T. vulgaris* EO in the concentration used in our experiment (caecum-coagulase-negative staphylococci: C = 4.97 ± 0.28; E1 = 5.49 ± 0.15, E2 = 4.02 ± 0.60; E3 = 4.23 ± 0.65 $\log_{10}$CFU.g^{-1}; lactic acid bacteria: C = 7.70 ± 0.28; E1 = 7.63 ± 0.35; E2 = 7.48 ± 0.40; E3 = 7.40 ± 0.33 $\log_{10}$CFU.g^{-1}; *Enterobacteriacae*: C = 5.57 ± 0.88; E1 = 6.23 ± 0.42; E2 = 3.76 ± 0.95; E3 = 3.42 ± 0.56 $\log_{10}$CFU.g^{-1}; faeaces *Enterobacteriacae*: C = 5.84 ± 1.43; E1 = 6.44 ± 0.75; E2 = 4.10 ± 1.48; E3 = 4.76 ± 1.24 $\log_{10}$CFU.g^{-1}).

Our experiment confirmed the antioxidant and antimicrobial properties of 0.05 % *Thymus vulgaris* EO as well as strong antioxidant properties of sodium selenite at a dose 0.4 mg.kg^{-1}.

ACKNOWLEDGEMENTS

The study was supported by the Slovak Scientific Agency VEGA projects 2/0010/10 and 2/0002/1 and the project No. 26220220152 supported by the Research & Development Operational Programme funded by the ERDF.

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FOLIA VETERINARIA, 56, Supplementum II: 48–49, 2012

NEW ENDOCRINE AND PHARMACOLOGICAL REGULATORS OF RABBIT REPRODUCTIVE FUNCTIONS

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ABSTRACT

We present review of original data concerning new extra- and intracellular regulators of rabbit ovarian functions. Effects of some recently discovered hormones (leptin, ghrelin), growth factors (IGF-I, EGF) and pharmacological regulators of some protein kinases (protein kinase A, MAP kinase, CDC2 kinase, tyrosine kinases) on the functions of ovarian cells (proliferation, apoptosis, secretory activity, expression of some protein kinases) and reproductive parameters (blood level of reproductive hormones, ovarian morphology, number of ovulations, embryo yield and quality, number and viability of offspring) and their possible interrelationships and practical application in rabbit breeding are reviewed.

Key words: hormone; ovarian; protein kinase; rabbit

INTRODUCTION

Progress in reproductive biology, assisted reproduction and animal production (including rabbits) is due to progress in understanding and application of the regulators of reproductive processes. The most known and potent regulators of reproduction are hormones, growth factors and mediators of their action – cyclic nucleotides, and protein kinases (8, 15). Rabbits have high reproductive potential, therefore they are widely used as a model in basic and applied reproductive biological studies. On the other hand, it is hard to imagine reproduction in large-scale rabbit farming without application of hormones. The best known inductors of rabbit ovarian follicle development and ovulation are analogues of follicle-stimulating and luteinizing hormones, pregnant mare serum gonadotropin (PMSG), human chorionic gonadotropin (hCG), as well as analogues of GnRH (2, 7, 8, 9, 13, 14.). Other hormones are less studied in this respect. The intracellular hormonal mechanisms were poorly studied, although their pharmacological and genomic regulators could be practically used for control of rabbit reproduction in addition or even instead of classical hormones (8, 15).

This review is a short summary of our recent findings concerning new regulators of rabbit ovarian cells (proliferation, apoptosis, secretory activity, expression of some protein kinases) and reproductive parameters (blood level of reproductive hormones, ovarian morphology, number of ovulations, embryo yield and quality, number and viability of offspring). Effects of some recently discovered hormones (leptin, ghrelin), growth factors (IGF-I, EGF) and pharmacological regulators of some protein kinases (protein kinase A, MAP kinase, CDC2 kinase, tyrosine kinases), are described.

MATERIALS AND METHODS

For both *in vivo* and *in vitro* experiments we used adult cycling female New Zealand rabbits that were kept in individual cages in the Animal Production Research Centre Nitra. For in-vitro experiments, we used cultured ovarian fragments, granulosa cells, zygotes and preimplantation embryos. For in-vivo experiments, we used female rabbits 4 months and 2 years of age. Markers of cell proliferation, apoptosis, embryonal development, release of peptide and steroid hormones, growth factors, protein kinases and transcription factors were evaluated by using microscopy, RIA, TUNEL, SDS PAGE-Western blotting and immunocytochemistry as it was described in the corresponding publications (1–14).

RESULTS AND DISCUSSION

We observed relationship between rabbit fertility and the concentration of leptin, IGF-I and steroid hormones in blood. Examination of effects of hormones leptin, ghrelin, growth factors IGF-I, EGF pharmacological preparations promoting accumulation of cAMP and protein kinase A (PKA) (3-isobutyl-methyl-xantin, IBMX, dibutyrhyl cAMP, cAMP) and pharmacological blockers of PKA, mitogen-activated protein kinases, MAPK and cyclin-dependent kinase, CDK (KT5820, PD98059 and olomoucine respectively) demonstrated that the basic ovarian functions (proliferation, apoptosis, sectetory activity, folliculogenesis, oogenesis and embryogenesis) are regulated not only by classical gonadotropins (1, 2, 7,12, 13, 14) but also by less known metabolic hormones leptin and ghrelin (1, 10, 11) and by growth factors IGF-I and EGF (1, 4, 5). Hormonal treatments caused changes in accumulation of some protein kinases (PKA, MAPK, CDK, tyrosine kinase). Changes in production or action of these protein kinases by pharmacological activators or inhibitors substantially altered ovarian functions (proliferation, apoptosis, secretory activity, protein kinase expression) and rabbit reproductive characteristics (blood level of reproductive hormones, ovarian folliculo- and oogenesis, number of ovulations, of produced embryos, their quality, number and viability of pups) (1, 3, 4, 6, 9, 12, 13, 14). Some treatments (ghrelín, IBMX, dbcAMP) were able to improve these rabbit reproductive characteristics both *in vivo* and *in vitro* (9, 11, 13, 14). Old females were less sensitive to administration of stimulators of reproduction then the young animals. The detailed description of our observations is presented in the corresponding publications (13).

These observations suggested, that rabbit ovarian functions could be regulated not only by well-known gonadotropins, but also by less known recently discovered metabolic hormones (leptin, ghrelin), growth factor (IGF-I) and intracellular mediators of their action (cyclic nucleotides and protein kinases). These molecules and their pharmacological regulators could be useful for characterization, prediction, regulation and improvement of ovarian and reproductive functions and for the treatment of reproductive disorders.

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FOLIA VETERINARIA, 56, Supplementum II: 50–52, 2012

POSSIBLE BRAIN OXIDATIVE DAMAGE OF THE RABBIT DUE TO EXPOSURE TO CARBAMATE INSECTICIDE

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ABSTRACT

Free radicals are generated in living organisms under physiological conditions by controlled stimulation and participate in regulation of many processes. Environmental pollutants can generate production of oxygen radicals. These radicals can influence and damage biomolecules and thereby induce oxidative stress. We investigated the effect of bendiocarb on the antioxidative enzyme system of brain of female rabbits. A significant reduction in the activity of superoxide dismutase (SOD) in the brain of females was observed on days 3 and 10 of the experiment. Catalase activity was increased in parallel. It is assumed that the SOD activity is inhibited by own production of H_2O_2 or its production by other metabolical pathways. Glutathione peroxidase activity was increased significantly on days 10 and 30. Activity of the auxiliary enzyme glutathione reductase was significantly decreased on day 3. Changes in specific activity of glutathione-S-transferase were very similar. Inhibition of the activity of certain enzymes could be ascribed to accumulation of reactive oxygen species (ROS). Oxidative damage to brain was confirmed by increased content of thiobarbituric acid reactive substances. The changes observed involve mechanism of ROS elimination and compensatory response of the tissue.

Key words: antioxidative enzymes; brain; carbamates; oxidative stress

INTRODUCTION

Bendiocarb is a carbamate insecticide effective against a wide range of pests and disease vectors. The toxicity of bendiocarb is primary related to the inhibition of acetylcholine esterase (5). According to toxicological studies, bendiocarb does not accumulate in the body. It undergoes rapid metabolic transformation and then it is rapidly eliminated by kidneys (5). Some intermediates can be toxic at certain conditions (4). It was confirmed that bendiocarb can generate production of reactive oxygen species (ROS). These radicals increase its primary toxic effect (12). The brain is the organ with the highest oxygen consumption. Its defence mechanisms against oxidative stress are insufficient (1). Superoxide dismutase (SOD) and ascorbic acid are responsible for the main antioxidative capacity of nerve tissues. Low activity of catalase and glutathione peroxidase was observed in the nervous tissue (6).

The aim of this study was to observe the effect of bendiocarb on the antioxidative enzyme system of brain of female rabbits.

MATERIAL AND METHODS

The experiment was carried out on clinically healthy female domestic rabbits (*Oryctolagus cuniculus domesticus*) obtained from an accredited animal farm (Nitra, SR). Experimental animals (6 in each group) were administered bendiocarb (96 % Bendiocarb, Bayer) *per os* at a dose of 5 $mg.kg^{-1}$ b.w. per day. Owing to the adverse side effects of bendiocarb after 10 days of the experiment, the dose mentioned was administered every 48 h. The animals were sacrificed on days 3, 10, 21 and 30 of the experiment. The study was carried out in agreement with the requirements of the institutional ethical authority.

The brain of the experimental animals was used to prepare 25 % (w/v) homogenates in 5 $mmol.l^{-1}$ TRIS-HCl buffer, pH 7.8. After centrifugation (105 000 g, 1 h, 4 °C) the total proteins were deter-

Table 1. Specific activities of antioxidative enzymes and TBARS levels in the brain of rabbits

Specific activity	SOD [U.mg^{-1}]	CAT [U.mg^{-1}]	GPxcum [U.mg^{-1}]	GPxH_2O_2 [U.mg^{-1}]	TBARS [A_{535}.mg^{-1}]
Control	**90** ± 1	**4.1** ± 0.7	**0.11** ± 0.01	**0.069** ± 0.008	**0.0175** ± 0.0007
Day 3	**4** ± 2*	**5.2** ± 0.9	**0.13** ± 0.01	**0.10** ± 0.02	**0.027** ± 0.002**
Day 10	**2.1** ± 0.9**	**9** ± 2*	**0.16** ± 0.02**	**0.085** ± 0.007*	**0.023** ± 0.005
Day 21	**7.4** ± 0.8	**5.5** ± 0.4*	**0.13** ± 0.02	**0.09** ± 0.03	**0.030** ± 0.008*
Day 30	**6** ± 2*	**3.7** ± 0.5	**0.142** ± 0.008**	**0.10** ± 0.01*	**0.016** ± 0.002

The values presented are means ± SD (n = 6).
Significance of differences determined by the Student *t*-test: * – $P < 0.05$; ** – $P < 0.01$

mined in supernatants by the method of Bradford (3). The determination of SOD was based on spectrophotometric measurement of the inhibition rate of cytochrome (c) reduction (550 nm). The activity of CAT was measured as a decrease in H_2O_2 in the reaction mixture at 240 nm. The glutathione peroxidase activity was determined by the kinetic measurements of the consumption of NADPH+H^+ for the reduction of glutathione produced at the removal of peroxides (340 nm). The TBARS, products of lipid peroxidation, produces coloured substances with thiobarbituric acid with absorption maximum at 535 nm. The specific activity of enzymes was expressed in U.mg^{-1} protein. The Student *t*-test was used for statistic assessment of results.

RESULTS AND DISCUSSION

Free radicals are generated in a living organism under physiological conditions by controlled stimulation and participate in regulation of proper course of many processes. The increased steady-state concentration of ROS constitutes the chemical basis of oxidative stress. The natural endogenous protection of the organism against the generation of ROS is the antioxidative enzyme system. Changes in their activities involve specific cells responding to contaminants exposure.

A significant reduction in the activity of SOD in the brain of female rabbits was observed on days 3 and 10 of our experiment (Table 1). Catalase activity was increased in parallel. SOD provides primary antioxidative protection of cells against ROS. The extent of the decrease in SOD activity depends on the degree of oxidative stress (10). The inhibition of the SOD activity may result from increased production of superoxide anion radical (12). Inhibition of SOD causes accumulation of superoxide anion radicals and leads to damage to the mitochondrial membrane and to apoptosis (8). Chronic inhibition of SOD induces degeneration of spinal neurons (11). Lower activity of SOD was found in rat brain in relation to age. Superoxide production in the brain increases with age together with decreased SOS activity. It was shown that SOD is inhibited by H_2O_2 (7). Inactivation of SOD is caused by the reduction of copper ions in the active centre of the enzyme.

Catalase and glutathione peroxidases are the predominant enzymes regulating and controlling intracellular H_2O_2 concentrations. Catalase is especially effective at high H_2O_2 concentration and glutathione peroxidase is capable to utilize hydroperoxides and to metabolize H_2O_2 at its low concentration (9). Because H_2O_2 is continuously produced by several enzymes, it can be assumed that the SOD activity was inhibited by own H_2O_2 production or by H_2O_2 production by other metabolical pathways. A correlation between decreased SOD activity and increased lipid peroxidation in different parts of the rat brain was shown (2). Oxidative damage to rabbit brain was confirmed by increased content of thiobarbituric acid reactive substances found in our experiment.

The alterations in the activities of antioxidative enzymes and increased TBARS values showed that the brain of rabbits was exposed to the action of free radicals. Toxicity and possible oxidative damage to brain depends on concentration of the pesticide and length of exposure.

ACKNOWLEDGEMENTS

The study was supported by the project KEGA 020UVLF-4/2012 of the Institute of Medical Chemistry of UVMP in Košice.

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FOLIA VETERINARIA, 56, Supplementum II: 53–55, 2012

GROWTH CHARACTERISTICS OF INDIGENOUS VAGINAL MICROORGANISMS OF HEIFERS AND COWS

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ABSTRACT

The aim of this study was to compare the growth characteristics of indigenous vaginal microorganisms of heifers and cows measured by three different methods. Absorbance values (A_{540nm}) were measured off-line by an UV-Vis spectrophotometer. Absorbance values (A_{450nm}, A_{540nm}, A_{630nm}, A_{950nm}) were measured also by means of the microplate reader. When expressed in absolute values, higher levels of absorbance were measured at lower wavelengths. Growth decrease occurred after 16 hours of cultivation and was caused by pH decrease and consumption of substrate. The amount of acids produced by vaginal microorganisms was determined indirectly by measuring pH of the culture supernatant with pH meter. An inverse relationship between turbidity and pH was confirmed.

Key words: absorbance; curve; growth; microorganism; pH; spectrophotometry; turbidity

INTRODUCTION

Lactobacilli have a number of properties which render them highly suitable for probiotic therapeutics that are of pharmaceutical interest. They play a significant role in harmonization and maintaining equilibrium in different microenvironments (4). The use of probiotic products does not cause adverse effects and could prevent the undesirable consequences of antibiotics (7). Lactobacilli are the productive microorganisms most frequently used for the preparation of probiotics. *Lactobacillus* spp., a part of microflora of the genital tract, can help to maintain its balance and stimulate the immune system (1). The knowledge of both technological and probiotic characteristics is important when selecting strains for probiotic purposes (5).

The aim of this study was to compare the growth characteristics of indigenous vaginal microorganisms obtained from heifers and cows, measured by three different methods.

MATERIALS AND METHODS

Strains used in the experiment were isolated from the vagina of healthy heifers and cows from four localities in Slovakia. The vulvar area was washed with povidone-iodine and water and a disposable speculum was inserted into the vagina to swab the posterior area. Vaginal swabs were placed into Amies agar gel with charcoal (DispoLab, Copan Italia, Brescia, Italy). The samples were diluted with saline solution (Imuna Pharm a. s., Šarišské Michaľany, Slovak Republic). The strains were grown in de Man, Rogosa and Sharpe agar (MRS; Carl Roth GmbH+Co. KG, Karlsruhe, Germany) for 48 h at 37 °C under anaerobic conditions (Gas Pak Plus, BBL Microbiology systems, Cockeysville, USA). Morphological characteristics were evaluated according to selection criteria. Single colonies were picked into the tubes with MRS broth (MRS; Carl Roth GmbH+Co. KG, Karlsruhe, Germany) and cultivated for 20 hours at 37 °C under aerobic conditions.

Turbidity measurement by spectrophotometer

Ten µl of the sample cultivated for 20 hours at 37 °C under aerobic conditions in the MRS broth (Carl Roth GmbH+Co. KG) was transferred to 50 ml of the MRS broth (Carl Roth GmbH+Co. KG) and vortexed. The samples were then continuously shaken on a roller mixer (Stuart® SRT9D, Bibby Scientific Ltd., Staffordshire,

UK) at 37 °C, the shaking interrupted by sampling every two hours. Turbidity was measured in the Plastibrand™ standard disposable cuvettes (PS, semi-micro; Brand GmbH+Co. KG, Wertheim, Germany) against distilled water by a UV-Vis spectrophotometer Cintra 202 (GBC Scientific Equipment, Hampshire,USA) every 2 hours, 16 times, at A540 nm.

Turbidity measurement by microplate reader

Measurements were made using a Synergy™ 4 Multi-Mode Microplate Reader (BioTek Instruments Inc.,Vermont, USA) and microtitration 96-well plates (Greiner ELISA 8 Well Strips, 350 µl, Flat Bottom, Cruinn Diagnostics Ltd., Dublin, Ireland). We used as a sample 290 µl of the MRS broth (Carl Roth GmbH+Co. KG) with 10 µl of the MRS broth (Carl Roth GmbH+Co. KG) with multiplied strain, cultivated for 20 hours at 37 °C under aerobic conditions. Distilled water was used as a blank. MRS broth (Carl Roth GmbH+Co. KG) was used as a control. Measurements were made every 15 min during two 23-hour cycles at different wavelengths: 450 nm, 540 nm, 630 nm and 950 nm (near-infrared spectrum). Altogether 1 127 measurements of 20 strains were made.

Acid production

The amount of acids produced by indigenous vaginal microorganisms was indirectly determined by measuring the pH of the culture supernatant with pH meter (pH 340; WTW, Weilheim, Germany). Measurements were made every two hours during three 16-hour cycles.

RESULTS AND DISCUSSION

When designing the probiotic products, two of the essential characteristics are studied: growth conditions and technological performance of the selected microorganisms (3). It is of primary interest to obtain the highest biomass and viability of the selected microorganisms. To study the optimum growth conditions and the characteristics of these conditions for such probiotic micro-organisms (with potential applications from the technological point of view), it is necessary to determine growth curves (2). During off-line spectrophotometric measurements by UV-Vis spectrophotometer Cintra 202 the growth was considerably influenced by interruptions every 2 hours, caused by preparation of samples for the measurements in cuvettes. Moreover, these measurements were time and staff demanding.

The Synergy™ 4 Multi-Mode Microplate Reader is equipped with an incorporated mounted shaking and thermostatic unit, so the measurements can be made more frequently and in various spectrums without interruptions. Measurements were made every 15 minutes during the 23-hour period. The absorbance was measured at 450 nm, 540 nm, 630 nm and 950 nm. When comparing the spectrum suitability we observed that the shape of the curves at 450 nm, 540 nm and 630 nm was to a large extent equal to the absolute shift in the measured turbidity values. Higher turbidity values were measured at lower wavelengths. The shape of the curves measured at 950 nm was similar, but in absolute absorbance values was displayed the subtraction of the absorbance of the plastic cuvette and water from the blank sample. Water and plastic material are highly absorbable in the near-infrared spectrum and for this reason the curves' shapes acquired also negative values. Absorbance values in individual measurements showed minimal variance. Microbial contamination in one of the quadruplet wells (errors in pipetting samples, etc.) was demonstrated in 15–20 % of the repeated measurements of the individual strains. Contamination was obvious from the shape of the growth curve. Curves which displayed explicit marks of contamination were not evaluated. After 12-hour measurements some curves have shown slightly sawtooth shape, which is probably caused by the aggregation of bacteria into larger clusters. Each strain of micro organism must be evaluated individually. Even though some of the used strains were classified as the same species because they showed the same phenotypic characteristics, they presented differences in their growth curves and biochemical properties. These results agree dwith the conclusions of different researchers, who stated that each bacterial strain shows different growth characteristics (6, 2).

Lactic acid, which is the characteristic fermentative product of lactobacilli, can reduce pH to the level at which the growth of pathogenic flora is inhibited. Low pH leads to decrease in the activity of metabolic enzymes and its denaturation. Only few bacterial strains are able to grow in pH lower than is the threshold for lactobacilli. However, pH measurement is not a reliable indicator of growth or growth inhibition of bacteria. pH can only give a partial idea of the organic acids' concentration in the medium, because a large proportion of these acids remains undissociated (4). Inverse relationship between turbidity and pH was confirmed.

In conclusion, measurement of the growth curves by microplate reader completes very effectively and exactly the evaluation of strains' growthin a liquid selective media. This method can be used as one of the preliminary steps in the selection of probiotic micro-organisms.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Education of the Slovak Republic VEGA (Project No. 1/0498/12).

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FOLIA VETERINARIA, 56, Supplementum II: 56–57, 2012

ANTIOXIDATIVE PROTECTION OF SQUALENE VACCINATION ADJUVANTS

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ABSTRACT

The study investigated photo-oxidative injury of squalene (SQ) and the possibilities of its prevention by antioxidants (α-tocopherol, β-carotene or rutin) in *in vitro* systems exposed to UV-radiation radiation at doses of 16, 48, 96, 144 and 192 kG. We measured peroxide number in squalene oil as well as in oil emulsions. Squalene was very sensitive to UV radiation, already the lowest dose (16 kG) which caused significant increase in its peroxide number ($P \leq 0,01$). α-tocopherol (αT) acted as a pro-oxidant, it had a weak antioxidant activity only when added to squalene after irradiation. Squalene oxidation was reduced most efficiently by β-carotene. Lower concentration of β-carotene (0.2 %) appeared to be more suitable at lower radiation doses while 0.5 % concentration was needed to reduce oxidation damage to squalene at higher doses. The antioxidant properties of rutin could not manifest itself in this system. In emulsion form, squalene was even more sensitive to UV-radiation.

INTRODUCTION

Squalene (SQ) is a polyprenyl compound naturally occurring in the animal and plant world (1). SQ in emulsion form is used for preparation of oil-in-water adjuvants to potentiate the effectiveness of inactivated parenteral vaccines.

Squalene, a poly-nonsaturated lipoid compound, is susceptible to oxidation changes. The primary products of its oxidation are SQ-monohydroxyperoxides. Such changes are undesirable in pharmaceutical products as they may result in health problems or disrupt emulsion stability (11). Recently publications dealt with SQ adjuvant emulsions with added α-tocopherol (αT) (2, 10).

Since SQ itself is an antioxidant (AO), we wondered whether the addition of other AOs (α-tocopherol – αT, β-carotene – βC, rutin – R) could affect photo-oxidation induced by various doses of UV radiation in the *in vitro* systems.

MATERIALS AND METHODS

Photo-oxidative injury of SQ and its potential prevention by AOs (αT, βC or R) were investigated in the *in vitro* systems exposed to various doses (16, 48, 96, 144 and 192 kG) of UV-radiation, induced by 30 W germicidal lamp. SQ samples were irradiated in oil and also in emulsion form, both in duplicate. The oxidation degree was measured by determination of the peroxide number (PN) of SQ in oil and also in emulsion form; twice for all samples.

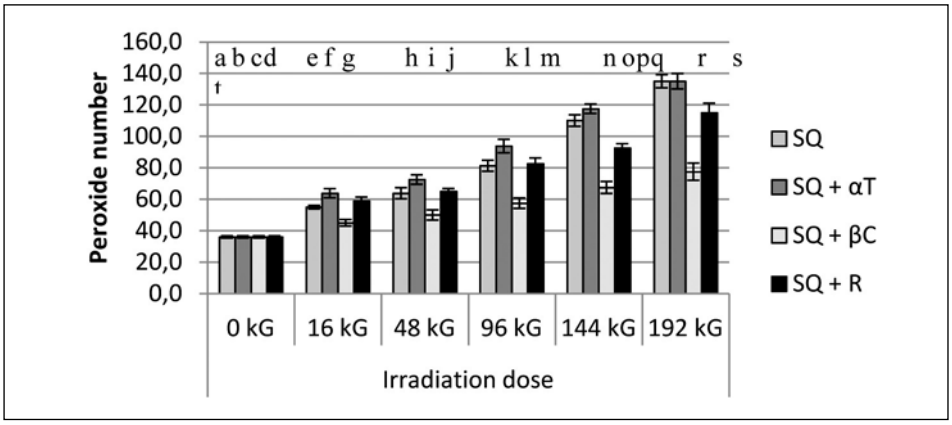
All samples with AOs were irradiated with the same doses as pure SQ. The dependence of the protection effect on concentration (0.05 %; 0.2 %; 0.5 %) was investigated in SQ-βC and SQ-R mixtures. The protective effect of αT was observed in SQ-αT (0.5 %) mixture because this is the concentration used in adjuvants. Considering the unexpected increase in PN in this system we decided to add the αT after UV-irradiation of SQ at a dose of 96 kG. The antioxidant effectiveness was then observed in relation to αT concentration (from 0.05 % to 0.5 %). Samples were tested after 10, 30 and 60 min after αT addition.

Statistical evaluation of results was carried out by means of paired Student's *t*-test.

Table 1. Peroxide number of SQ and SQ-AO emulsions after UV-irradiation

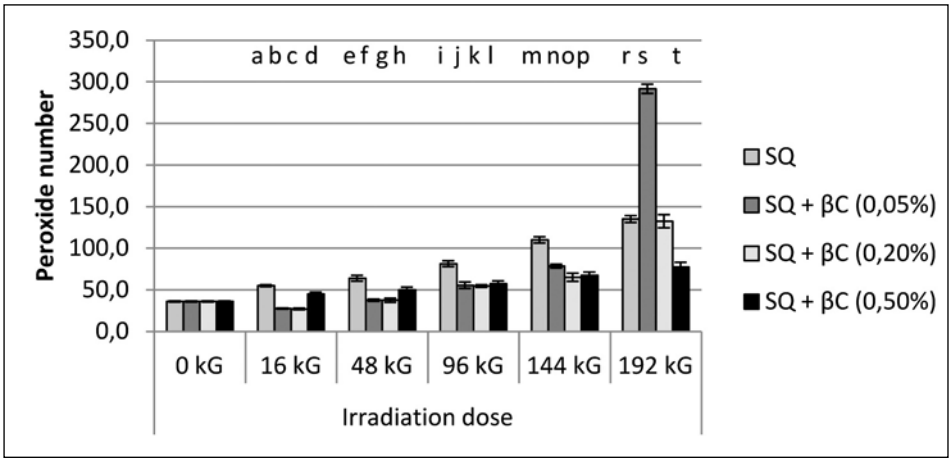
Dose of UV radiation	0 kG	16 kG	48 kG	96 kG	192 kG
emulsion (SQ)	4	200	250	460	970
emulsion (SQ + αT)		220++	370	530	650
emulsion (SQ + βC)		100	120	150	180
emulsion (SQ + R)		180	200	300	650

SQ – concentration in emulsion: 2.5 %; AO – concentration in emulsion: 0.5 %



Significant differences at P ≤ 0.05: h-i; n-o;
at P ≤ 0.01: a-e; a-h; a-k; a-n; a-r; e-f; e-g; h-j; k-l; k-m; n-p; n-q; r-s; r-t

Fig.1: Peroxide number of oil SQ and SQ-AO mixtures (0.5 % concentration) after UV-irradiation



Significant differences at P ≤ 0.01: a-b; a-c; a-d; e-f; e-g; e-h; i-j; i-k; i-l; m-n; m-o; m-p; r-s; r-t
SQ – squalene; AO – antioxidant; PN – peroxide number; αT – α-tocopherol; βC – β-carotene; R – rutin

Fig. 2. Peroxide number of oil SQ and SQ-βC mixtures (various concentrations) after UV-irradiation

RESULTS AND DISCUSSION

SQ was very sensitive to UV radiation already at the lowest dose (16 kG) which caused a significant increase in PN ($P \leq 0.01$; Fig. 1). AOs added to SQ in 0.5 % concentration affected the oxidation. Surprisingly, αT had a pro-oxidant effect after UV-irradiation at all doses except for the highest one (192 kG) at which the same value of PN was measured as in pure irradiated SQ determined. This was alarming because due to well known antioxidant properties of αT, it is often added to products containing lipids (adjuvants, dermatological and cosmetic creams). The increased PN could be even caused by oxidation products of αT (3). Therefore, it should be noted that although αT is known as the most active interrupter of chain reaction in human tissues (8), in *in vitro* systems, where reparative mechanisms are absent, it is necessary to ensure its regeneration. Kohno *et al.* (5) and Psomiadou and Tsimidou (9) on the basis of their photo-oxidation studies reported that SQ regenerates αT and not conversely. In our experiment αT showed low antioxidant activity if it was added to the system only after irradiation. In such case we determined the lowest value of PN 30 min after addition of αT to irradiated SQ.

The SQ oxidation was reduced most efficiently by βC (Fig. 1). Lower concentrations of βC (0.2 %) appeared to be more suitable at lower radiation doses, while 0.5 % concentration was needed to reduce oxidation injury of SQ at higher doses (Fig. 2). βC has a similar mechanism of action as SQ, both are potent quenchers of singlet oxygen (4, 12). Carotenoids can also directly scavenge free radicals (6).

Rutin showed lower antioxidant activity, especially at higher irradiation doses.

SQ in emulsion form is considerably more sensitive than the SQ oil. During the emulsion preparation the samples are aerated and the increased temperature used at homogenization promotes production of peroxides. The PN of non-irradiated emulsion was indeed 9-fold lower then of oil SQ (Tab. 1) but it should be noted that oil content in emulsion was 40-fold lower (2.5 %). After irradiation we observed a high increase in PN, depending on the radiation dose. These selected AOs affected the emulsions analogous to oil samples. Our results are in correlation with the statement of Mueller and Boehm (8), who compared the antioxidant activity of βC and αT in *in vitro* systems, and found that βC was characterized by a significantly better antioxidant activity than αT.

In any case, results of this study indicate that it is necessary to consider whether it is appropriate to add additional ingredients into SQ emulsions. Although some compounds could have a positive effect on various living systems, their action in the *in vitro* systems should be verified. This is important with regard to stability of emulsions as addition of ingredients to emulsion systems may cause their disruption (7). According to Herbert (3) "More is sometimes better, sometimes worse, but always more expensive."

ACKNOWLEDGEMENT

The study was supported by the scientific project KEGA (SR), No. 020UVLF-4/2012.

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FOLIA VETERINARIA, 56, Supplementum II: 59–61, 2012

THE EFFECT OF DIFFERENT APPLICATION OF OXYHUMOLIT ON NUTRIENTS UTILIZATION IN BROILER CHICKS

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ABSTRACT

The aim of the present study was to investigate the effect of oxyhumolit on body weight, feed, nitrogen and metabolisable energy conversion ratio, fermentation process in *caecum* and quality of droppings of broiler chicks. The supplementation of oxyhumolit did not influence significantly the body weight of chicks but improved feed, nitrogen and metabolisable energy conversion ratio ($P < 0.001$), mostly when fed throughout the trial. A significantly higher concentration of acetic ($P < 0.01$; $P < 0.05$), propionic ($P < 0.01$) and butyric acid ($P < 0.001$) was found in the group where supplementation started in the 3rd trial week. Quality of droppings was not affected significantly.

Key words: chicks; dropping; feed conversion; natural humic compounds

INTRODUCTION

The oxyhumolit is an oxidised brown coal with extremely high amount of humic substances (up to 85 %) (5). Humic substances are natural organic compounds that originate from the decomposition of plant and animal remains (7). In the veterinary medicine they are used in horses, ruminants, swine and poultry for the treatment of diarrhoea, dyspepsia and acute intoxications (3).

The aim of the present study was to investigate the effect of oxyhumolit on body weight, feed, nitrogen and metabolisable energy conversion ratio, fermentation process in caecum and the quality of droppings of broiler chickens.

MATERIAL AND METHODS

A total of 150 unsexed one-day-old broiler chicks (Ross 308) were weighed and randomly divided into three groups (n = 50). The birds were fed complete mixed feed in the mash form according to growth phases (phase 1: weeks 1–2; phase 2: weeks 3–5; phase 3: week 6) *ad libitum*. The test group diets were supplemented with natural humic compounds (oxyhumolit – total humic acids 68 %, free humic acids 48 %, minerals 18 %): in the first test group (O_{3-6}) during phases 2 and 3 of the fattening, in the amount of 5 $g.kg^{-1}$ of the diet and in the second test group (O_{1-6}) during phase 1 of the fattening in the amount of 3 $g.kg^{-1}$. The birds were individually weighed and feed consumption was recorded weekly. The content of caecum for determination of pH (potentiometric) and short-chain fatty acids (acetic, propionic, butyric and lactic acid; isotachophoresis) was obtained from 6 chicks from each group on day 35 of the trial. Samples of droppings were collected during weeks 2–5 of the trial and were analyzed for dry mater and crude protein by the AOAC methods (1). One-way ANOVA (Tukey's multiple comparison test) was used for statistical evaluation.

RESULTS AND DISCUSSION

The oxyhumolit supplementation did not affect significantly the body weight of chicks throughout the experiment (data not shown). The final body weight of the chicks in both test groups was insignificantly higher in comparison to the control group (by about 1.33 % in O_{3-6} and 1.71 % in O_{1-6}). The highest final body weights were found in the group O_{1-6}.

Feed, nitrogen and metabolisable energy conversion ratio

Table 1. Feed, nitrogen and metabolisable energy conversion ratio (x ±)

	Week	Control	$O_{3–6}$	$O_{1–6}$
Feed conversion ratio (kg.kg^{-1} weight gain)	1–2	1.27 ± 0.013	1.26 ± 0.015	1.27 ± 0.011
	3–5	1.70 ± 0.005^{a}	1.67 ± 0.010	1.66 ± 0.011^{d}
	6	2.47 ± 0.041^{a}	2.23 ± 0.024^{c}	2.22 ± 0.031^{c}
	1–6	1.79 ± 0.006^{a}	1.74 ± 0.002^{b}	1.72 ± 0.005^{b}
Nitrogen conversion ratio (g.kg^{-1} weight gain)	1–2	269.9 ± 2.79	270.8 ± 3.29	270.3 ± 2.38
	3–5	361.7 ± 1.09	354.1 ± 2.10	357.4 ± 2.42
	6	530.9 ± 8.87^{a}	470.2 ± 4.99^{b}	466.5 ± 6.55^{b}
	1–6	382.5 ± 1.30^{a}	368.7 ± 0.50^{b}	367.8 ± 1.12^{b}
Metabolisable energy conversion ratio (MJ.kg^{-1} weight gain)	1–2	15.0 ± 0.16	15.0 ± 0.18	15.0 ± 0.13
	3–5	20.5 ± 0.06^{a}	20.1 ± 0.12	19.9 ± 0.14^{d}
	6	29.3 ± 0.49^{a}	26.4 ± 0.28^{c}	26.6 ± 0.37^{c}
	1–6	21.4 ± 0.07^{a}	20.8 ± 0.03^{b}	20.6 ± 0.06^{b}

Letters in superscript indicate significance at ^{ad} – $P < 0.05$; ^{ac} – $P < 0.01$; ^{ab} – $P < 0.001$

Table 2. The pH values and content of fatty acids in the caecum content (x ±)

	Control	$O_{3–6}$	$O_{1–6}$
pH	7.12 ± 0.08	6.59 ± 0.23	7.05 ± 0.12
Lactic acid [mmol.l^{-1}]	19.30 ± 3.44	21.80 ± 0.84	16.31 ± 3.51
Acetic acid [mmol.l^{-1}]	90.48 ± 6.15^{c}	145.70 ± 11.03^{a}	108.25 ± 7.43^{d}
Propionic acid [mmol.l^{-1}]	20.49 ± 3.52^{c}	37.18 ± 3.22^{a}	20.89 ± 3.05^{c}
Butyric acid [mmol.l^{-1}]	8.57 ± 1.02^{b}	18.89 ± 1.81^{a}	8.45 ± 0.66^{b}

Letters in superscript indicate significance at ^{ad} – $P < 0.05$; ^{ac} – $P < 0.01$; ^{ab} – $P < 0.001$

values in the respective phases are shown in Table 1. In the group $O_{1–6}$ significantly lower values of feed and metabolisable energy conversion ratio were found in the second ($P < 0.05$) and third phase ($P < 0.01$) of the trial, and in the third phase significantly lower values of nitrogen conversion ratio ($P < 0.001$) compared to the control group. In the group $O_{3–6}$ significantly lower values of these parameters on comparison with control chicks were found only in the third phase of the trial ($P < 0.001$ in nitrogen conversion ratio, $P < 0.01$ in feed and metabolisable energy conversion ratio). In the first feeding phase the feed, nitrogen and metabolisable energy conversion ratio were not significantly influenced. The values of these parameters monitored throughout the trial were significantly lower in both test groups compared to the control ($P < 0.001$), whereas lower values were found in the group $O_{1–6}$. Similar to our study, better feed conversion was also observed by El-Husseiny *et al.* (2) and Ozturk *et al.* (4).

The caecum content pH was not affected significantly by the oxyhumolit supplementation (Table 2). A significantly higher concentration of the acetic acid ($P < 0.01$, $P < 0.05$), propionic acid ($P < 0.01$) and butyric acid ($P < 0.001$) were found in the group $O_{3–6}$ compared to the control and the $O_{1–6}$ group. Higher concentration of these short-chain fatty acids in caecum content indicates increased microbial fermentation (6). The content of dry matter and crude protein in droppings was not affected significantly (data not shown).

In conclusion, our results showed, that the addition of oxyhumolit to the diets of broiler chicks resulted in better utilization of nutrients while enhanced effect was achieved by feed supplementation throughout the fattening. This is supported by the insignificantly higher body weight of chicks in the group O_{1-6}. Oxyhumolit supplementation throughout the shorter fattening period may lead to enhanced microbial fermentation in the caecum.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Education of the Slovak Republic through the research project VEGA No. 1/0878/11.

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FOLIA VETERINARIA, 56, Supplementum II: 62–64, 2012

EFFECT OF MILKING MACHINE ON BEHAVIORAL MANIFESTATIONS OF MILKING COWS

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ABSTRACT

The study observed the influence of automatic milking systems (AMS) on behaviour of milking cows and potential different influence of milking machines on dairy cows after the first and the following lactations. The activities of all dairy cows in the herd were observed during 24 hours. We focused on their need of food, drinking and lying down during 30 minutes after machine milking. We evaluated the data from milking machine Lely Astronaut – average daily milk yield of each cow and the herd as a whole, number of visits of milking machine, the length of preparation for milking and the time of milking. We compared results from dairy cows after the first lactation and those after two and more lactations. A need for drinking was observed in 40.4 % of cows after the first lactation (Group 1) and in 41.7 % of cows after two and more lactations (Group 2). A need for food intake was identified in 75 % of cows from Group 1 and in 88.6 % of cows from Group 2. Twelve percent of Group 1 cows showed need to lie down in 30 minutes after milking while 13 % of those from Group 2 needed the rest. Complete preparations for milking lasted 2:04 min in dairy cows after the first lactation and 1:54 min in cows from Group 2.

Key words: behaviour of cows; dairy cows; dairy cows after the first lactation; machine milking

INTRODUCTION

Many different investigations of milking by milking machines showed that various experts do not unequivocally agree with the contribution of automatic milking systems (AMS) to dairy farming. However, it is a fact that on smaller family farms the milking by machines saves time and labour. Cows get used to milking machines rather quickly (in 2 to 7 days). From the point of view of welfare, the AMS are very beneficial, too – the cows are disturbed only during the mucking out and feeding. AMS also help to decrease noise in the housings and reduce stress, because the cows are not driven into the milking parlour.

The aim of our study was to determine the behaviour of dairy cows after the first and following lactations after leaving the AMS. The detected data were compared with the references in the literature and on the internet which stated that the longest period for cow's rest falls to the time between 10 p.m. and 4 a.m. (1, 3), the length of preparation for milking ranges between 2 and 2.5 minutes (2) and that there is necessary to attach the milking machine within one minute (2) because of the proper physiology of milking and the limited period of releasing the hormone oxytocin. The main motivation for the food intake is feeling hungry (3).

MATERIAL AND METHODS

The ethological monitoring lasted always 24 hours and included 60 Holstein dairy cows, 20 after the first lactation and 40 after two and more lactations. Cows in this herd had the highest yield in the Czech Republic in the year 2010, namely 13 380 kg of milk. The fodder was supplied once a day by a mixing feeding cart and the composition of the rations was optimised according to animal groups. The milking cows were given extra mixed feed in machine (0.4 kg.l^{-1} milk). The cows were housed free in one stable. The AMS from

Holland company Lely (Lely Astronaut A3) was installed in 2007. Individual dairy cows were observed always during 30 minutes after leaving the machine and the need for drinking, food intake and lying down was recorded. The monitored data were processed by Microsoft Excel software, in which graphs and tables were created. The data about the milk yield, length of milking and preparation for milking, the number of visits and movement activities were detected by means of the computer programme of the automatic milking system Lely.

Statistical significance of differences between the groups were determined by the Student *t*-test using software Statistika.

RESULTS AND DISCUSSION

The data on movement activities showed that the animals were most active at about 8 p.m. From 8 p.m. the activity decreased till 4 a.m., when the values were the lowest, from 4 a.m. till 10 a.m. the activity increased gradually and from 10 a.m. till 2 p.m. another decrease was observed. From 2 a.m. the activity increased till 8 p.m. when it culminated, the reason of which could obviously be the loading of the fresh fodder. According to many authors the longest period of cattle relaxation falls between 10 p.m. and 4 a.m. (1, 3) as observed also in our study.

The movement activity of the dairy cows after the first lactation was significantly higher during the day with 95 % probability compared to the activity of the cows after additional lactations. However, the curves of the movement activity were very similar. The highest activity of dairy cows was observed during the summer months, the lowest in winter.

During the 24 hours, each cow was observed for 30 minutes after leaving the milking machine. Altogether 148 milkings were observed and of that after 18 the dairy cows laid down within 30 minutes, i.e. 12.16 % of all observed dairy cows. The percentage difference between the cows after the first lactation and the others was small. The highest number of lying down within 30 minutes (50 %) was observed during the period from 0 a.m. to 6 a.m. In the course of the day the cows laid down more or less accidentally. When we compared the relationship between the need for drinking and time of the day, we found that the highest frequency of drinking after milking was in the period from 6 a.m. till 12 a.m., which corresponds to the highest number of visits of AMS in this period. The water intake within 30 minutes after milking was observed in 40.4 % of cows from Group 1 and in 41.7 % of cows from Group 2.

Solid fodder was given already during the milking. Afterwards, 75 % of cows from Group 1 and 88.6 % from Group 2 sought the a manger with silage fodder within 30 minutes. This implied that the frequency of the fodder intake is significantly higher than that of water intake. The highest fodder intake was detected in the period from 6 p.m. to 12 p.m., which was related to loading of the fresh fodder in evening hours.

The need for fodder and water within 30 minutes after leaving the milking machine was observed after 35 milkings (23.63 %) from the total of 148 milking. Only in two cases (1.35 %) we detected no need for drinking or fodder intake.

From the total number of 148 milkings at 60 dairy cows we found an average milk yield 15 kg per 1 milking, the average frequency of AMS visits being 2.5. The average daily milk yield was 37.5 kg. From the total number of 52 milkings of the dairy cows after the first lactation we found an average milk yield of 13.22 kg per 1 milking, the average frequency of AMS visits equal to 2.6. The average daily milk yield was 34.4 kg. From the total number of 96 milkings of the dairy cows after more than one lactation we found an average milk yield of 15.9 kg per 1 milking, the average frequency of AMS visits equal to 2.4. The average daily milk yield was 38.2 kg. Comparison of observations showed that the periods of milking of individual quarters were shorter.

We also compared the length of preparation for all milkings during the day, namely in 10 dairy cows from the first group and 10 from the second group. We considered that 48 seconds was the average time needed for attaching the equipment. The mean preparation time for milking of dairy cows after the first lactation was 2:04 min and of those after two and more lactations 1:54 min. We recorded a shorter time of preparation than 2–2.5 min which is the time reported by some authors (2).

CONCLUSIONS

Comparison of movement activity of the cows from the first and second group showed higher activity in cows after the first lactation throughout the day. The curve of activity was almost the same in both groups with only small differences. This comparison proved that the medium movement activity in the first group was significantly higher with 95 % probability than that in cows from the second group.

The need of lying down within 30 minutes after milking was detected in 11.5 % of cows after the first lactation and in 12.5 % of cows after two or more lactations. Fifty percent of all lying down occurred in the period from 0 a.m. till 6 a.m.

The need for water intake within 30 minutes after milking was detected in 40.4 % of cows from the first group and in 41.7 % of cows from the second group. The need for fodder intake within 30 minutes after milking was detected in 75 % of cows from the first group and in 88.6 % of cows from the second one. The need for both water and fodder intake was detected in 23 % of cows. Only in two cases from the total 148 milkings no need for drinking or fodder intake was observed.

The average daily milk yield was 34.4 kg in the first group and 38.2 kg in the second group. We detected longer milking in the first group in comparison with the second. The average time of preparation for milking including the time from entering the milking machine till the beginning of milking was 2:04 min in the first group and 1:54 min in the second group.

The results of the present study do not indicate significant differences between the groups related to the need of lying down and water intake in the period of 30 minutes after milking. Higher differences with respect to the influence of milking are evident in the need for fodder within 30 minutes

after milking which is obviously caused by higher efficiency of cows after two and more lactations.

ACKNOWLEDGEMENTS

The study was supported by the project NAZV QH91260.

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FOLIA VETERINARIA, 56, Supplementum II: 65–67, 2012

INFLUENCE OF SOME FACTORS ON THE BEHAVIOUR OF FEMALE DOMESTIC GUINEA PIGS DURING LABOUR AND THE NUMBER OF SUFFOCATED YOUNG

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ABSTRACT

The aim of this study was to examine selected factors that influence the behaviour of female domestic guinea pigs (*Cavia aperea f. porcellus*) during giving birth to the young. Sows do not treat up to 45 % of the born young that subsequently die due to asphyxiation in embryonic covers. The following determinants were observed: sex of the young, birth weight, condition of the young (alive/dead), litter size, season, daytime. In the overall evaluation, a relation between the alive/dead young and other observed values was assessed using Pearson's chi-squared test. A statistical significance was unambiguously proved in the case of daytime, which clearly reflected the influence of light and darkness on a loss of maternal behaviour and instinctive urge of female guinea pigs. Another parameter in which statistical significance was clearly proved was litter size. Some sows do not manage to clean all their young after giving birth to a large litter, they are more exhausted and the impact is fatal on the newborn young. The ethological observation has also led to a finding that the females devote special care to their firstborn young and neglect the others, this phenomenon also often leads to the late rupture of embryonic covers and suffocation of the young. Birth weight of newborns is another indicator that has an effect on mortality of the young at birth. It is closely associated with litter size. The statistical significance was not proved in the other evaluated indicators, such as sex and season. In conclusion, the study proved a relationship between selected parameters which can affect behaviour of female domestic guinea pigs during labour with an impact on giving birth to the live young.

Key words: behaviour; birth; guinea pig; young

INTRODUCTION

Animal reproduction is one of the main objectives of animal breeding. To achieve the greatest success possible, it is crucial to know the physiology of guinea-pigs thoroughly and to ensure the most suitable hygienic conditions and welfare for their breeding.

Sex dimorphism has not developed in guinea-pigs. The male can be distinguished from the female by different shape of genitals (1). Young females are sexually mature around four weeks of life. They reach breeding maturity after the fifth month of age when they are ready to breed; of course, with respect to good health condition and stature of a female. Minimum weight to mate a female guinea pig is around 700 g (4). The female should give first birth before one year of age. In that period cartilage ossification starts in females and bones lose their elasticity (8). Male guinea pigs reach sexual maturity after the sixth week of life but they are used for mating after the fifth month of age. This is crucial from the aspect of proper sperm production (4). Oestrus is repeated in a female after 14–17 days and usually lasts several hours only (5). The pregnant sows need for vitamin C also increases but the guinea-pig is not able to synthesize it itself and so the animal is dependent on its intake from food. If high-quality food with sufficient saturation of vitamin C is not administered daily, vitamin supplementation is necessary (7). Decreased vitamin C content results in insufficient weight gain of the young in the lactation period (6). The movement of the female in the last two weeks before labour is cumbersome, she is often resting and the movements of the young in the belly are discernible. Several days before labour the female's pelvic bones become wider and open and if they are spread about a finger wide, labour will occur within 24 hours (8). Labour occurs spontaneously at night or

during the day after 59–72 days of gestation, around day 65 on average. Labour is short and should not take more than an hour. The young are born head first, in several-minute intervals, within ten to thirty minutes, Mother cleans them from foetal envelopes. Embryonic envelopes of a guinea pig are very firm, the young are not able to rupture them themselves and they fully depend on mother's or tender's help (5). Up to 45 % of the young are suffocated at birth due to the late rupture of membranes by mother (9). There may be 17 newborns per litter, the most frequently it is 2–3. Birth weight is one of the parameters that can be measured immediately after the young is born. Further development of the young can be evaluated indirectly on the basis of birth weight. The birth weight of domestic guinea pig is from 60 to 120 g (5). Guinea pig newborns are fully furred, with developed teeth, sense of hearing and eyesight. The eyes of the young open wide in mother's womb 14 days before birth (2). The first oestrus of a female occurs within several hours after labour. Mother is nursing the young for 4–5 weeks (5).

MATERIAL AND METHODS

The study was conducted in 2009–2011 period at a breeding station of exhibition guinea pigs. The herd consisted of about thirty females and ten males. They were used for breeding at the age of minimum six months and at minimum weight of 800 g. Maximum age of a female was four years. No inbreeding occurred in this herd thanks to a high number of breeding lines, therefore this factor was excluded from the evaluated factors of potential influence. The short-furred breed English Crested was studied. Guinea-pigs were bred exclusively for specific exterior characteristics and very good health condition. All litters observed over the three-year period were evaluated statistically. Only those litters were excluded from the study in which the onset of physiological labour was earlier than usual and in which the young already died in mother's body before regular labour. The reason was that we wanted to assess exclusively the mother's influence at birth as exactly as possible. Birth weight was recorded in all these newborns. The young were weighed after birth, always within 12 hours of age at the latest. Live weight was measured on a digital scale. The sex of newborns was determined along with weighing. Birth date and daytime were also recorded and litters were included in the respective groups according to the number of newborns: small litter 1–2 newborns; medium-size litter 3–4 newborns; large litter 5–6 newborns.

All results were processed statistically by Pearson's chi-square test.

RESULTS AND DISCUSSION

A total of 78 litters comprising 219 newborns were evaluated, which corresponds to two to three newborns per litter, as reported by Henwood (2). The mortality rate of the young was 39 % and this relatively high figure almost corresponded to data of Wagner (9), who reported in his study up to 45 % of the suffocated young during labour. The lowest measured weight of the young was 46 g and the maximum weight was 138 g, the average weight was 95 g. These values were described by Tejml (5). The number of newborns per litter was from one to six, in agreement with Peaker (3). The relationship between the stillborn/born alive young and sex, daytime, year season, number of newborns per litter and birth weight was evaluated. The relationship between the stillborn/born alive young and daytime, number of newborns per litter and weight of the young was significant (Table 1). The influence on the weight of newborns was mostly directly proportionate to the number of newborns per litter. The young of higher birth weight complicated the female's labour because the labour was more exhausting and suffocation of the young was more frequent. Multiple-birth litters also caused the female's exhaustion during labour. As stated by Tejml (5), litter size may influence the course of labour and pregnancy length. The daytime and night time of labour should not be influenced by worse light conditions but by a probable loss of females' maternal instinct that could be oppressed by the breeding process. No statistical significance was found for the other relations.

In conclusion, our results allowed us to state that some factors influence the females of domestic guinea pig during labour and their result is higher mortality of newborns.

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Table 1. The relationship between the stillborn/born alive guinea pig pups and daytime, number of newborns per litter and weight of the young

Day/night in relation to stillborn/born alive	x^2 = 19.2567 critical value (0.05; 1) = 3.841459149
Number of the young per litter, stillborn/born alive	x^2 = 14.93150 critical value (0.05; 5) = 11.0705
Stillborn/born alive in relation to weight of the young	x^2= 17.2724 critical value (0.05; 3) = 14.0671

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FOLIA VETERINARIA, 56, Supplementum II: 68–69, 2012

MICROFLORA OF THE BAT GUANO

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ABSTRACT

Thirty randomly selected mesophilic isolates from two guano samples, six years old (Slovenska Lupča village) and two years old (Rybník village) samples, from mixed *Myotis myotis* and *M. blythii* summer roosts colonies were identified by MALDI TOF analysis. No typical representatives of mammalian gastro-intestinal microflora were detected and coagulase negative staphylococci were found to be dominant in both samples: *Staphylococcus nepalensis* in Slovenska Lupča village sample and *S. sciuri*, *S. lentus*, *S. fleurettii* accompanied by *Providencia rettgeri* and *Alcaligenes faecalis* in Rybník village sample. The antibiotic resistance analysis revealed high prevalence of tetracycline, erythromycin, and chloramphenicol resistance in *S. nepalensis* but not in other staphylococci. Similarly high frequency of antibiotic resistance to tetracycline and ampicillin was observed in *P. rettgeri*, a recognized human pathogen. The experiments showed that typical bat faecal microflora was rapidly replaced by environmental (mainly ureolytic) bacteria and the guano that accumulated near or directly in human dwellings and buildings may present a potential risk to human health.

Key words: antibiotic resistance; bats; coagulase negative staphylococci; guano; microbial communities

INTRODUCTION

Bats (*Mammalia: Chiroptera*) are very interesting, mysterious and even today little known animals with mostly nocturnal activity. During the day bats use a diverse range of natural and anthropogenic shelters. With many years of repetitive use of the same shelter bat's excrements (urine and faeces) gathers on the bottom and form guano. Composition of guano may vary depending on the diet of bats (residue of insects, beetles and moth, etc.), foraging biotope and other factors. Nowadays there are limited data on gastrointestinal flora of bats and guano microflora (1, 2).

Given that bats and bat guano may serve as reservoirs or vectors of several zoonoses we aimed this study on extending the knowledge on cultivable bacteria from the bat guano.

MATERIAL AND METHODS

Guano of bats used in our experiments was obtained from two the summer colonies of bats from the church towers in Slovak Ľupča village and Rybník village. The samples of guano were collected in December 2010 (Slovak Ľupča village) and October 2010 (Rybník village) and were analyzed for the presence of cultivable bacteria. The pH of guano was determined according to ISO standard. To the representative samples of guano (0.5 g) 10 ml of sterile PBS solution was added and after 30 min of intensive mixing aliquots were spread on non-selective agar medium (Nutrient agar no. 2, Oxoid, USA). Cultivation was conducted under aerobic conditions at 37 °C for 24 hours. The production of coagulase was tested using the coagulase test from Fluka-Analytical (Fluka, Switzerland).

The selected isolates were identified by MALDI-TOF mass spectroscopy using a Maldi Biotyper instrument (Bruker Daltonics, Germany) and Gram staining.

The selected isolates were tested for resistance to ampicillin (10 μg), erythromycin (15 μg), tetracycline (5 μg), kanamycin (30 μg), chloramphenicol (30 μg), neomycin (30 μg), streptomycin

(10 μg), gentamycin (10 μg), vancomycin (5 μg) and sulfamethoxazol (0.25 μg) by the disk dilution method on Mueller Hinton Agar (Becton Dickinson, MA, USA) according to NCLSS standards.

RESULTS AND DISCUSSION

Bats and bat guano could serve as reservoirs or vectors of several zoonoses (3), however, microflora of bat guano from Paleoarctic region is basically unknown. In our experiments we analysed cultivable mesophilic bacteria from two guano samples, six years old (Slovenska Lupča village) and two years old (Rybník village) samples, from either mixed *Myotis myotis* and *M. blythii* or summer roosts colonies (4). Guano was made up of brown to black pellets of size about 4–7 mm and the pH of the guano was 7.7 (Slovenska Lupča village) and 5.0 (Rybník village). By cultivation on non-selective agar similar counts of cultivable bacteria (2×10^5 per gram of guano) were found in both samples. Thirty randomly selected mesophilic isolates from both samples were identified by MALDI TOF analysis. In both samples coagulase negative staphylococci (CoNS) were found to be dominant: *Staphylococcus nepalensis* (94 % of cultivable bacteria in Slovenska Lupča village sample) and *S. lentus* (43 %), *S. sciuri* (13 %), *S. fleurettii* (3 %) accompanied by *Providencia rettgeri* (17 %) and *Alcaligenes faecalis* (3 % of cultivable bacteria) in Rybník village sample. About 6 % isolates from Slovenska Lupča and 20 % isolates from Rybnik samples could not be identified by the method used. No typical representatives of mammalian or bat gastro-intestinal microflora e.g. enterobacteria or enterococci (1, 2) were detected. All species detected were recognized as highly active ureolytic bacteria. Staphylococci occur frequently in the gastrointestinal tract or on mucous membranes of warm-blooded animals (5) but their predominance in bat guano has not been recorded as yet. *S. nepalensis*, and *S. saphrophyticus* cluster member recently described from goats, as well as *S. lentus*, *S. sciuri*, and *S. fleurettii* belonging to the *S. sciuri* cluster are principally animal species generally considered to be bacteria of doubtful pathogenicity. However, they may colonize humans and several recent studies indicated significance of some CoNS species that may cause serious infections in humans (6).

The treatment of staphylococcal infections is frequently impeded by resistance of staphylococci to the antibiotics. The antibiotic resistance analysis of selected isolates revealed high prevalence of tetracycline, erythromycin, and chloramphenicol resistance (in about 80 %, 100 %, and 60 % of isolates, resp.) in *S. nepalensis* but not in *S. sciuri* cluster staphylococci. Similarly, high frequency of antibiotic resistance to tetracycline and ampicillin (in more than 90 % of isolates) was observed in *P. rettgeri*, a recognized human pathogen (7).

CONCLUSIONS

The study showed that coagulase negative staphylococci are the dominant cultivable bacteria in both bat guano samples. The antibiotic resistance analysis revealed high prevalence of tetracycline, erythromycin, and chloramphenicol resistance in *S. nepalensis* but not in *S. sciuri* cluster staphylococci. Our data indicate that typical bat faecal microflora is rapidly replaced by environmental (mainly ureolytic) bacteria and the guano accumulated near or directly in human dwellings and buildings may present potential risk to human health.

ACKNOWLEDGEMENTS

This study was supported by the Project No. ITMS 26220120066, of the Research & Development Operational Programme funded by the ERDF.

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FOLIA VETERINARIA, 56, Supplementum II: 70–72, 2012

OXIDATIVE CHANGES IN KIDNEY INDUCED BY SELECTED NATURAL SUBSTANCES

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ABSTRACT

The liver and kidneys are highly metabolically active bodies, calculated per gram of tissue and time unit even by the kidney. All metabolic processes are associated with the formation of reactive oxygen species. Since these are involved in the degradative processes, together with antioxidant system abnormality can alter glomerular permeability and overall health. Their generation can be positively or negatively affected by feed supplementation. In this experiment, we pointed out that horse chestnut extract alleviates the negative effect of higher intake of n-6 polyunsaturated fatty acids on kidney oxidative status, but less effectively than with the addition of n–3 in linseed oil.

Key words: kidney; mitochondria antioxidants; high fat diet; horse chestnut

INTRODUCTION

The kidney have important physiological functions including maintenance of water and electrolyte balance, synthesis, metabolism and secretion of hormones, and excretion of the waste products from metabolism. In addition, the kidneys play a major role in the active metabolism and excretion of drugs, hormones, and xenobiotics. Renal pathways for the biotransformation involve cytochrome P450 dependent mixed function oxidase systems that could be directly induced by aromatic compounds or not, but affecting fatty acid and steroid metabolism (5). Especially, arachidonic acid ω/ω–1 oxygenase activity has been related to hypertension (6). Renal pathways generally lead to a product that is more easily excreted in the urine, or may be reabsorbed, at least in the form of a more active and toxic product but is also associated with the formation of free radicals. Imbalance between oxidants and antioxidants activity causing oxidative injury degrades the glomerular basement membrane and reduces de novo synthesis of proteoglycans that affects glomerular permeability (2).

Supplementation of feed with plant extracts with antioxidant properties may affect the status of the metabolising organ and thus affect directly the functional and structural integrity of the body as well.

Our experiment focused on monitoring of changes in selected antioxidants after feeding high fat diet in combination with horse chestnut extract.

MATERIAL AND METHODS

This study was carried out on sixty three Sprague-Dawley rats aged four months, body weight range 300–400 g. The animals were obtained from the Animal Facility of the Pavol Jozef Šafárik University. They were kept under standard laboratory conditions and during the trial had access to feed and water *ad libitum*. The rats were randomly divided to seven equal groups: Group C (control) received only normal diet (rat chow) for six months. Group G1 received high fat diet with 20 % share of sunflower oil with a wide ratio of ω–6 : ω–3 polyunsaturated fatty acids (PUFAs) (n–6 : n–3 ratio 610 : 1). The diet of group G2 was enriched with extract of horse chestnut dry extract (CALENDULA ojsc, Slovakia) in an amount of 2 %. The G3 group received the same high fat diet as G2 together with linseed oil. The experiment was carried out in accordance with the guidelines of the Animal Care and Use Com-

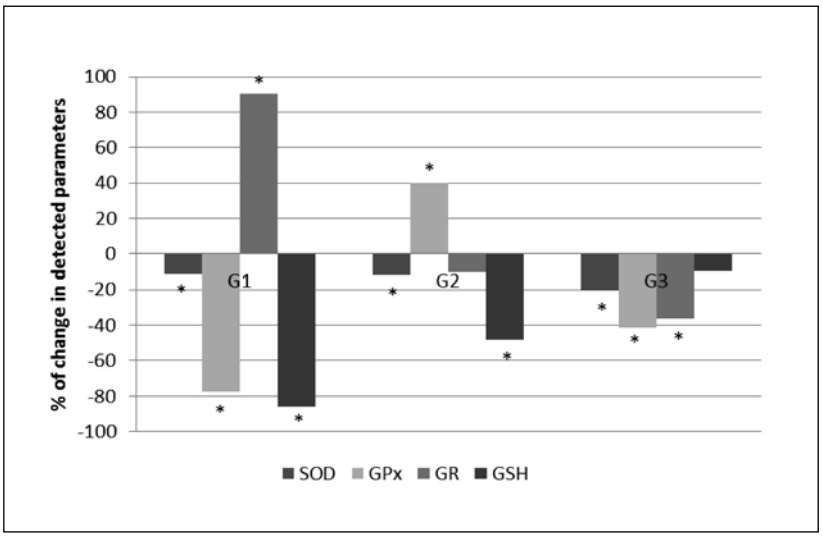

Fig. 1. Activities of enzymatic antioxidants and levels of non-enzymatic antioxidants in rat kidney mitochondria (* – P < 0.05)

mittee of Pavol Jozef Šafárik University in Košice. By the end of the 6-month experimental period, all rats were sacrificed by cervical dislocation and kidney samples were collected. Kidney mitochondria were isolated according Fernández-Vizzara (3). The activity of glutathione reductase (GR, E.C.1.6.4.2) was measured by a modified method previously described by Carlberg and Mannervik (1); that of glutathione peroxidase (GPx, E.C. 1.11.1.9) was measured as described by Flohe and Gunzler (4) and calculated per mg of mitochondrial proteins (mg P); activity of superoxide dismutase (SOD, E.C. 1.15.1.1) by means of the SOD-Assay Kit-WST (Fluka, Japan) in accordance with the user manual. Proteins were quantified using bicinchinonic acid. Values of the measured parameters were expressed as means ± SD and the difference between the groups was assessed using unpaired Student's *t*-test; and the significance was considered at $P < 0.05$.

RESULTS AND DISCUSSION

Kidney generally metabolize endogenous and exogenous chemicals to compounds with reduced biological activity. There are several instances in which metabolism will produce a toxic intermediate that may result even in mutagenesis or cell necrosis (5). We assessed the changes in antioxidants after supplying different types of diet. The addition of oils and extract of horse chestnut caused decrease in the activities of SOD as compared to the control, which may be due to the nature of the added substances. The extract of horse chestnut contains bioflavonoids, like quercetin, kaempherol and their diglycosyl derivatives and other antioxidants (proanthocianidin A2) and coumarins (aesculin and fraxin), which preventse formation of superoxide radicals (7). High-fat diet contained mainly polyunsaturated fatty acids, which may be very sensitive to oxidation and when oxidized they form peroxyl radicals, starting itself a chain reaction of oxidation, which forms substrate peroxides and cyclic peroxides. The G1 group showed GSH depletion, not sufficiently ensured by increased activity of GR. Moreover, all peroxidases were inhibited by excess of peroxides, that together led to a significant decrease in the activity of GPx. The negative effect of G1 diet was partially reduced by adding horse chestnut extract to feed supplied to G2. The GSH level was low, although significantly, but changes in the activities of GR and mainly GPx indicated primarily the elimination of peroxides. The overall positive effect on kidney mitochondrial antioxidant status was assessed after the addition of linseed oil to the diet in G3. The activities of enzymes were decreased in comparison with the control group and the level of GSH did not show significant changes.

The results indicate that although the addition of natural plant extracts with antioxidant effect may dampen the negative effect of higher intake of n-6 microbial PUFAs on oxidative status of the kidneys, they are less effective than improvement of the ratio of n–6 : n–3 fatty acids.

ACKNOWLEDGEMENT

The study was supported by Projects VEGA 1/0372/10 and 1/1236/12.

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FOLIA VETERINARIA, 56, Supplementum II: 73–74, 2012

POSSIBILITIES OF USING NONCONVENTIONAL METHODS AND DIETARY SUPPLEMENTS IN PREVENTION AND HEALTH CARE OF CALVES

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ABSTRACT

It is important to take care of calves and their health in order to have good results in cattle breeding. The aim of this study was to evaluate the effect of dietary supplements Lactovita and Biopolym (the hydrolyzate of brown seaweed *Ascophyllum nodosum*) on the frequency of diarrhoea in calves. The experiment included two experimental and one control group of calves. The experiment was conducted in a calving barn under ordinary operating conditions in a conventional management system. Holstein calves were used in the experiment.

Key words: biopolym; calves; diarrhea; lactovita; probiotics

INTRODUCTION

Biopolym is a hydrolyzate of the brown seaweed *Ascophyllum nodosum*, which is harvested in cold coastal waters mainly in the proximity of Iceland and also in littoral zones of Norway and Canada. It supports regeneration of the organism, improves health status and overall fitness of animals (4).

Lactovita is a food supplement. It contains vitamin B complex and bacteria of lactic acid fermentation which help to maintain the intestinal microflora balance (1).

R o s m i n i (3) stated that in an intensive management system of farm animals, especially when calves are kept separately from cows, the natural acquisition of indigenous microflora is drastically reduced, which alters the intestinal environment, making it easy for pathogens to colonize the intestinal microflora.

Frequent and serious problems are infectious diarrhoeas that develop in weakened calves due to dyspepsia or occur primarily in conditions of low-hygiene management or insufficient care of calves. The main causes of diarrhoeas are mixed infections of viruses and bacteria, protozoans and moulds (2).

MATERIAL AND METHODS

In total, 124 experimental calves and 62 control calves were included in our experiment that was conducted in a conventional management system under ordinary operating conditions on Staré Hobzí Farm. The calves were divided after birth to three groups: Lactovita group, Biopolym group and control group. In addition to colostrum, the Lactovita experimental group was applied one tablet of probiotics *per os*. The Biopolym experimental group received with colostrum 5 ml of hydrolyzate from the brown seaweed *per os*. Both experimental groups were given these dietary supplements once a day at the second feeding. Both products were administered to experimental groups for the first seven days after birth. The control group received feed rations without supplementation. Control and experimental groups were monitored for four weeks from their inclusion in the experiment. Disease occurrence was recorded regularly and data were presented in Table and Figure.

RESULTS AND DISCUSSION

The number and percentage of healthy and infected cows are summarised in Table 1.

The one-sided P-value for results presented in Table 1 was 0.03747. This value proves statistical significance of positive effects of the application of Lactovita and Biopolym products.

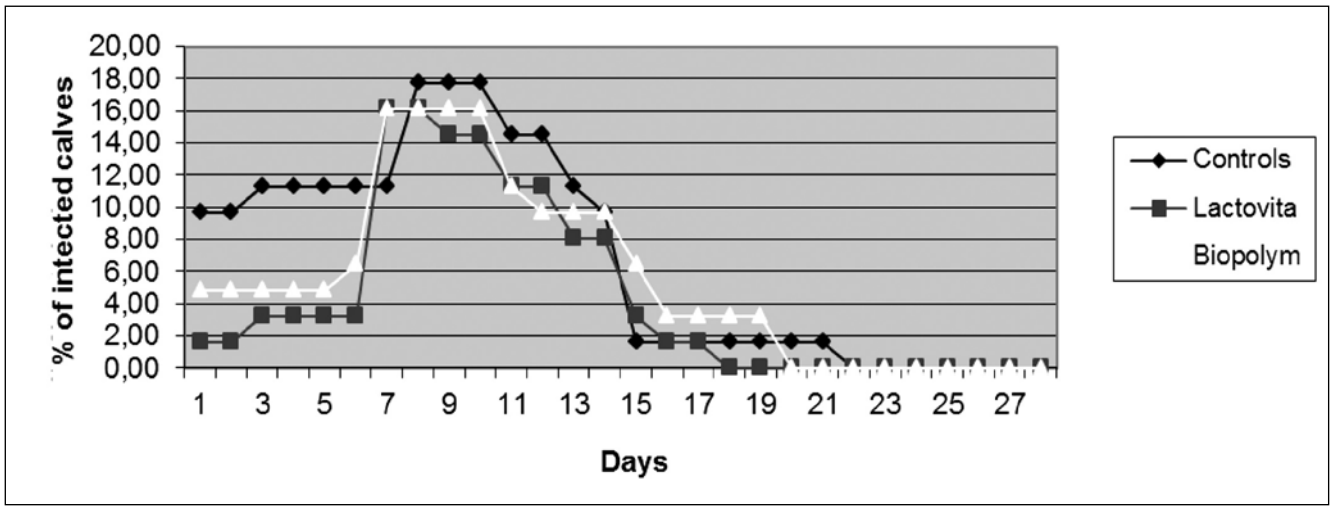

Fig. 1. Comparison of calf morbidity on the particular days after birth

Table 1. Percentages of healthy and infected calves

Group	Lactovita	Biopolym	Control
No. of calves	62	62	62
Healthy	83.87 %	77.42 %	66.13 %
Infected	16.13 %	22.58 %	33.87 %

Percentage difference in the morbidity between groups C and L was 17.57 %, in favour of the calves from the of Lactovita group. Percentage difference in the morbidity of groups C + B was 11.29 %, in favour of the calves from Biopolym group. Percentage difference in the morbidity of groups B + L was 6.45 %, in favour of the calves from the Lactovita group.

Fig. 1 documents that diarrhoea occurrence was evaluated in the period of days 1–30 after birth. While in the Lactovita experimental group the peak of diarrhoea occurrence was reached on days 7–8 after birth, in the Biopolym experimental group the highest occurrence of diarrhoea was observed on days 7–10 after birth and in the control group the peak of diarrhoea occurrence was reached on days 8–10 after birth. Statistical significance of differences was evaluated by the Friedman test. The calculated P value (0.0001) proved that the application of Lactovita and Biopolym supplements reduced the occurrence of diarrhoeas compared to the control group without supplementation.

ACKNOWLEDGEMENTS

The study was supported by the NAZV QJ 121044.

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