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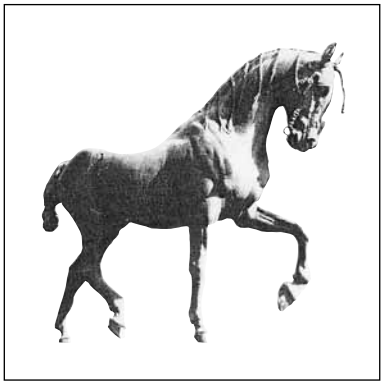

52nd STUDENT SCIENTIFIC CONFERENCE

April 28th, 2009

The aim of the 52st Student scientific conference (ŠVOČ) organised in the academic year 2008/2009 was to present results of scientific investigations carried out by undergraduate students and young scientists. The papers were presented in the following four sections:

1. Preclinical – 2. Clinical
3. Hygiene of food and the environment
4. Young scientists

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MONITORING OF OCCURRENCE OF SARCOCYSTOSIS IN HOOFED GAME IN EASTERN SLOVAKIA

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ABSTRACT

In 2006–2009 we investigated prevalence and intensity of infection with *Sarcocystis* spp. in samples of heart and skeletal muscles from hoofed game (n = 46) hunted down in eastern Slovakia. The total prevalence in the investigated hoofed game (deer, roe-deer, fallow-deer, moufflon, wild-boar) reached 87 % while in 2 dear the prevalence was 50 %, in 8 roe-deer, 3 fallow-deer and 3 moufflons it reached 100 % and in 30 wild-boars 83.3 %. Samples of heart and skeletal muscles were examined also for infection with microcysts of *Sarcocystis* spp. Gender-related investigations revealed higher intensity of infection in males compared to females (buck of roe-deer 361 and the female 59 microcysts per 1 gram of sample, buck of fallow-deer 56 and the female 23 microcysts per 1 gram of sample, moufflon male 67 and female 7 microcysts per 1 gram of sample). In the wild-boar the intensity of infection was higher in females than in males (female 46 and male 25 microcysts per 1 gram of sample). Animals younger than one year showed higher intensity of infection with *Sarcocystis* spp. than animals of age above one year (roe-deer 30 g^{-1}, wild-boar 24 g^{-1}).

Key words: developmental cycle; hoofed game; prevalence; *Sarcocystis* spp.

INTRODUCTION

Sarcocystosis is one of the most spread muscle parasitoses of domestic and free living herbivores and carnivores (6). There were reported also intoxications of people with specific thermolabile sarcosporidium toxin (sarcotoxin). It is a protozoan disease induced by species of the genus *Sarcocystis* with obligate two-host developmental cycle with gametogonous and sporogonous stages occurring in the definitive host and the schizogonous stage taking place in intermediate host (5). Circulation of *Sarcocystis* spp. in free nature has sylvatic character.

Examination of deer *(Cervus elaphus)* showed presence of species *Sarcocystis hofmanni, S. capreolicanis* and *S. grueneri*. Red-deer *(Capreolus capreolus)* harboured *Sarcocystis hofmanni, S. capreolicanis* and S. gracilis. Species *Sarcocystis hofmanni, S. grueneri* and *S. jorrini* have been detected in fallow-deer *(Dama dama)* while moufflon *(Ovis musimon)* is the intermediate host of species *Sarcocystis ovicanis* and *S. arieticanis*. Species *Sarcocystis miescheriana, S. porcifelis* and *S. suihominis* were found in wild-boars *(Sus scrofa)* (4, 8). Cysts (located intracellularly) have been found in heart and skeletal muscles. Various species have their own predilection localisation, in the heart, oesophagus and tongue, but may be present also in the brain, kidneys, spinal cord, spleen and other organs (3).

In the man as their final host, sarcocysts cause oedemas, nausea, inappetence, abdominal pain, vomiting, diarrhoea, respiratory difficulties and slow pulse (2). In animals there were recorded inflammatory reactions characterised by massive perivascular infiltration of mononuclear cells and multiorgan petechial haemorrhage associated with weakness, fever and sometimes also death. Key prevention step is inspection of animals at slaughter. Intestinal form in humans may be prevented by cooking or freezing of meat, thus so avoiding to consumption of raw or insufficiently heat-treated meat is an effective preventive measure.

MATERIAL AND METHODS

During the hunting season of 2006–2009 we examined 46 samples of skeletal and heart muscles obtained from hoofed game (deer, roe-deer, fallow-deer, moufflon, wild-boar) hunted down in hunting grounds of eastern Slovakia. Of the total number of 46 heads of hoofed game of varying age (6 months – 10 years) examinations for the presence of microcysts of *Sarcocystis* spp. were carried out in 2 deer (males), 8 red-deer (4 males and 4 females), 3 fallow-deer (1 male and 2 females), 3 moufflons (2 males and 1 female) and 30 wild-boars (22 males and 8 females). The specimens collected were frozen (–18 °C) as it was impossible to examine them immediately after collection. They were allowed to thaw before examination and were stored for 14–24 h at room temperature.

Digestion method: 15 g of sample were cut to small pieces and briefly mixed with 40 ml phosphate buffer containing 0.06 % trypsin. Trypsinization was carried out for 30 min at constant mixing with an electromagnetic mixer. After trypsinization the content was transferred to a centrifuge tube and centrifuged for 5 min at 1000 r.p.m. The supernatant was discarded, distilled water was added to the sediment and centrifuged for 2 min at 1000 r.p.m. Several drops of suspension were transferred to a microscopic slide, covered with a cover slip and observed under a microscope at 200–400 magnification.

Quantitative method: determination of the number of microcysts in 1 gram of sample.

RESULTS

Of 46 samples from hoofed game (deer, roe-deer, fallow-deer, moufflon, wild-boar), examined in 2006–2009, forty samples were infected. Our results indicated high prevalence (87.0 %) of muscle cysts of *Sarcocystis* spp. in hoofed game hunted down in Eastern Slovakia. The prevalence in individual game species was as follows: 50 % prevalence in 2 examined deer, 100 % in 8 roe-deer, 3 fallow-deer and 3 moufflons and 83.3 % in 30 examined wild-boars.

The heart and skeletal muscles were examined also for intensity of infection with microcysts of *Sarcocystis* spp. Intensity of infection was higher in males compared to females (roe-deer: male 361, female 59 microcysts.g^{-1} sample; fallow-deer: male 56, female 23 microcysts.g^{-1} sample; moufflon: male 67, female 7 microcysts.g^{-1} sample). In wild-boars intensity of infection was higher in females (female 46, male 25 microcysts.g^{-1} sample). Animals younger than one year showed higher intensity of infection with *Sarcocystis* spp. than animals of age above one year (roe-deer 30 g^{-1}, wild-boar 24 g^{-1}).

DISCUSSION

Forty of forty six hoofed game hunted down in Eastern Slovakia was infected. The total prevalence reached 87 %.

On the basis of results obtained in Poland prevalence reached 94.3 % in deer, 88.7 % in red-deer and 24.7 % in wild-boar (10). Comparable results were reached in Germany with 86 % prevalence in deer and 87.3 % in red-deer (9). The prevalence in deer in Lithuania reached 70.2 % (3), in Spain 63 % (7) and in the Czech Republic 76 % (1). High prevalence of sarcocystosis was observed particularly in red-deer probably due to more frequent occurrence of red-deer close to human dwelling and the related risk of consumption of growth contaminated with faeces of final hosts containing oocysts and sporocysts of *Sarcocystis* spp.

The intensity of infection expressed as number of microcysts per gram of muscle sample was significantly higher in males compared to females. Animals before one year of age shower lower infection intensity than older individuals. Our results agree with those of Kutkiené (4), Malakauskas (6) and Spicksen *et al.* (9).

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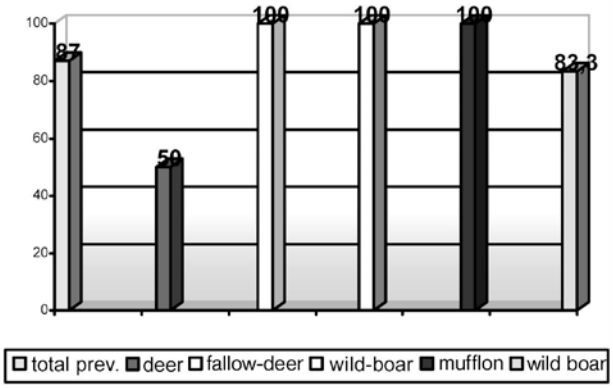

Fig. 1. Prevalence of sarcocystosis in hoofed game (%)

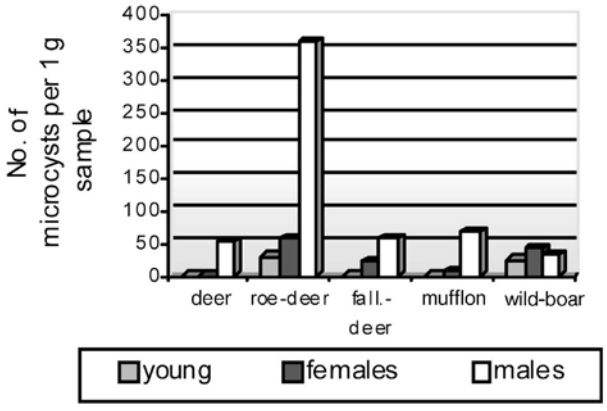

Fig. 2. Intensity of infection

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OCCURRENCE AND SPECIES DIVERSITY OF BITING MIDGES *(CULICOIDES)* – THE VECTORS OF THE VIRUS OF CATARRHAL FEVER IN SHEEP IN SLOVAKIA

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ABSTRACT

Catarrhal fever is a dangerous infection of ruminants that is transmitted by biting midges from typical tropic endemic regions. Occurrence of *Culicoides imicola*, the vector of the virus of catarrhal fever has been recently recorded in the countries of the Mediterranean region. However the function of the vector can be carried out also by the so-called cold loving species of midges, such as *Culicoides obsoletus*, *C. pulicaris, C. nubeculosus, C. deWulfi,* and others. To understand the spreading of vector-transmitted diseases it is necessary to obtain detailed data about their multiplication, occurrence and seasonability over a year in different environments and climatic zones. The entomologic investigation of the fauna of midges was performed from May 2008 in selected sheep herds in eastern Slovakia. Midges were captured regularly in weekly intervals using light catchers. Up to the end of November 2008, more than 18 thousand insects were caught by catchers of which there were more than 10 thousand (59 %) midges. The most numerous (more than 25 %) were the species belonging to the Complex *Obsoletus*. From the Complex *Pulicaris* 10.8 % species were diagnosed. Proportions of additional potential vectors from the Complexes *Schultzei* and *Nubeculosus* ranged between 2.6 and 0.1 %. Regarding the seasonal dynamics, most numerous populations populations were captured in weeks 25–26, i.e. between June 18th and 30th. In this period, the mean daily temperatures in the investigated region ranged between 16 and 25 °C (Ø 19.5 °C), and relative humidity between 58 and 87 % (Ø 66 %).

Key words: *Culicoides*; seasonal dynamics; species composition

INTRODUCTION

Midges are minute blood suckling insects belonging to the family Ceratopogonidae. Catarrhal fever is a very dangerous infection of domestic and free-living ruminants. Originally, it occurred in Africa, Australia, America and Asia whereas in Europe its occurrence was very rare before 1998. At first, it spread only to southern Europe but since 2006 the disease has also been recorded in the countries of northern Europe, such as the Netherlands, Belgium and Germany and since 2007 also in the Czech Republic (1). Herds in Slovakia are also in direct danger as a case of imported catarrhal fever has been recorded in cattle. The agent of catarrhal fever is BTV virus from the genus *Orbivirus*. Currently we recognize 24 serotypes of BTV. BTV 8 serotype was identified in northern Europe which till then had occurred only in Africa and America. The vectors of the virus are midges, especially *C. imicola*. They occur in Africa and southern Europe. As a thermophilic species they require mean environmental temperature higher than 12 °C and thus they do not occur in our territory. However, other species of midges can serve as vectors, such as *C. obsoletus*, *C. nubeculosus*, *C. pulicaris*, *C. punctatus*, *C. deWulfi* and others. In Slovakia till now sufficient attention has not been paid to the entomologic investigation of midges. So that Slovakia may meet the requirement of the Commission of European Community (2003/828/EC) on protection zones and observations in relation to catarrhal fever of sheep, complex investigation of these potential vectors has been initiated within the project AV/4/2041/08 and basic research at NRL for pesticides at the University of Veterinary Medicine in Košice.

Table 1. Total number of insects and midges captured on the investigated farm

Date	Week	N/H	*Culicoides* total		Complex *Nubecul.*		Complex *Pulicaris*		Complex *Obsoletus*		Complex *Schultzei*		*Culicoides* others	
			N	%	N	%	N	%	N	%	N	%	N	%
May 25	21	415	138	33.2	0	0	21	15.2	47	34.1	2	1.4	68	49.3
June 2	23	1842	707	38.4	7	1	16	2,3	191	27	26	3.7	467	660
June 18	25	1226	785	64	0	0	38	4.8	221	28.2	7	0.9	519	66.1
June 21	25	301	233	77.4	2	0.9	0	0	35	15	0	0	196	84.1
June 30	26	5618	4971	89.5	0	0	738	14.8	1317	26.5	207	4.2	2709	54.5
July 6	27	627	41	6.5	0	0	4	9.8	9	21.9	0	0	28	68.3
July 10	28	1861	579	31.1	0	0	44	7.6	76	13.1	3	0.5	456	78.8
July 14	29	3006	1001	33.3	0	0	133	13.3	231	23.1	0	0	637	63.6
July 22	30	308	143	43.5	0	0	8	5.6	33	23.1	4	2.8	98	68.5
Aug. 13	33	172	32	18.6	0	0	0	0	16	50	0	0	16	50
Aug. 20	34	117	55	47	0	0	1	1.8	17	30.9	0	0	37	67.3
Sep. 24	39	35	31	88.6	0	0	2	6.5	16	51.6	0	0	13	41.9
Sep. 29	40	30	20	66.7	0	0	3	15	13	65	0	0	4	20
TOTAL		17085	9754	57.1	11	0.1	1048	10.8	2478	25.4	256	2.6	5963	61.1

N/H – Total number of captured insects; N – number of captured midges

MATERIAL AND METHODS

Our entomological investigations started in 2008 in the sheep herd in eastern Slovakia. Midges were captured regularly in weekly intervals by means of a light catcher "Model 1212", which was placed at the entrance of the stable and the collecting container was located under a light source at the height of minimally 1.5 m (4). On the day of capture we measured also air temperature, relative humidity and airflow using manual thermohydrometer. The insects captured in the collecting container were transported to the laboratory either in a vessel containing 70 % ethanol, or "in a dry way". Each collection was marked and stored in a fridge or freezer until examination. After registration in the laboratory the insects were analysed and subjected to species identification using a binocular magnifying glass, stereo microscope and available keys (5, 2).

RESULTS AND DISCUSSION

Up to the end of August, 2008, 17,085 insects were captured by catchers, out of that 9,754 (57.1 %) midges (Tab. 1). More than 25 % were species belonging to the Complex *Obsoletus* and 10.8 % species were diagnosed as Complex *Pulicaris*. Additional potential vectors from the Complexes *Schultzei* and *Nubeculosus* were present in proportions ranging from 2.6 to 0.1 %. More than 61 % of captured midges were from the group of the so-called "other" *Culicoides* spp. midges that are not considered vectors of the virus of catarrhal fever. From the point of view of seasonal dynamics, the majority of potential midges was captured in weeks 25–26 with continuation up to week 29, i. e. in the period from June 18 up to July 14. The activity of midges was influenced by air temperature, light, relative humidity, and airflow. The optimum temperature for *C. obsoletus* is 7–6 °C and for *C. punctatus* 10–16 °C. The daily rhythm of blood suckling is influenced by periodical changes in the temperature and light intensity. Most of the midges are active at semidarkness and night, however, some species are also active during the day (3). In the period of our investigations, the mean daily temperatures ranged from 16 do 25 °C (Ø 19.5 °C) and relative humidity from 58 to 87 % (Ø 66 %).

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DETECTION OF *ICA* GENE ENCODING THE BIOFILM FORMATION IN *S. aureus* ISOLATES

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ABSTRACT

The aim of the study was to determine the prevalence of *icaADBC* locus encoding polysaccharide intercellular adhesin, poly-N-glucosamin (PIA-PNSG), responsible for biofilm formation in *Staphylococcus aureus*. PCR analysis using *icaADBC* specific primers of 45 *S. aureus* isolates from raw cow and sheep milk and sheep cheese showed that 11 (24.4 %) were *ica* positive. The highest prevalence of *ica*+ samples (8/23, 34.8 %) was detected in the group of isolates from sheep milk. In the group of 13 *S. aureus* isolates from sheep cheese 2 (15.4 %) were *ica* positive. In the group of 9 isolates from cow milk only one (11.1 %) sample was *ica* positive.

Key words: biofilm; *icaADBC* locus; *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus is an opportunistic pathogen whose ability to persist and multiply in a variety of environments causes a wide spectrum of diseases in both humans and animals. Many chronic infections induced by *S. aureus* are associated with its ability to produce biofilm. Two major surface components have been implicated in biofilm formation by *S. aureus*: (i) the product of the *icaADBC* operon which encodes proteins involved in the synthesis of polysaccharide intercellular adhesin (PIA), the composition of which is poly-N-glucosamin (PIA-PNSG) (1); and (ii) Bap surface protein (2). Bap promotes both primary attachment to inert surfaces and intercellular adhesion, whereas PIA/PNAG seems to be involved in intercellular adhesion alone. The *bap* gene has only been found in bovine mastitis isolates (2).

The purpose of this study was to use PCR amplification to investigate the presence of *icaADBC* locus in *S. aureus* isolates (n = 45) originating from raw cow and sheep milk and sheep cheese.

MATERIALS AND METHODS

Bacterial strains and culture media: *S. aureus* strains (n = 45) used in this study were collected at the Department of microbiology and immunology from raw cow milk (n = 9), sheep milk (n = 23) and sheep cheese (n = 13). Staphylococci strains were cultured in brain heart infusion (BHI) broth at 37 °C for 16–18 h.

Nucleic acid amplification – PCR: Nucleic acid amplification was performed on *S. aureus* genomic DNA isolated according to Hein *et al.* (4). PCR amplification of the part of *ica* gene was performed using primer pairs icaF: 5´-TAT ACC TTT CTT CGA TGT CG-3´) and icaR (5´-CTT TCG TTA TAA CAG GCA AG-3´) with an initial denaturation step at 94 °C for 2 min followed by 40 cycles at 94 °C for 20 s, 46 °C for 20 s, and 72 °C for 50 s, with final extension at 72 °C for 5 min (3). The size of the PCR products (616 bp) was analyzed by electrophoresis on 1.0 % ethidium-bromide-stained agarose gels.

RESULTS AND DISCUSSION

PCR analysis using the *icaADBC* specific primers of 45 *S. aureus* isolates originating from raw cow and sheep

milk and sheep cheese showed that 11 (24.4 %) were *ica* positive. The highest prevalence of *ica*+ samples (8/23, 34.8 %) was detected in the group of sheep milk isolates. In the group of sheep cheese 2 of 13 samples (15.4 %) were *ica* positive. In the group of *S. aureus* isolates from cow milk only one (11.1 %) was *ica* positive (Table 1).

Table 1. Prevalence of *ica* gene in *S. aureus*

Origin	No. of samples	*ica* positive isolates	
		N	%
Raw cow milk	9	1	11.1
Raw sheep milk	23	8	34.8
Sheep cheese	13	2	15.4
Total	45	11	24.4

The ability of biofilm formation is one of many factors affecting pathogenicity of *Staphyloccocus aureus.* Bacteria growing in a biofilm resist to action of antibiotics. Biofilms may be detected by genotypic and phenotypic methods. Genotypic methods are based on demonstration of genes responsible for adhesion of microbial cells to surfaces (*spa* gene), or genes involved in synthesis of extracellular matrix (*ica* gene). Potential ability of staphylococci to produce biofilms can be proved on the basis of presence of genes of *ica* operone responsible for production of the key component of biofilms – polysaccharide intercellular adhesin (PIA), most frequently by PCR. Interpretation of results is complicated because it is necessary to ascertain whether these genes are expressed and whether the examined isolate really forms a biofilm. Therefore, in order to prove the biofilm formation ability of staphylococci one must also use *in vitro* cultivation of these bacteria on abiotic surfaces (e.g. 96-well polystyrene microtitration plate) with subsequent confirmation of biofilm presence by its staining and spectrophotometric evaluation (5).

CONCLUSION

Staphylococcus aureus is one of the most important pathogens causing mastitis in farm animals. Its ability of biofilm formation is an important factor affecting long-term persistence of these bacteria in the mammary gland that eventually results in chronic mastitis. Moreover, biofilm formation decreases effectiveness of antibiotic therapy. Because of that the virulence of *S. aureus* is confirmed also by presence of genes participating in biofilm formation. However, the PCR method reveals only genetic predisposition for biofilm formation and expression of this gene and thus the real biofilm formation must be confirmed by additional phenotypic methods.

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INCORPORATION OF PROSPECTIVE ANTICANCER DRUG HYPERICIN INTO FATTY-ACIDS-CONTAINING SERUM ALBUMINS

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ABSTRACT

Serum albumins (SA) constitute quantitatively the biggest group of blood plasma proteins and affect significantly transport and regulation body processes. They bind to a wide range of substrates and thus can be transported by blood (1). One of such substrates is hypericin (HYP), the potential drug used for the treatment of oncogenic diseases by photodynamic therapy. The study investigated incorporation of hypericin into structure of fatty-acids(FA)-containing albumins by determining binding constants of the respective complexes and the time of incorporation of drug into the macromolecule of serum albumins (bovine serum albumin – BSA and human serum albumin – HSA). When comparing the binding constants of complexes HYP/SA without FA and HYP/SA with FA, higher binding constant was determined for the latter which indicated that fatty acids increase affinity of HYP to SA. Thus hypericin will bind preferentially to molecules with higher lipophility. Kinetics of incorporation of HYP into SA with FA differs by its character for albumin from different sources (HSA and BSA) which should be considered regarding their potential use in biological organisms.

Key words: binding constant; fluorescence intensity; hypericin; serum albumins

INTRODUCTION

Photodynamic therapy (PDT) is based on administration of a photosensitive substance which accumulates preferentially in tumour cells and upon irradiation causes their destruction. Hypericin (HYP) (Fig. 1) is a potential photosensitive substance for use in the treatment of oncogenic diseases by the PDT method. Its natural source is St. John's Wort (*Hypericum perforatum*) (2). Serum proteins: lipoproteins (LDL, HDL) and serum albumins (SA) act as natural transport systems of photosensitive substances (including HYP) in the blood. The aim of our study was to determine binding constants and kinetics of complexes of HYP with fatty-acids-containing serum albumins and thus characterise partially transport of the drug in an organism.

MATERIAL AND METHODS

HYP containing complexes HYP(KRD)/SA (Sigma-Aldrich) with constant concentration of 10^{-7} M and variable concentration of SA were prepared in phosphate buffer, pH 7.4; HYP was dissolved in dimethylsulphoxide (its content did not exceed 1 %). Fluorescence emission spectra of HYP in complexes were measured at excitation wavelength of 550 nm and the fluorescence intensity was measured as a function of i) concentration of SA (for binding experiments) and ii) time (for incorporation kinetics). Spectra were processed by means of software Microcal Origin, version 6.0. The relationships were fitted to a Langmuir equation which served to determine dissociation and subsequently the binding constants. Kinetics of incorporation were fitted to diexponential function.

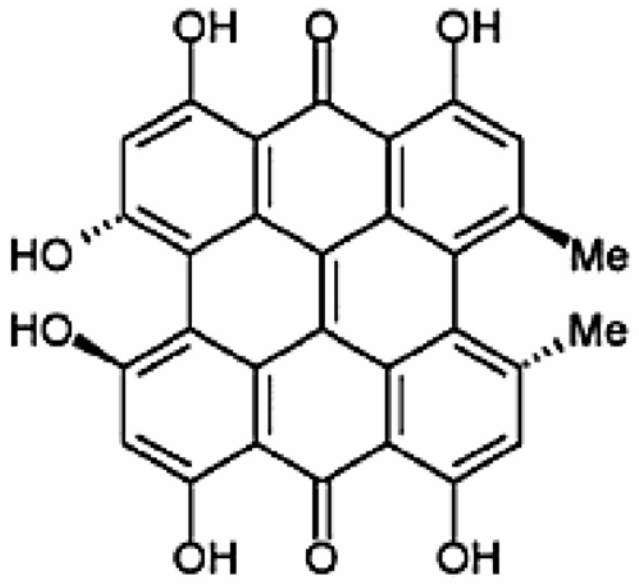

Fig. 1. Chemical structure of hypericin

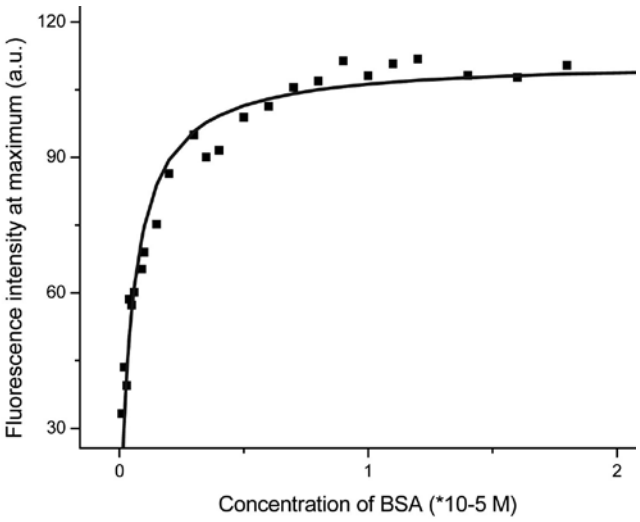

Fig. 2. Binding curve of the complex HYP/BSA

RESULTS

The binding curve shown in Fig. 2 served to determine for the first time the binding constant of the complex HYP/BSA with FA: $K_B = 20 . 10^5 M^{-1}$ which could be compared to the binding constant of the complex HYP/BSA without FA (3).

Kinetics of the complex HYP/BSA allowed us to determine two half-times of binding that were in intervals 0.1–1 min and 1–10 min.

Study of the complex HYP/HSA showed a shift in fluorescence maximum wavelength with time.

DISCUSSION AND CONCLUSION

The results obtained point to differences in the character of incorporation of HYP into SA with FA in comparison with incorporation of the drug into SA without FA (3). The higher binding constant of HYP/BSA with FA compared to the binding constant of HYP/BSA without FA suggests increased affinity of HYP to BSA with FA. Fatty acids in the structure of BSA increase lipophilicity of the macromolecule which facilitates stronger incorporation of HYP into BSA with FA. This allowed us to assume that the binding site for HYP was not occupied by FA.

Kinetics of incorporation of HYP into SA differs for albumins from various sources (HSA and BSA). Kinetics of HYP/BSA involves probably two stages in a real time and in addition to that one may anticipate also very swift kinetics.

The shift in fluorescence maximum with time indicates increased hydrophobicity of HYP surroundings after incorporation into molecule HSA without FA (4).

ACKNOWLEDGEMENT

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A COMPARATIVE STUDY OF THERAPY OF RUPTURED *LIGAMENTUM CRUCIATUM CRANIALE* BY TPLO AND TTA METHODS – A PRELIMINARY STUDY

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ABSTRACT

Rupture of the *ligamentum cruciatum craniale* (LCC) is a common multi-etiological disease of the stifle joint in dogs of medium and large breeds. Surgical intervention at the knee joint appears to be the most suitable therapy of LCC rupture. Up to this date many therapeutic methods of varying successfulness have been developed. The aim of the present study was to observe and compare clinical therapeutic results of two modern methods, TPLO (*Tibial Plateau Leveling Osteotomy*) and TTA (*Tibial Tuberosity Advancement*) that do not involve replacement of the ruptured cruciate ligament but aim to change the biomechanical forces acting at the stifle joint. Their neutralization is ensured by changing the angle between tibial plateau and patellar ligament or the tibial axis to 90°. Observation of patients for up to 6 months after the operation allowed us to conclude that the TTA method appeared more suitable as it resulted in minimum peri-operative morbidity and permitted almost complete restoration of leg's function and disappearance of lameness.

Key words: dogs; rupture of the cranial cruciate ligament; treatment; TPLO; TTA

INTRODUCTION

Rupture of the cranial cruciate ligament (*ligamentum cruciatum craniale* – LCC) is one of the most common diseases of the stifle joint in dogs and is manifested by acute onset of limping. Its develops due to overcoming the ligament elasticity when increased forces act at the stifle joint or weakening of the ligament and failure to support the knee. It can result from trauma, ligament degeneration, abnormal angles of pelvic limbs, developed arthritis, age, body weight of dog and similar (2).

The selection of method and suitability of therapy depends on animal size, practical experience of veterinarian, owner solvency and available instruments (5, 7). The primary aim is to restore the function of ruptured LCC. Because the ligament cannot be replaced physically but only morphologically, novel therapeutic methods have been developed, such as LCC, TTA and TPLO, and became a subject of investigations regarding successfulness of relevant surgical therapy and overcoming the problem (6).

The aim of the present study was to evaluate and compare practical usability and effectiveness of TPLO and TTA modern intra-articular therapy of LCC rupture in dogs.

MATERIAL AND METHODS

Relevant patients were treated and observed at the Clinic of small animals of UVM in Košice, Section of surgery, orthopaedics and roentgenology in the period of 2006–2008. TTA and TPLO methods were used to treat 8 dogs (TTA – 5; TPLO – 3) of large breeds: Labrador retriever, Doberman, Rottweiler, German shepherd and Caucasian sheepdog, 1.5 to 8 years old, weighing 30 to 58.5 kg.

The examination of dogs included anamnesis, clinical examination, specific tests (tibial-compression, drawer, sitting), evaluation of lameness and X-rays (neutral, stress). Surgery was carried out after evaluation of health state.

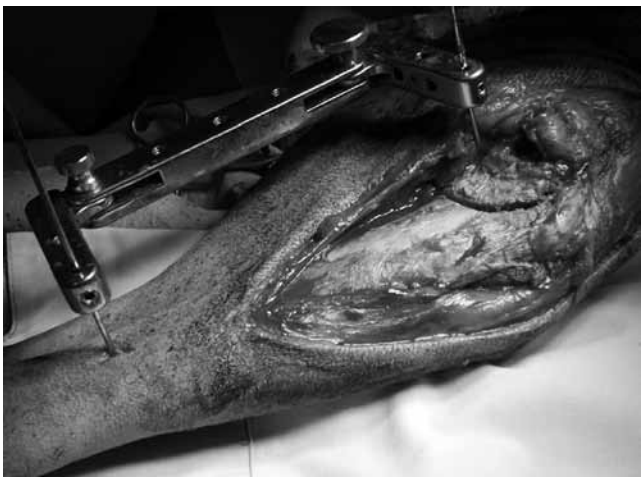

Fig. 1. Bone segment after osteotomy of tibia

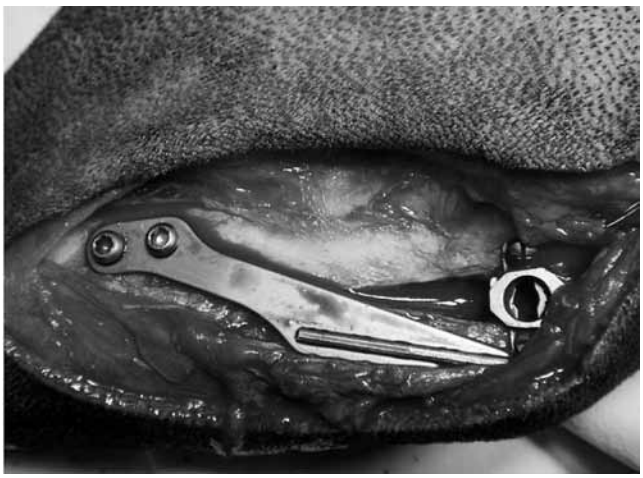

Fig. 2. TTA cage with a plate

TPLO surgical treatment of cranial cruciate ligament rupture

The TPLO method was developed by Slocum and Devine in 1993 (9). It is based on rotation of the obtained bone segment of tibia following its osteotomy by predetermined distance determined from a radiograph to level it at 90° angle between tibial plateau and long tibial axis. During the surgery the bone segment is fixed to tibia using a TPLO jig (Fig. 1). The altered slope of tibial plateau is stabilised by a TPLO plate of required shape which is fixed to tibia with bone screws.

TTA surgical treatment of cranial cruciate ligament rupture

The TTA method was developed by Tepic and Montavon in 2002. The authors presented a biomechanic analysis stating that shear forces acting at LCC can be eliminated by advancing tibial tuberosity to achieve a perpendicular relationship between the tibial plateau and patellar ligament axis (8). The 90° angle is achieved by moving away the bone segment of *tuberositas tibiae* after its osteotomy by the distance read from a radiograph. The bone segment is then fixed by means of a TTA plate using a special fork and stabilised by a TTA cage (Fig. 2). A bone graft is then used to fill up the defect after osteotomy.

RESULTS AND DISCUSSION

The condition of patients subjected to therapy of LCC rupture by TTA and TPLO methods was as follows:

1) In the majority of patients lameness disappeared within 6 months after the surgery,

2) the dogs put weight on operated limbs within 4 days after the surgery with the TTA method and within 7 days after the TPLO method,

3) Development of arthritic changes was recorded only with TTA in two patients within 6 months after the surgery. The successfulness of therapy characterised by cessation of limping and restoration of leg function within 6 months following the surgery reached 80% with TTA and 66% with TPLO.

TPLO and TTA methods are relatively new alternatives of surgical therapy of LCC rupture. However, with both methods there is a risk of damage to meniscus in the post-operative period (TPLO 2%; TTA 9–10%) (1).

Good results with the TPLO method were achieved by Vezzoni (10) with successfulness reaching 90%. Lower successfulness (69%) was recorded by Hulse and Hudson (4).

The TTA method appears to be a more advantageous alternative of LCC rupture surgical treatment. It reduces operation time and peri-operative morbidity of patients and shortens return to weight-bearing after the surgery (3, 4, 8). Successfulness of therapy in the study by Hulse and Hudson (4) reached 90%.

Despite the relatively small number of patients we can support the view that the TTA method is more advantageous compared to TPLO due to earlier return to weight-bearing, lower iatrogenic damage and lower number of recorded complications. Both methods constitute a contribution to the complex solution of LCC rupture. Their application is justified particularly in large breeds and in dogs in which the previous therapeutic methods were unsuccessful, or dogs with bigger than 26° angle of tibial plateau.

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DAILY DYNAMICS OF HOOF TEMPERATURE

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ABSTRACT

Under normal conditions considerable thermal symmetry prevails in the body of horse and therefore abnormal or asymmetric temperature commonly indicates a problem. Palpation is unable to identify temperature differences smaller than 2 °C (1). Thermography allows one to identify diseases of hoofs and other organs already two weeks before the clinical symptoms begin to appear (2). Decreased circulation may occur in affected tissues due to swelling or thrombosis which can be manifested by decreased temperature. The aim of the study was to investigate external and potentially also internal influences on temperature of hoof in a group of 8 horses.

Key words: hoof; pododermatitis; temperature; thermography

INTRODUCTION

All environmental factors, various pathological processes, injuries, split hooves, decubiti and other hoof traumas affect its functional properties. Thermometry of hoof surface temperature reflects changes in blood supply from deeper hoof tissues and thus enables to identify zones affected by pathological processes. Tissues affected by inflammatory processes exhibit increased circulation and appear as hyperthermic zones. On the other hand, tissues with insufficient blood supply are manifested as hypothermic.

MATERIAL AND METHODS

The experimental group (EG) of 8 horses of American Quarter Horse (AQH) breed consisted of 5 mares, 1 gelding and 2 stallions of mean age 4.31 ± 3.15 SD years (min. 0.003 and max. 11 years) and of varying use. Temperature of hoofs (Tk) was measured at the medial line of dorsal surface of hoof capsule, 3 cm below coronet band, using infrared, non-contact digital thermometer TN2 with laser pointer (Electronic Temperature Instruments Ltd., UK). In the neonatal subject we had to consider the hoof size so the temperature was measured in the point according to the respective ratio of distances from the coronet band of the hoof (approx. 7–8 mm). To monitor the daily temperature dynamics of hoofs we measured Tk every morning and evening or before and immediately after the load. We recorded in parallel body temperature of individual horses (Tt), environmental temperature (Tpros) and temperature of the support (Tpod) on which the hoof temperature presumably depended. The data obtained were recorded in experimental protocol and processed by mathematic-statistical methods in numeric and graphic form.

RESULTS

Altogether 1024 measurements of hoof temperature were made in EG. The hoof temperature was affected most significantly by morning temperature of the support shoeing decreases sensitivity) and the environment (Fig.1).

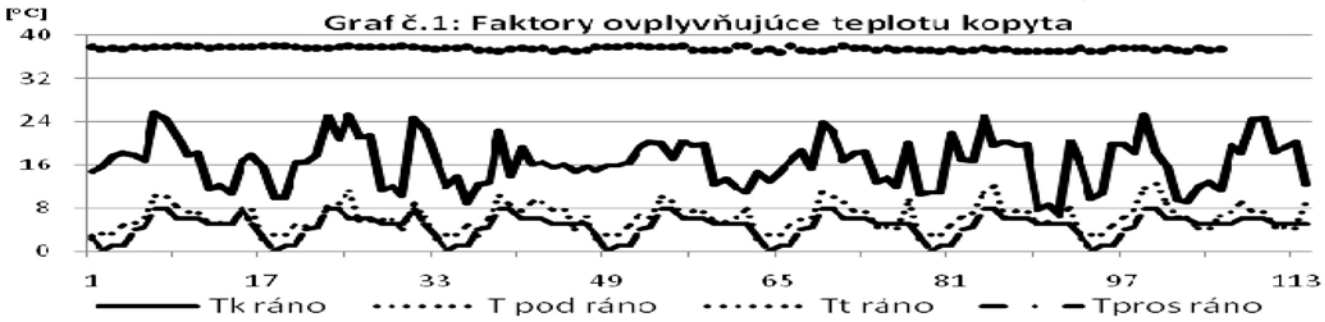

Fig. 1. Factors affecting hoof temperature in the morning

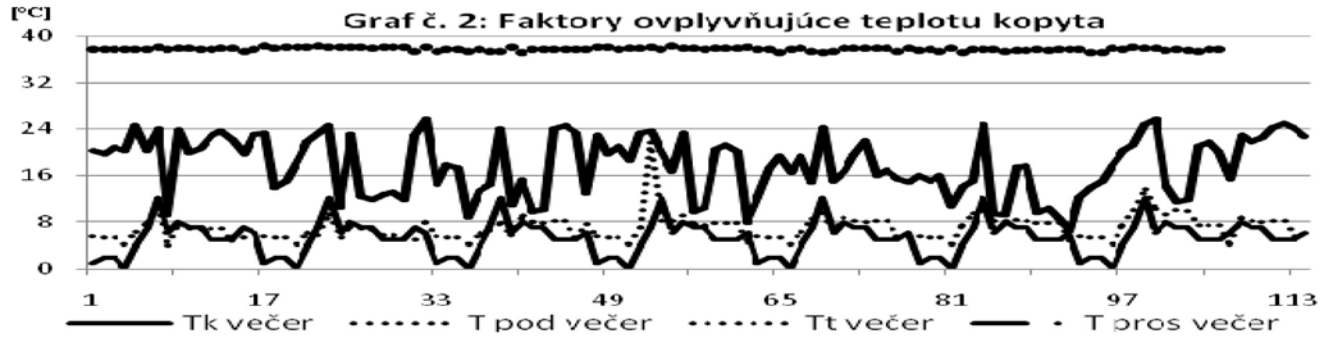

Fig. 2. Factors affecting hoof temperature in the evening

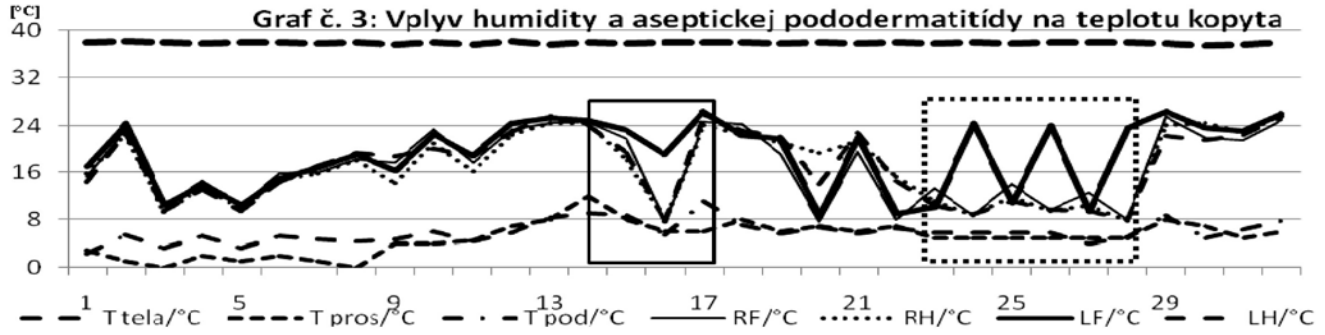

Fig. 3. Effect of humidity and aseptic pododermatitis on hoof temperature

The evening body temperature and environmental temperature had no significant effect on the temperature of hooves (Fig. 2). A more marked effect of other tested environmental factors on temperature dynamics of hoofs was recorded only sporadically.

A significant Spearman correlation was observed between morning and evening hoof temperature and and the difference between both test criteria was highly significant ($P < 0.0001$) according to paired t-test. Increased humidity of the terrain in horse run resulted in a significant decrease in hoof temperature in all experimental horses (Fig. 3, measurements 15–17, delineated by solid line). During the experiment we recorded aseptic pododermatitis on the left thoracic limb in one horse. It was manifested by significantly increased temperature of the affected hoof (Fig. 3, measurements 23–28, delineated by dashed line). The horse was subjected to NSAIDs treatment and was shoed which resulted in a return of hoof temperature back to symmetric values.

Stereotypical daily regimen of horses affects positively the thermal symmetry of individual hoofs. Analysis of presented Figures indicated that physical loading of observed horses had a significant positive effect on immediate dynamics of Tk, i.e. in the majority of cases the temperature after loading was significantly higher than before loading (Fig. 4).

The graphic results were also confirmed by statistical evaluation using paired t-test which showed that the difference between the two test criteria (Tk before loading and after loading) were significant at the level of $P < 0.05$.

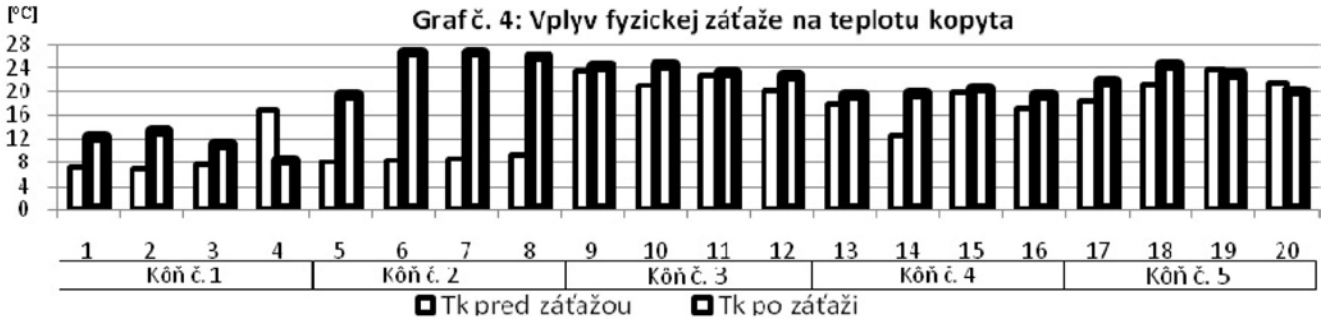

Fig. 4. The influence of physical loading on hoof temperature. Horses 1–5, Tk before loading, Tk after loading

DISCUSSION AND CONCLUSION

Thermography is a non-invasive diagnostic method allowing one to measure the surface temperature which reflects health of soft tissues and bone structures located close to horse body surface and facilitates early diagnosis of potential pathological changes that may prove decisive for successful treatment. Observation of hoof temperature changes in orthopaedic patients is useful for diagnosis, prognosis and evaluation of damage to hoof frog. We investigated the influence of environmental, support and body temperature, physical load and potential pathological processes in locomotory apparatus on daily temperature dynamics of hoof as well as the usability of pyrometry in horse shoeing. The mean temperature of hoof of experimental horses was 17.2 °C (min. 3.6 °C, max. 27.2 °C, SD 5.47 °C). Our results showed a significant influence of morning support temperature ($P < 0.001$), morning environmental and body temperature, evening support temperature and physical load ($P < 0.05$) on daily dynamics of hoof capsule temperature while significant effect on hoof temperature of other investigated factors was observed only sporadically. Pyrometry appeared to be a suitable method for detection of temperature changes in hoof capsule from both practical and economical point of view.

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COMMON LAMENESS IN WESTERN HORSES

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ABSTRACT

The origin of Western horseback riding, a new riding discipline in Slovakia, dates back to the early nineties of the last century. Since then it has developed rather abruptly, from weekend enthusiastic amateur events up to the current professional sport. With increasing number of equestrians and horses competing at international events, the diseases typical of this type of sport also began to appear.

The most frequent causes of front limbs lameness are palmar pain syndrome, desmitis of the proximal interosseous muscle insertion, chip fractures, carpal diseases and degenerative diseases of interphalangeal joints. In back limbs the most frequent are degenerative diseases of interphalangeal joints, inflammation of distal intertarsal and tarsometatarsal joints, osteochondrosis of femoropatellar and femorotibial joints, desmitis of the the proximal interosseous muscle insertion and chip fractures of pastern bone. A frequent problem affecting dorsal region is kissing spine, involving thoracic and lumbar spinous processes.

Key words: chip fractures; desmitis; kissing spine; osteochondrosis; palmar pain syndrome; Western horseback riding

INTRODUCTION

Preferred breeds in western sports are American quarter horse, Paint horse and Appaloosa, all animals of lower height, approximately 145–155 cm, active, psychically stable, capable of optimum performance even under considerable stress.

Good results in equine sports are based on breeding of successful lines which resulted on the one hand in extremely agile and fast horses but, on the other hand, in horses with small body and hoofs that participate in development of diseases in the distal region of limbs.

Diseases of the podotrochlear apparatus are frequent in Western horses (1). They occur commonly in reining and cutting horses in which the small hoof compared to body size is a predisposition factor.

Fatigue participates in development of desmitis either of collateral ligaments or interosseous muscle. Additional predisposition factors are improper shoeing, painfulness of back limbs and unsuitable surface (1).

Degenerative diseases of interphalangeal joints include ringbone, i. e. osteoarthrosis of the pastern or coffin joint and DJD.

Chip fractures involve most frequently *processus extensorius* of the coffin bone, proximal edge of the pastern carpal joint, particularly C3 fracture and individual phalanges of the digit. Relatively frequent is the chip fracture of the short pastern.

In back limbs the most frequently affected are hock and knee joint. Distal tarsal joints are joints with small range of movement exposed to action of various torsion forces so the question is not whether but when the inflammation is going to occur. Usually the first sign is not lameness but decrease in performance or worsened performance of some manoeuvres. Painfulness frequently subsides spontaneously after ankylosis. Ankylosis may be stimulated partially either by injection of sodium monoiodoacetate (2) or surgically by destruction of distal tarsal joint cartilage with a drill inserted through a joint fissure (3).

Frequent diseases of the knee joint include osteochondrosis which affects most commonly the medial regions of femoropatellar and femorotibial joints.

With kissing spine the lameness results from increased pressure at thickening of proximal ends of dorsal spinous processes. It is usually located between Th 12 – L 3. The condition is frequently a consequence of injury which may manifest itself 2 – 3 years later (4) or develops as a result of *spondylosis deformans* after loss of ventral and ventrolateral support structures of *anulus fibrosus* (5).

MATERIAL AND METHODS

The present study focused on cases examined at the Clinic for horses and in field practice, their comparison with published data and impact of diagnosed diseases on further use of horses for work and sport. We examined altogether 5 horses, of them 3 stallions, 1 gelding and 1 mare.

Four of them were reining horses that competed at top European events and one was barrel horse. Changes were observed by X-ray examination using apparatuses HF 80 and Chirax 70, with digitalisation units Agfa CR 30 and Orex PcCR 1417.

We used latero-medial, latero-lateral and dorso-palmar projections. Diagnosis of kissing spine was supported by scintigraphic examination. All horses were examined clinically for signs of lameness.

Damage to soft tissues was diagnosed by ultrasonography by means of a Mindray 6600, using 7.5 and 10 Mhz linear probes.

RESULTS

The study was carried out on 5 horses of breed American quarter horse, 4 of them reining and 1 barrel horse. The horses were 8 to 13 years old.

Desmitis of colateral tendons of coffin joints was diagnosed as a cause of lameness in two patients, namely in 8 and 11 years old geldings, competing in reining for more than 5 years. The process was located in the region of fetlock and pastern joint or coffin joint. One horse was treated with NSAID (phenylbutasone) and the other was administered corticoids (betametazone, 3 doses every 3 weeks) locally and preparation Cortaflex™ perorally.

Chip fracture was diagnosed in two cases, 8-year old barrel mare had fracture located at the dorsal surface of carpus LFL in the C3 region, while the 13-year old reining stallion suffered fracture of palmar lateral ligament tuber of pastern. Conservative therapy was selected using locally applied corticosteroids (betametazone or dexametazone) and pause in training for 12 months.

The last patient was 12-year old stallion competing in reining for 9 years. The primary symptoms were head shaking and head held low which culminated in fall of the horse together with the rider. Series of examination resulted in diagnosis of kissing spine in the region Th 14–16, affecting 3 vertebrae. Training of the horse was discontinued for 12 months during which NSAID were applied and the horse underwent physiotherapy consisting of riding and lungeing with neck kept low to relieve the dorsal muscles.

DISCUSSION

When diagnosing orthopaedic problems in Western horses one should keep in mind that the horse does not show signs of lameness in the initial stages of disease. Complaints of the owner regarding changed behaviour are frequently the only reason for examination of the horse. X-ray examination of the contralateral limb should be a part of the diagnostic process. In the early stage, when only soft tissues are affected and the process is not detectable by X-rays, the examination should be repeated after 3–4 weeks. This applies particularly to degenerative processes. An alternative method in the acute stage is nuclear scintigraphy (6). This worked out in two cases in which decreased performance preceded the lameness.

Desmitis of collateral ligaments is a potential differential diagnosis in case of lameness located in the region of distal interphalangeal joint (7). Desmitis was the definitive diagnosis in two cases in which X-ray examination either failed to show changes in bone basis or the changes were clinically insignificant. Changes in ligaments were confirmed by ultrasonographic examination.

Chip fractures at proximal end of the large pastern of front limbs are relatively frequent. The majority of these fractures affect the dorsal surface. Their occurrence in other locations is relatively scarce. In the observed case the fracture was located at the lateral edge of the distal end of the large pastern but was clinically insignificant. In another case we detected avulsion fracture of palmar margin of proximal joint surface which caused lameness.

Avulsion fracture of the palmar margin of the proximal joint surface of pastern has been frequently subjected to successful conservative treatment (4). In our case the horse was allowed to rest for 12 months during which fixation of chip occurred. Chip fracture of carpus affects most frequently the radial carpal bone, 3rd carpal bone (C3) and medium carpal bone (4). In our case we observed fracture of the dorsal surface of C3.

CONCLUSION

The study investigated 5 Western horses involved in top sport competitions for 5 and more years, all of them of American quarter horse breed, bred for this type of sport. They started with their career at the age of 2 years.

In the majority of them the trainers observed decreased performance, worsened performance of some manoeuvres or reluctant movement before manifestation of clinical symptoms and lameness. All these signs could be explained by an effort to protect the affected structures against more serious damage.

This is the reason why we should consider these signals as indications of potential problems and together with trainers pay increased attention to such patients.

This will enable to initiate effective therapy already in the early stage of the disease.

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COMPARISON OF INNOVATED OESTRUS SYNCHRONIZING METHODS APPLIED AT THE BEGINNING OF BREEDING SEASON IN IMPROVED WALLACHIAN SHEEP

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ABSTRACT

Production of milk lambs for Easter market with required economic effect calls for adequate preparation of ewes in the mating season. Therefore, one must select optimum method and procedure of intervention in the reproduction cycle. The goal of the study was to verify and compare the effect of breeding and biotechnological methods of stimulation and synchronisation of oestrus in improved Wallachian ewes. Both the effect of innovated methods of controlled reproduction and the effect of breeding methods in combination with biotechnological methods were observed with focus on production of slaughter lambs for early Easter market. Our efforts resulted in reproductive parameters highly exceeding the breeding standards and in subsequent successful sale of weight-balanced lambs for the early Easter market.

INTRODUCTION

The methods of assisted oestrus are divided to natural (breeding) and pharmacological. The measures taken by the breeder or management include formation of sheep groups according to the stage of sexual cycle, abrupt increase in feed rations (flushing), introduction of test rams into the flock of sheep and stimulation of the sexual cycle by light regimen. The medicamentous synchronisation of oestrus can be divided to the methods using preparations prolonging the sexual cycle and those that result in its shortening. The aim of the present study was to validate the innovated methods of assisted oestrus for early beginning of mating according to the requirements of Easter market.

MATERIAL AND METHODS

We compared the effect of GnRh in combination with D-cloprostenol according to the OvSynch protocol with the effect after treatment with innovated Chronogest ® CR in combination with eCG and the effect of pheromones of rams in the flock (ram effect). Altogether 500 ewes were mated. The first group with 300 ewes was exposed to the effect of pheromones of rams in the flock. The second group of 170 ewes was treated with preparations Chronogest®CR (cronolone, 20 mg) and Sergon (eCG, 500 I.U.). The third group of 30 ewes was treated with preparation Supergestran (lecirelin, 12.5 μg) in combination with preparation Remophan (D-cloprostenol, 37.5 μg) according to the OvSynch protocol.

RESULTS AND DISCUSSION

The results obtained were processed statistically and were evaluated by chí (χ^2) quadrate test.

The reproduction parameters in the group subjected to innovated progesterone treatment in combination with eCG were comparable and higher than those in other breeds (11, 12) and also higher than those at out-of-season mating (8). Reproductive parameters in the group treated according to the OvSynch protocol exceeded significantly the hitherto results (2, 7). The effect of rams on the production of LH in ewes in all stages of the oestrus cycle was confirmed (5) as well as their positive effect on fertility and ovulation ratio in time after withdrawal of progesterone sponges (sudden introduction of rams) (4).

Table 1. Basic reproductive fertility parameters

Group	Number of treated ewes	Number of lambed ewes	Fertility (%)	Fecundity (%)	Natality (%)
"ram effect"	300	281	93.7[d]	108.0[a,c]	115.3[a,b]
Crono-lon+eCG	170	167	98.2[c,d]	168.2[a]	171.3[a]
Lecirelin+ D-cloprostenol (OvSynch)	30	27	90.0[c,d]	126.7[a,c]	140.7[a,b]

[a] – P < 0.001, [b] – P < 0.01, [c] – P < 0.05, [d] – P > 0.05

Table 2. Parturitions and the number of produced lambs

Parameter	Groups of animals					
	Ram effect		Cronolon+eCG		OvSynch	
	N	%	N	%	n	%
Ewes with 1 lamb	238	84.7	50	29.9	19	70.4
Ewes with 2 lambs	43	15.3[a,c]	115	68.9[a]	8	29.6[a,c]
Ewes with 3 lambs	0	0	2	1.2	1	3.7

[a] – P < 0.001, [c] – P < 0.005

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EVALUATION OF HAEMOGLOBIN AND MYOGLOBIN IN POULTRY SLAUGHTERED BY STUNNING AND KOSHER SLAUGHTER

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ABSTRACT

The study was conducted to evaluate the differences in haemoglobin and myoglobin values in poultry slaughtered after previous stunning, improperly bled chickens and through the kosher slaughtering process. All poultry products were homogenized and haemoglobin and myoglobin levels were estimated from the homogenate for their respective levels. The results were compared and evaluated to determine which process was more effective in removing the blood.

Key words: blood; haemoglobin; kosher slaughter; myoglobin; poultry; stunning

INTRODUCTION

Assays for quantification of myoglobin in striated muscle must distinguish this protein from haemoglobin of blood since the two tissues can not be completely separated. These proteins may be physically or chemically distinguished on the basis of differences in their absorption spectra after derivation, molecular weight, size, isoelectric points or antigenicity. The separation is accomplished by use of electrophoresis, chromatography, differential salt extraction, absorption spectroscopy or immunologic assays (1).

The aim of this research was to evaluate the differences in haemoglobin and myoglobin values in poultry slaughtered by stunning, improperly bled chickens and after the kosher slaughtering process.

MATERIALS AND METHODS

Three groups of six chickens each were analyzed for their residual blood level in muscle. Samples where taken from both light (breast) and dark (thigh) meat. Group 1 were poultry slaughtered under regular conditions after stunning. Group 2 were poultry that were slaughtered according to ritual rites and subsequently koshered (residual blood was removed by salting of meat). Group 3 was poultry excluded from human consumption on the basis of insufficient bleeding.

For myoglobin and haemoglobin extraction from all meat samples we used modified method published by O'Brien *et al.* (1). All the chickens in each category had their thigh and breast removed. The specimens were frozen to prevent denaturing during the homogenizing process. After freezing, the whole breast and the whole thigh were blended into smaller pieces. 5 g of sample and 15 ml of 80 Mm KCl was mixed with 50 Mm Tris-HCL pH 8.0 buffer in a test tube. The buffer mimics intracellular ion concentration and prevents denaturation of myoglobin by acid produced during glycolysis (1). The samples were than homogenized for 2 minutes at 3500 rpm, rinsed with additional 5ml of the buffer and centrifuged at 3500 rpm at 21 °C for 30 min to clarify the suspension. The supernatant in each sample was used as a stock sample solution for further evaluation.

Haemoglobin and Myoglobin Evaluation

A modified method according to Goyal and Basak (2), was used: haemoglobin (or haem) acts as a chemical catalyst to

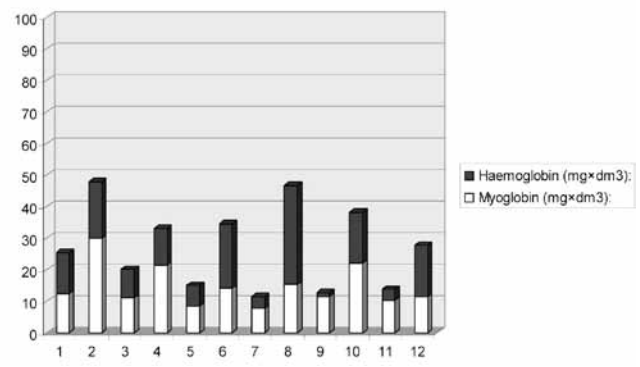

Fig. 1. Group 1 – stunned chickens

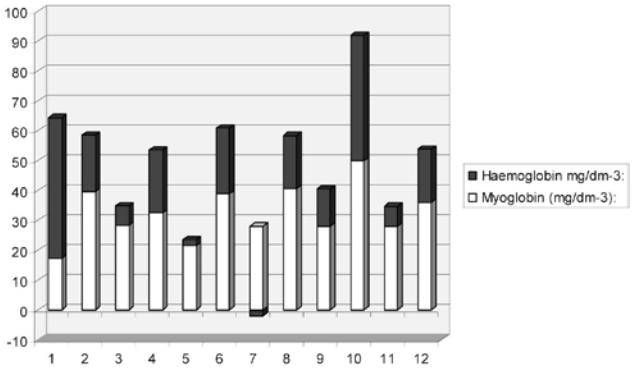

Fig. 2. Group 2 – Kosher chickens

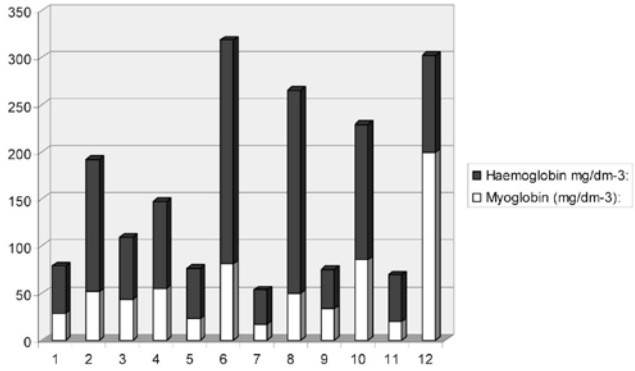

Fig. 3. Group 3 – Improperly bled chickens

break down hydrogen peroxide into water and nascent oxygen. Nascent oxygen oxidizes o-tolidine to give oxidized product which is of green-blue colour. The rate of colour development is measured at 630nm which is directly proportional to haemoglobin concentration.

o-Tolidine stock solution: was prepared by dissolving of 2 g of o-tolidine in 100 ml of solvent (20 ml of glacial acetic acid (GAA) and 80 ml of ethanol) to make a stock solution. The working reagent (0.4 g.dl^{-1}) was prepared by diluting stock solution with the same solvent (1:5). 100 µl Triton-X-100 was mixed with 100 ml of working reagent to increase the linearity of kinetic reaction.

Hydrogen peroxide solution: 2 % (v/v) hydrogen peroxide solution was prepared in deionized water and 2.26 g sodium acetate (100 ml) to create a buffering environment with GAA present in the final reaction mixture to maintain pH between 3.0 to 3.5 in the final reaction mixture. This solution may only be used for 6 to 8 hrs.

Haemoglobin standard solution: haemoglobin stock solution was prepared by diluting bovine blood haemoglobin (Sigma) in Tris-HCl buffer, pH 8.0, in concentration of 240 mg.l^{-1}. For the estimation of the enzymatic reaction kinetics, the stock solution was diluted with 80 mmol Tris-HCl buffer, pH 8.0, to make final concentrations 4.0, 8.0, 12.0, 16.0, 20.0, and 24.0 mg.l^{-1} of haemoglobin, respectively.

Haemoglobin assay procedure: 1.0 ml of working solution and 1.0 ml of H_2O_2 solution were pipetted into test tubes, mixed well and allowed to stand for 5 min. 10 µl of each sample was added and 10 µl of Tris-HCl buffer solution was used as a reagent blank. Absorbance at 630 nm (A_{630}) was measured after 120 sec.

Myoglobin Evaluation: From each sample 2 ml of the supernatant were saturated with 75 % ammonium sulphate (0.525 g.ml^{-1}) to precipitate the haemoglobin while keeping the myoglobin in the solution (1). Precipitated haemoglobin was separated by centrifugation at 2000 rpm at 21 °C for 45 min. This solution was used for evaluation of myoglobin using the modified kinetic method with o-tolidine as described above.

The results were processed statistically using software "Statgraphic Plus". The dependence of A_{630} on sample concentration was linear and the calculated relation was: **mg.l^{-1} = -0.0804722 + 14.6076 . A_{630}** (correlation coefficient; r = 0.992784)

Total haeme levels (myoglobin + haemoglobin) were calculated by multiplying the results obtained by the above formula by 5 (dilution of the sample at homogenization/extraction). Myoglobin levels were calculated as total haeme but the resulting value was multiplied by 1.061 to compensate for loses at salting out by ammonium sulphate. These loses were estimated at 49 % (1).

RESULTS AND DISCUSSION

Standard bleeding of the stunned chickens is highly efficient and the residual blood would not exceed the level of blood of kosher chickens. During the sample preparation, however, blood pockets were found in muscles showing that a major source of the residual blood in muscles was due to haemorrhages caused ante mortem by improper handling.

Due to the previously stated problems it seems necessary to ensure better handling with the chickens so that standard koshering ensures expected effective residual blood removal as haemorrhages were not dealt with in this process completely.

CONCLUSION

The removal of haemoglobin is essential for the quality and consistence of poultry products. Blood components,

especially haemoglobin, are powerful promoters of lipid oxidation and may decrease the shelf life of meat products (3). Studies have also shown that blood can cause cancer and is a carrier of food-borne pathogens and parasites. Therefore it is crucial that further studies be conducted in order to evaluate and refine the methods used for slaughtering by Stunning and Kosher processes and all areas of possible human error should be eliminated or greatly improved upon. Slaughter using the stunning method should further refine the process used for selection of poultry in relation to size in order to reduce the amount of improperly bled chickens.

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ABUDANCE OF BIOGENIC AMINES IN OUR FOOD

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ABSTRACT

Biogenic amines (BA) are anti-nutritional food components. They are produced and degraded by plant, animal and microbial metabolism. Biogenic amines have various biological effects and are naturally present in low concentrations in the majority of food. However, consumption of higher quantities of BA in food may cause problems, such as high or low blood pressure, migraine, allergic manifestations, erythema, nausea or diarrhoea.

From the point of view of food hygiene, the most important BA include the following: histamine (HIS), the most investigated BA, tyramine (TYR), putrescine (PUT), cadaverine (CAD), spermidine (SPD) and spermine (SPM). BA can be found at higher levels in fish, hard cheeses, fermented meat products, vine and beer. The occurrence of BA is related to the quality of the input raw material and hygiene of technological lines and processing. Thus they serve as indicators of microbial contamination or indicators of food quality

The presence of 40 mg BA in one meal produces no problems for healthy people. Such level of BA is commonly inactivated by amino oxidases acting in the digestion tract. However, sick people treated with drugs inhibiting amino oxidases should not take up more than 6 mg BA in one meal. Similar inhibitory effects on the mentioned enzymes have been recorded also for coffee, tea, alcohol and cigarettes. The aim of the study was to calculate the quantities of some foods rich in BA that can be consumed by a healthy man who smokes and drinks coffee and alcohol because these are exactly the factors that can increase the health risk resulting from uptake of higher levels of BA.

INTRODUCTION

Biogenic amines (BA) are part of normal nutrient exchange and metabolism of humans, animals, plants and micro-organisms. They are present in food.

BA play an important role in an organism. They are a source of reserve substances, participate in proteosynthesis and function as hormones. They are important for normal functioning of the nervous system, affect intestinal motorics, control body temperature and blood circulation. However, if present in food in higher levels they can produce problems. BA taken up with food may cause migraine, high or low blood pressure, food intoxication and are precursors of carcinogenic nitrosamines (5).

Humans have natural detoxication mechanisms in intestinal mucosa. Inactivation of amines by amine oxidases occurs as follows: monoamine oxidase (MAO) decomposes, for example, tyramine and tryptamine and diamine oxidase (DAO) degrades histamine, putrescine, cadaverine, spermidine and spermine (1). Health problems occur when detoxication mechanisms fail to ensure deamination of BA in the body after consumption of food with higher level of amines or the detoxication ability of the individual is in some way reduced, e.g. in allergic persons. Decreased activity or even failure of amino oxidases may result from genetic predisposition, gastrointestinal diseases or amino oxidase inhibitors, such as coffee, alcohol, some drugs or smoking (4). Thus some people may consume food rich in amines without difficulties while for other such food may present a problem (1).

A healthy person should be able to consume a meal containing 40 mg BA without problems (2). For sick people treated

with amino oxidase inhibiting medications the acceptable level is much lower, namely 6 mg in one meal (2).

The aim of the study was to indicate that health problems may occur even in healthy people consuming food with higher level of BA if they smoke, drink coffee and alcohol.

Statistical data oriented on lifestyle of students, collected in the period of 1998–2003 (8), showed that approx. 70 % of females drank coffee, almost 90 % of them drank occasionally alcohol and about 20 % smoked. Because these factors are exactly those which inhibit mono and diamine oxidases we used the mentioned data in our considerations.

Statistical data indicate that females are subject to a higher risk because proportion of smokers among females and males is almost the same and females keep with males even in occasional drinking of alcohol. Although in comparison with women only about half of the men drink coffee, men consume more meat and meat products (11, 7) which are a rich source of BA and can result in various health risks or problems.

MATERIAL AND METHODS

Various types of food from market network were analysed for the content of BA by thin layer chromatography (TLC). We investigated 4 amines: histamine, tyramine, putrescine and cadaverine. Food samples were extracted with 5 % trichloroacetic acid and extract aliquots were applied to chromatographic plates together with three different concentrations of respective standards (100 $\mu g.ml^{-1}$, 50 $\mu g.ml^{-1}$ and 20 $\mu g.ml^{-1}$ of each investigated amine). The plates were developed in a mixture of chloroform : methanol : ammonium (2 : 2 : 1). The spots were detected using 0.3 % solution of ninhydrine in ethanol (9).

RESULTS

The spots of samples on TLC plates were compared with those produced by standard mixtures. Concentrations of amines in some foods are shown in the Table 1.

The results were compared with the levels published in other studies (10, 8) which reported similar but also much higher levels of BA (above 1 000 mg. kg^{-1}). Dičáková and Bystrický (3) reported mean levels of BA in 33 thermally unprocessed meat products: histamine 39.8 $mg.kg^{-1}$; tyramine 132.7 $mg.kg^{-1}$, putrescine 86.8 $mg.kg^{-1}$, cadaverine 7.8 $mg.kg^{-1}$ and the sum of BA 267.0 $mg.kg^{-1}$. Similar sum of BA, exceeding 200 $mg.kg^{-1}$, was calculated for three analysed, thermally unprocessed meat products shown in the Table.

When using statistical data, i.e. that male consumer consumes up to 49 % of the recommended daily dose of meat (157 g) in the form of meat products, his total daily intake of meat products comes to 76.9 g (7, 11). If they are meat products from the considered group, he consumes more than 12 mg BA. And to this we must add amines in other food consumed during one day. Higher levels of amines were detected also in cheeses, fish and beer. One package of bryndza (125 g) means uptake of more than 25 mg amines and 2 beers correspond to 50 mg amines.

CONCLUSION

TLC analysis of selected food showed different levels of amines in them. The highest levels were found in thermally unprocessed meat products and bryndza.

From the health point of view one should not take up more than 40 mg BA in one meal (2). For sick people, treated with medications inhibiting amino oxidases, the recommended level is much lower (6 mg in one meal). Similar inhibitory effect on the enzymes mentioned has frequent drinking of coffee, smoking and alcohol drinking (5).

Because of that for people in the selected group (students) with certain lifestyle (and also inhibition of amino oxidases by certain risk substances) we can recommend that they subdivide the food consumed in

Table 1. Levels of biogenic amines in food determined by TLC ($mg.kg^{-1}$)

Food	histamine	tyramine	putrescine	cadaverin	Sum of BA
Broccoli	50	<20	<20	< 20	> 50
Bryndza*	>>100	> 50	20	> 50	>>200
Beer	50	<20	<20	<20	> 50
Salami Nitran	>>100	<100	<20	<20	>>150
Malokarpatská salami	>>100	100	<20	<100	>>200
Meat sausage	>>100	> 50	20	> 20	>>200
Carp	20	<20	<20	<20	> 20
Smoked mackerel	>100	<20	> 20	> 50	> 200
Canned tunafish	>100	<20	> 20	> 20	> 150

one day to several meals in such a way that in one meal they take up no more than 80 of fermented salami, half a package of bryndza or one beer, which comes to the uptake of 12 to 25 mg of biogenic amines.

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COMPARISON OF WATER CONTENT AND ELECTRIC CONDUCTIVITY IN HONEY OF VARIOUS ORIGIN

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ABSTRACT

During the years 2008 and 2009 the analyses of honey samples coming from various regions of the central and eastern Slovakia were carried out. Totally 59 samples of honey from the honey harvest of 2008 were examined. Three samples (2 from the market chain and 1 directly from a bee-keeper) contained higher percentage of water than that set by the Food Codex of SR. The highest electric conductivity was measured in rape honey (market chain 1.829 mS.cm^{-1}) which confirmed high content of mineral substances in flower honey, however, it did not meet the requirement of the Food Codex for electric conductivity (max. 0.8 mS.cm^{-1}). Out of eight samples labelled as "forest honey" four had the value of electric conductivity below 0.8 mS.cm^{-1}, and four exceeded the value of 0.8 mS.cm^{-1}. The Food Codex of SR stipulates that the value of electric conductivity for flower honey should be max. 0.8 mS.cm^{-1} (with the exception of honeydew and chestnut honey and their mixtures, min. 0.8 mS.cm^{-1}), seven samples had unsuitable value of electric conductivity which indicated incorrect labelling of honey samples (six samples were from home producers). According to our results the mean electric conductivity was 0.65 ± 0.43 mS.cm^{-1} and mean water content reached 7.98 ± 1.4 %. In the samples from market chain the mean water content was 17.07 % and in those from home producers 18.61 %. Honeydew honey showed the mean electric conductivity of 1.057 mS.cm^{-1} and flower honey 0.523 mS.cm^{-1}.

Key words: electric conductivity; honey; water content

INTRODUCTION

Honey is natural sweet substance produced by bees (*Apis mellifera*) from plant nectar, secretions of live parts of plants or insect suckling live parts of plants which bees collect and enrich with their own specific substances, deposit, make thicker, store and leave in honeycombs to ripen (4).

Bees produce honey from nectar or honeydew which are natural sweet juices. Nectar and honeydew are basically water solutions of sugars containing 15 to 95 % water. Watery sweet solutions are not long-lasting and are very quickly fermented in nature as they are perfect substrates for multiplication of ubiquitous yeasts. In order to prevent the spoiling, bees change nectar or honeydew to honey containing 14 to 19 % water and in this way ensure its perfect preservation. Besides evaporation the water content in honey decreases also due to action of enzymes participating in honey ripening. Ripened honey with low content of water can be stored almost infinitely because it is highly concentrated and no microorganisms can multiply in it. So, it can be said that the less water content the honey has, the better the quality of honey. Ripened honey is an oversaturated solution of sugars and therefore it has a tendency to crystallize and take up water from the surrounding environment. Therefore, honey must be stored in tight containers (1).

Water content of honey should comply with the Food Codex of the Slovak Republic (4) generally it should not exceed 20 %; in honey from the common heather (*Calluna*) and in baker honey in general the maximum acceptable is 23 % and in baker honey from the common heather max. 25 %. According to the Food Codex of SR (4) electric conductivity of flower honey should not exceed 0.8 mS.cm^{-1}, for honeydew and chest-

nut honey and their mixture the maximum acceptable is 0.8 $mS.cm^{-1}$. Exceptions are: the strawberry tree (*Arbutus unedo*), bell-heather (*Erica*), eucalyptus, linden (*Tillia* spp.), common heather (*Calluna vulgaris*), manuka or gelatinous shrub (*Leptospermum*) and bottle-brush (*Melaleuca* spp.).

The electric conductivity of honey depends on the content of mineral substances in honey (2). The higher it is, the more ion particles are in honey and the higher its content of mineral substances. Due to high content of mineral substances in honeydew honey this type of honey has higher electric conductivity than flower honey. The electric conductivity is expressed in siemens (S) (3). The specific electric conductivity of flower and mixed honey is $1.2–10.5 \times 10^4$ $S.cm^{-1}$. The specific electric conductivity of honeydew honey is always higher than 10.5×10^4 $S.cm^{-1}$. An exception is chestnut honey with specific electric conductivity reaching up to 13.7×10^4 $S.cm^{-1}$ (2).

MATERIAL AND METHODS

Fifty-nine honey samples originating from the market chain (n = 18) and from home producers (n = 41) were analysed. The honey samples were bottled in 2008 (n = 30), 2007 (n = 24) and from the 2006 honey harvest (n = 5). In the individual honey samples both the water content and electric conductivity were measured.

Water content was determined by a manual refractometer Honey tester (Meopta Přerov, Czech Republic) with automatic compensation for temperature within the range of 10–30 °C.

Electric conductivity was determined using a conductometer Vario kond, according to STN 570190, in 20 % honey solution in deionised water at 20 °C. The value of electric conductivity was read from the conductometer in $mS.cm^{-1}$. The honey solution used for measurement contained 20 % dry matter in 100 ml distilled water and the measurements were carried out using an electric conducting cell (double platinum electrode). Determination of the electric conductivity is based upon the measurement of electric resistance. electric conductivity is the inverse value of electric resistance.

RESULTS AND DISCUSSION

Both the water content and electric conductivity were determined in the samples. The results are presented in the Table.

The mean value of water content in the samples was 17.98 % (range 15–21.5 %). Three samples (2 from market chain and 1 from a bee-keeper) contained higher level of water than that permitted by the Food Codex of SR (4). Mean water content in honey samples obtained directly from bee-keepers reached 17.71 % and in those

Table. Water content and electric conductivity of honey samples

Sample No.	Type of honey	Origin	Date of filling	Water content (%)	Electric conductivity ($mS.cm^{-1}$)
1	Acacia	Košice vicinity	2006	18	0.156
2	Acacia	Market chain	2007	17.5	0.155
3	Acacia	Detva	2008	16	0.174
4	Acacia	Jasov	2008	19.5	0.794
5	Acacia	Košice vicinity	2008	18	0.315
6	Acacia	Košice vicinity	2008	18	0.185
7	Flower	Košice vicinity	2006	19.5	0.73
8	Flower	Košice vicinity	2007	20	0.456
9	Flower	Market chain	2007	19	0.42
10	Flower	Market chain	2007	17	1.093
11	Flower	Market chain	2007	17.5	0.387
12	Flower	Market chain	2007	18	0.357
13	Flower	Market chain	2007	21	0.164
14	Flower	Revúca	2007	19.5	0.436
15	Flower	Revúca	2007	18.5	0.357
16	Flower	Rožňava	2007	18.5	0.43
17	Flower	Rožňava	2007	17.5	0.434
18	Flower	Rožňava	2007	17.5	0.508
19	Flower	Rožňava	2007	17.5	0.549
20	Flower	Svidník	2007	18	0.589
21	Flower	Košice vicinity	2008	19.5	0.67
22	Flower	Košice vicinity	2008	16	0.445

Sample No.	Type of honey	Origin	Date of filling	Water content (%)	Electric conductivity (mS.cm^{-1})
23	Flower	Market chain	2008	17.5	0.311
24	Flower	Market chain	2008	18	0.292
25	Flower	Market chain	2008	19	0.275
26	Flower	Rožňava	2008	16	0.311
27	Flower	Rožňava	2008	17.5	0.457
28	Forest	Market chain	2006	17.5	0.647
29	Forest	Košice vicinity	2007	16	1.124
30	Forest	Market chain	2007	19	0.982
31	Forest	Market chain	2007	19.5	0.36
32	Forest	Market chain	2008	18	1.338
33	Forest	Košice vicinity	2008	16	1.028
34	Forest	Market chain	2007	19	1.36
35	Linden-honeydew	Detva	2007	15	0.993
36	Linden	Market chain	2008	18	0.442
37	Raspberry-linden	Detva	2006	16	0.644
38	Honeydew	Košice vicinity	2007	17	1.046
39	Honeydew	Košice vicinity	2007	17	1.046
40	Honeydew	Košice vicinity	2007	17.5	1.17
41	Honeydew	Market chain	2007	19	1.194
42	Honeydew	Revúca	2008	17.5	0.96
43	Honeydew	Košice vicinity	2008	18.5	1.145
44	Honeydew	Košice vicinity	2006	18.5	1.29
45	Rape	Košice vicinity	2008	18	0.274
46	Rape	Košice vicinity	2008	20	0.285
47	Rape	Košice vicinity	2008	17.5	0.227
48	Rape	Market chain	2008	19	0.208
49	Rape	Market chain	2008	21.5	1.585
50	Flower	Detva	2008	18.5	0.222
51	Flower	Detva	2008	16.5	0.28
52	Acacia	Dvorníky	2008	16	1.207
53	Forest	Košice vicinity	2008	18.5	0.541
54	Acacia-rape	Košice vicinity	2008	17	1.368
55	Honeydew	Detva	2008	20.5	0.608
56	Flower	Dvorníky	2008	17.5	0.342
57	Flower	Hlinisko, Tehelňa	2008	20	0.306
58	Flower	Zámutov	2008	15.5	1.829
59	Acacia	Detva	2008	16.5	1.329
Mean				**17.98**	**0.658**

from market chain 18.61 %, i. e. slightly higher. Measurement by the manual refractometer has its advantages, because the water content in honey can be determined very simply and quickly, i.e. directly in the honeycomb taken from hive closely before straining (1).

The mean value of electric conductivity of all honey samples was 0.658 mS.cm^{-1} (0.155–1.829 mS.cm^{-1}). Electric conductivity of honeydew samples was 1.057 mS.cm^{-1} and of flower and mixed honey 0.595 mS.cm^{-1}. The highest electric conductivity was measured in rape honey from a home producer (1.829 mS.cm^{-1}) which did not comply with the requirements of the Food Codex of SR (4) for electric conductivity. Of eight samples labelled as "forest honey" three showed electric conductivity lower than 0.8 mS.cm^{-1} and in four samples the conductivity exceeded 0.8 mS.cm^{-1}. Four flower honey samples did not meet the requirements, their conductivity was higher than 0.8 mS.cm^{-1}. One sample originated from the market chain and three from home producers; incorrect labelling of honey was assumed. The lowest values of electric conductivity were measured in acacia honey followed by flower and rape honey. Despite the highest values in rape honey even after repeated measurements, incorrect labelling was assumed. Higher values were measured in honeydew and forest honey indicating the dependence of electric conductivity on origin of honey.

CONCLUSION

Out of 59 analysed samples of honey three (2 from market chain and 1 from a bee-keeper) had higher content of water than that stipulated by the Food Codex of SR (4). The highest electric conductivity was measured in rape honey (from home production), 1.829 $mS.cm^{-1}$, which did not meet the requirement of the Food Codex of SR (4) for electric conductivity (up to 0.8 $mS.cm^{-1}$). Out of eight samples labelled as "forest honey" three showed electric conductivity lower than 0.8 $mS.cm^{-1}$ and 4 above 0.8 $mS.cm^{-1}$.

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THE EFFECT OF DIETARY SUPPLEMENTATION OF PIGLETS WITH POLYUNSATURATED FATTY ACIDS AND LACTOBACILLI ON OXIDATIVE STABILITY OF PORK

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ABSTRACT

The study investigated the effect of feeding linseed (alone or in combination with probiotic bacteria) on oxidative stability and sensory properties of pork (leg and loin) stored in refrigerator (4 °C). Supplementation of feed with linseed (source of polyunsaturated fatty acids – PUFA) increased content of fat in muscles. Results of determination of decomposition changes in fat (thiobarbituric acid reactive substances, TBARs) indicated that linseed supplementation affected significantly ($P < 0.05$) the oxidative processes during storage in comparison with control. Sensory evaluation showed marked differences in taste and meat aroma compared to the control.

INTRODUCTION

The principal contribution of fat to nutrition is based on the content of essential polyunsaturated fatty acids (PUFA). It was proved that consumption of n-3 PUFA has a positive effect on human organism (2). It has been recommended to decrease consumption of saturated and trans-unsaturated fatty acids and increase intake of PUFA.

A number of scientific studies focused on increasing proportion of PUFA in animal products by supplementation of animal feed with vegetable oils. The content and composition of fatty acids affects significantly the quality of fatty tissues of pigs (1). Linseed is a rich source of linolenic acid (C18 : 3, n-3) and its dietary supplementation to poultry (6) changed markedly the PUFA/SFA ratio and increased content of n-3 acids in meat.

The present study investigated the effect of dietary supplementation of pigs with linseed alone and in combination with lactobacilli on oxidative stability and organoleptic properties of produced meat during its storage in a refrigerator.

MATERIAL AND METHODS

The experiment included 36 piglets, 14 days old. They were divided to three groups and fed 10 days before weaning and 35 days after weaning as follows: the first group (FA) was fed commercial mixed feed OŠ-02 NORM TYP (Spišské Vlachy, SR) supplemented with linseed variety Flanders (10 % in rations, 56.8 % linolenic acid). The second group (LFA) was fed mixed feed OŠ-02 NORM TYP supplemented with linseed (10 % in rations) and probiotic strains (L81 – *Lactobacillus plantarum* and 2I3 – *Lactobacillus fermentum*) in the form of probiotic cheese at a dose of 4 g/pig/day. The control group (C) was fed commercial mixed feed. The piglets were slaughtered on day 35 of life in accordance with relevant legislative provision and under veterinary observation. Immediately after slaughter and dressing the meat was deboned and skin removed. Meat samples (leg and loin – *m. longissimus dorsi*) were wrapped and stored in a refrigerator (4 °C) for 11 days.

Fat and proteins were determined in meat samples according to veterinary laboratory methods (5). Oxidation of fat was determined by 2-thiobarbituric (TBA) method according to Marcinčák *et al.* (335). Individual determinations were carried out on days 1, 3 and 11 of storage in a refrigerator.

Sensory analysis of meat (leg) was carried out 24 h after dressing. Samples were evaluated by a cooking test. A 5-point evaluation system was used (4).

Statistical software Graph Pad Prism 3.0 (1999) was used for statistical processing of results. Results were compared using one-way ANOVA test. Statistical differences between measured values were evaluated by Tukey comparison test and $P < 0.05$ was used as the level of significance.

RESULTS AND DISCUSSION

The results obtained are presented in tables as means ± standard deviations (x ± SD). Tab. 1 presents chemical composition of muscles and shows that dietary supplementation of linseed as a source of PUFA resulted in increased content of fat in groups FA and LFA.

Tab. 2 shows the results of TBA determination in samples stored in a refrigerator (4 °C, 11 days). Control group exhibited significantly lower increase in the level of malonedialdehyde ($P < 0.05$) which indicated considerably reduced oxidative stability of meat after dietary supplementation of linseed and linseed plus lactobacilli. The results agree with the previously published ones (1, 2) which stated that pork from pigs supplied linseed in the rations showed lower oxidative stability and higher levels of decomposition products during storage. Therefore, at dietary supplementation of vegetable oils with higher level of PUFA it appears inevitable to supplement also effective quantities of antioxidants.

Sensory evaluation of leg muscle samples was conducted by a panel of professionals. Already after dressing of pig carcasses there were significant differences between control and experimental groups (Tab. 3). Sensory evaluation of meat stored for 11 days in a refrigerator using a 5-point scale showed higher rating of control meat ($P < 0.05$). The most pronounced differences were observed when evaluating aroma and taste of the meat – in experimental groups both properties were evaluated negatively (1).

Table 1. Chemical composition of leg muscles (%)

	C		FA		LFA	
	Loin	Leg	Loin	Leg	Loin	Leg
Fat (%)	2.91^{a} ± 0.63*	3.54^{c} ± 1.08	3.43^{b} ± 0.68	5.26^{d} ± 0.63	4.65^{b} ± 0.68	6.29^{d} ± 1.19
Proteins (%)	18.19^{c} ± 0.62	16.83^{a} ± 0.23	17.68^{b} ± 0.10	16.98^{a} ± 0.15	17.74^{c} ± 0.28	17.28^{a} ± 0.14

[a, b, c, d] – values with different superscripts are statistically different

Table 2. Results of determination of TBA expressed as malonealdehyde level (mg.kg^{-1}) in leg muscles stored at 4 °C for 11 days

		Day 1	Day 3	Day 11
C	Loin	0.089 ± 0.029^{a}	0.199 ± 0.028^{a}	0.334 ± 0.062^{a}
	Leg	0.130 ± 0.044^{d}	0.244 ± 0.048^{c}	0.571 ± 0.113^{c}
FA	Loin	0.132 ± 0.047^{b}	0.315 ± 0.026^{b}	0.969 ± 0.258^{b}
	Leg	0.235 ± 0.067^{e}	1.838 ± 0.641^{e}	3.01 ± 1.540^{e}
LFA	Loin	0.207 ± 0.048^{c}	0.364 ± 0.072^{b}	1.112 ± 0.107^{b}
	Leg	0.227 ± 0.031e	0.647 ± 0.141^{d}	2.385 ± 0.582^{d}

[a, b, c, d] – values with different superscripts are statistically different

Table 3. Total sensory evaluation on a 5-point scale

	After dressing	After storage in a refrigerator for 11 days
C	18.4 ± 1.2^{a}	16.8 ± 1.6^{a}
FA	17.6 ± 0.9^{b}	14.1 ± 1.9^{b}
LFA	17.4 ± 1.4^{b}	13.6 ± 2.2^{b}

[a, b,] – values with different superscripts are statistically different

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DETERMINATION OF SENSITIVITY OF STAPHYLOCOCCAL ISOLATES FROM FISH MEAT AGAINST SELECTED ANTIBIOTICS

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ABSTRACT

The goal of our study was to determine the sensitivity of staphylococcal isolates from fish meat against six antibiotics. Our results showed that out of the total number of 90 tested staphylococci isolates the highest sensitivity was observed against gentamicin (85 strains) and novobiocin (75 strains) and, on the contrary, the highest resistance was detected against penicillin (34%) and tetracyclin (32%). Twenty of the tested isolates of staphylococci (28%) were resistant to only one antibiotic, 34 (47%) to two antibiotics, and multiresistance was confirmed in 18 isolates (25%).

Key words: antibiotics; antimicrobial resistance; disc diffuse method; fish; staphylococci

INTRODUCTION

Recently, a dramatic increase in the resistance against antibiotics routinely used in human as well as in veterinary medicine has been recorded also in the members of the genus *Staphylococcus*. Development of resistant or multiresistant staphylococci strains causes considerable therapeutic problems.

MATERIAL AND METHODS

Samples for microbiological examination were collected from the muscles of 5 carps (*Cyprinus carpio)* from the fish plant Rybárstvo Zemplín, Ltd., farm Hrhov, from 7 rainbow trouts (*Oncorhynchus mykiss)* from the fish plant Rybárstvo Požehy, Ltd., and from 9 frozen filets from the Cold stores Poprad, Ltd. – of that 5 filets were from frozen Alaskan codfish (*Theragra chalcogramma*) and 4 from frozen Atlantic mackerel (*Scomber scombrus*). Staphylococci were isolated from the samples according to STN ISO 6888-1 (6). All staphylococci isolates were subjected to the test-tube plasma coagulase test (STAFYLO PK, IMUNA, Šarišské Michaľany). Sensitivity of individual strains of staphylococci to selected antibiotics was determined by disc diffuse method according to Kirby-Bauer (2) using commercially produced discs (OXOID, Great Britain) with known concentrations of antibiotics. The results were evaluated according to criteria determined by CLSI (NCCLS) for the disc diffuse test (3).

RESULTS

Altogether 90 isolates of staphylococci were obtained by culture microbial examination of the samples of frozen muscles of two freshwater and two sea fish species. The test-tube plasma coagulase test showed that all the isolates were coagulase-negative. The results of the disc diffuse method are summarized in Table 2.

Table 1. Survey of the number of staphylococcal isolates sensitive (s) and resistant (r) against selected antibiotics

Antibiotics	Alaskan codfish		Atlantic mackerel		Rainobow trout		Carp	
	R	S	R	S	R	S	R	S
Penicillin G (10 μg)	11	18	12	13	8	6	15	7
Tetracycline (30 μg)	11	17	0	24	1	13	1	19
Erythromycin (15 μg)	12	8	8	10	5	4	3	14
Novobiocin (30 μg)	2	25	1	23	0	12	6	14
Ampicillin (10 μg)	10	19	13	12	8	6	16	6
Gentamicin (10 μg)	0	26	0	25	0	14	0	20

Table 1 shows that the resistance against antibiotics was confirmed in 29 staphylococcal isolates from frozen filets of Alaskan codfish, of that 11 strains were resistant gainst penicillin, 11 against tetracycline, 12 against erythromycin, 2 against novobiocin and 10 against ampicillin. None of the strains was resistant to gentamicin.

Evaluation of sensitivity of 25 staphylococcal isolates from frozen Atlantic mackerel detected resistance to ampicillin in 13 strains, to penicillin in 12 strains, to erythromycin in 8 strains, and to novobiocin in 1 strain. None of the isolates from this group was resistant either to gentamicin or to tetracycline.

Examination of 14 isolates of staphylococci from rainbow trout by disc diffuse test (3) revealed that 8 strains were resistant to penicillin and ampicillin, 5 to erythromycin and 1 to tetracycline. Neither one was resistant to gentamicin or novobiocin.

Interpretative criteria for the disc diffuse method (3) allowed us to determine that out of 22 strains of staphylococci isolated from carp 16 strains were resistant to ampicilin, 15 to penicillin, 6 to novobiocin, 3 to erythromycin and one strain to tetracycline. Again no resistance to gentamicin was confirmed.

Our results showed that out of the total number of 90 staphyloccocal isolates from different species of fish the highest number was sensitive to gentamicin (85 strains) and novobiocin (75 strains) and the highest resistance was recorded against penicillin (34 %) and tetracycline (32 %). Out of the total number of staphylococcal isolate, 72 strains were resistant at least to one antibiotic tested; 20 strains (28 %) were resistant only to one antibiotic, 34 strains (47 %) to two antibiotics and 18 strains showed multiresistance, of that 14 strains (19 %) to three antibiotics and 4 strains (6 %) to four antibiotics.

DISCUSSION

Investigation of antibiotic resistance of staphylococcal isolates from fish meat showed that the resistance to penicillin and ampicilin was most common and some of them were resistant to more than one tested antibiotic.

A number of authors tested staphylococcal isolates from other commodities and reported similar observations involving resistance and multiresistance to the mentioned antibiotics. Shitandi and Mwangi (5) tested 216 strains of *Staphyloccoccus aureus* isolated from the milk samples collected from various regions of Kenya. They confirmed resistance to penicillin (72.2 %), trimetoprim + sulfametazin (59.2 %), tetracycline (57.9 %), erythromycin (21.3 %), chloramphenicol (46.8 %), and meticilin (7.8 %). Ebrahimi and Lotfalian (4) isolated 22 coagulase-positive staphylococci from honey samples and determined proportion of strains resistant to individual antibiotics. The highest resistance was recorded against penicillin (85.71 %) and erythromycin (50.0 %). Also the study of Bardoň *et al.* (1) confirmed considerable resistance to tetracycline (22.6 %) and erythromycin (19 %) in the strains of *Staphylococcus* spp. isolated from food of animal origin and food producing animals.

CONCLUSION

The recent increase in the resistance of staphylococcal isolates against some antibiotics is alarming. A rational use of antibiotics, complying with preventive measures in environmental hygiene and monitoring of existing resistance to antibiotics are very important weapons against spreading of antibiotic resistance.

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ANALYSIS OF POLYMORPHISMS OF PRION PROTEIN GENE IN SELECTED CATTLE BREEDS

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ABSTRACT

The aim of the present study was to examine variations of the *PrP* gene in different cattle breeds. We examined 215 samples, Slovak spotted cattle (n = 49), Simmental (n = 44), Holstein (n = 28) and crossbreeds (n = 94) for the 23-bp indel polymorphism in the putative promoter region, 12-bp indel polymorphism in the first intron of the *PrP* gene, variations in number of the octapeptide repeat units and the presence of the silent *AAC* → *AAT* transition in codon 192 within the protein-coding region of the *PrP* gene. Significant differences between examined groups were found only in allele and genotype frequencies of codon 192.

Key words: bovine spongiform encephalopathy; cattle; PCR; polymorphism; prion protein gene

INTRODUCTION

The susceptibility against transmissible spongiform encephalopathies (TSE) and/or incubation period are influenced by polymorphisms in the protein coding region of the prion protein gene (*PrP* gene) in sheep and human. None of the more than 60 polymorphisms found in the protein coding region of *PrP* gene in cattle is associated with BSE infection (5).

Table 1. Primers used in the study

Primer	Oligosequence 5' → 3'	References	PCR conditions
23indel-F	GTGCCAGCCATGTAAGTG	Sander (5)	35 x: 94 °C, 45 s 62 °C, 45 s 72 °C, 45 s
23indel-R	TGGACAGGCACAATGGG		
12indel-F	CTTCTCTCTCGCAGAAGCAG	Nakamitsu (3)	35 x: 94 °C, 45 s 59 °C, 45 s 72 °C, 45 s
12indel-R	CCCTTGTTCTTCTGAGCTCC		
PrP-CDS-F	CTAGGGTCCCCACAAGAACAAG	Designed in present study	35 x: 94 °C, 1 min 62 °C, 1 min 72 °C, 1 min
PrP-CDS-R	ACGGGGCTGCAGGTAGATA		
Tokt-F	TTTGTGGCCATGTGGAGTGACG	Designed in present study	35 x: 94 °C, 1 min 57 °C, 1 min 72 °C, 1 min
Tokt-R	CCCCTTGGTGGTGGTGGTGA		

Table 2. Genotype and allele frequencies of the polymorphisms among examined groups

Polymorphism	Sample	Total	Genotype frequency			Allele frequency	
		n	+/+	+/-	-/-	+	-
23-indel	SP	49	0.143	0.388	0.469	0.3367	0.6633
	S	44	0.114	0.500	0.386	0.3636	0.6364
	H	28	0.143	0.357	0.500	0.3214	0.6786
	Cb	94	0.181	0.415	0.404	0.3883	0.6117
				ns, p = 0.8248		**ns, p = 0.7454**	
		n	+/+	+/-	-/-	+	-
12-indel	SP	49	0.082	0.530	0.388	0.3469	0.6531
	S	44	0.091	0.568	0.341	0.3750	0.6250
	H	28	0.143	0.464	0.393	0.3750	0.6250
	Cb	94	0.191	0.511	0.298	0.4468	0.5532
				ns, p = 0.5205		**ns, p = 0.3559**	
		n	6/6	6/5	5/5	6	5
Octapeptide repetitions	SP	49	0.918	0.082	0.0	0.9592	0.0408
	S	44	0.955	0.045	0.0	0.9773	0.0227
	H	28	0.929	0.071	0.0	0.9643	0.0357
	Cb	94	0.957	0.043	0.0	0.9787	0.0213
				ns, p = 0.7644		**ns, p = 0.7724**	
		n	*AAC/AAC*	*AAC/AAT*	*AAT/AAT*	*AAC*	*AAT*
Codon 192	SP	49	0.796	0.163	0.041	0.8776	0.1224
	S	44	0.704	0.273	0.023	0.8409	0.1591
	H	28	0.1	0.0	0.0	1.0	0.0
	Cb	94	0.840	0.117	0.043	0.8989	0.1011
				$\chi 2$ calculations not valid		***, p = 0.0214**	

SP – Slovak spotted, S – Simmental, H – Holstein, cb – crossbreeds, ns – not significant

Possible influence of the number of octapeptide repetitions in the protein coding region on susceptibility to BSE was shown only in an experimental study on transgenic mice (1). A single nucleotide polymorphism (*AAC → AAT*) in codon 192 occurred only with six octapeptide repetitions (2). Two insertion/deletion polymorphisms found in the putative promoter region and in the first intron of the cattle *PrP* gene might be associated with BSE in German cattle (5). Insertion alleles are considered to be protective against developing BSE.

MATERIAL AND METHODS

We examined 215 samples of genomic DNA from different cattle breeds, Slovak spotted cattle (n = 49), Simmental (n = 44), Holstein (n = 28) and crossbreeds (n = 94). The DNA was isolated from blood leukocytes by a method of Sambrook (4).

All PCR reactions were carried out as follows: 5 min at 94 °C; amplification (Tab. 1); 10 min at 72 °C. We amplified 100 or 123 bp long products using 23indel-F and 23indel-R primers in the 23-bp indel polymorphism analysis. We ampli-

fied 414 or 426 bp long products using 12indel-F and 12indel-R primers in the 12-bp indel polymorphism analysis. Products were digested with *SacII* enzyme. We performed the nested PCR method for the octapeptide polymorphism analysis. In the first step we amplified 1256 or 1280 bp products using PrP-CDS-F and PrP-CDS-R primers. In the second step we amplified 555 or 579 bp products using primers Tokt-F and Tokt-R indicating 5 or 6 octapeptide repeat units. The 1256 and 1280 bp products were digested with *Hind*II enzyme for the purpose of the codon 192 silent mutation analysis. All PCR products and restriction fragments were analyzed on 2 % ethidium-bromide-stained agarose gel.

χ square test and Fisher's exact test were used for the analysis of alleles and genotypes frequencies in examined groups.

RESULTS AND DISCUSSION

Alleles and genotypes frequencies among examined groups are shown in Table 2. We found no significant differences in the protection alleles distribution among examined breeds. Similar, we found no significant differences in the octapeptide polymorphism. The analysis of silent transition in codon 192 showed significant differences ($P < 0.05$) in allele distribution among examined groups. The codon 192 allele distribution was statistically significant comparing Holstein to Slovak spotted cattle ($P < 0.01$), to Simmental ($P < 0.001$) and to crossbreeds ($P < 0.01$). Significant differences in codon 192 genotypes distribution were found comparing Holstein to Slovak spotted cattle ($P < 0.05$), Holstein to Simmental ($P < 0.005$) and crossbreeds to Simmental cattle ($P < 0.05$).

Analyses of examined polymorphisms performed on Japanese (3) or Polish cattle (6) showed alleles and genotypes distributions similar to our results. Further research should be focused on the *PrP* gene polymorphisms analysis in BSE-affected cattle in Slovakia and comparison with previous results.

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THE EFFECT OF β (1,3/1,6)D-GLUCAN ON SELLECTED NON-SPECIFIC AND SPECIFIC IMMUNOLOGICAL PARAMETERS IN DOGS AFTER VACCINATION

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ABSTRACT

The study investigated the immunostimulative effect of β(1,3/1,6)-D glucan (syrup Plerasan) in immunosuppressed dogs and its influence on effectiveness of anti-rabies vaccination. Administration of glucan in the form of syrup to puppies (group I.G) increased significantly the non-specific immunological parameters, such as functional activities of phagocytes (FALe, FILe, IMA) and lymphocytes (SIP), in comparison with the 0-sampling and the control group (II. K – without glucan). Puppies with confirmed immunosuppression at 0-sampling (II. K) exhibited on day 28 post-vaccination a significantly lower level of anti-rabies antibodies and failed to reach the required level at all following samplings which is a serious observation from the immunological point of view. Contrary to that, puppies from group I.G (administration of glucan) showed protective level of rabies antibodies already on day 14 post-vaccination (> 1 UE.ml^{-1}). The highest level of Ab ($P < 0.0001$) was reached on day 28 post-vaccination in glucan-treated dogs.

Key words: β(1,3/1,6) D-glucan; dogs; immunological parameters; vaccination

INTRODUCTION

Glucans are polysaccharide substances isolated from yeasts and mushrooms. Beta glucan isolated from mushrooms *(Pleurotus ostreatus, Hiratake)* has a pronounced anticancer and immunomodulative effects (5). It stimulates humoral and cell-mediated imunity and haematopoiesis (7). An important effect of glucans involves secretion of IL-1 by macrophages, i.e. the cytokine decisive for activation of T-lymphocytes in the process of presentation of antigen and for production of IL-2 (8). The aim of our study was to investigate specific and non-specific immunological parameters after immunostimulation by β(1,3/1,6) D-glucan.

MATERIAL AND METHODS

Animals

Group I.G: 12 dogs of various breeds and gender, approx. 4 months old, originating from a dog shelter. After sampling of blood on day 0 they were administered *per os* immunoglucan (syrup Plerasan, PLEURAN, Bratislava) at a dose of 2ml.5kg^{-1} for 2 months and were vaccinated against infectious diseases. Dogs from both groups (I.G and II.K) were subjected to primo-vaccination and subsequently were re-vaccinated in 2-week interval. They were examined for selected non-specific immunological parameters and titre of rabies antibodies. Group II. K: 12 dogs of various breeds and gender, approx. 4 months old, originating from a dog shelter. Dogs of this group were not administered immunoglucan and were vaccinated according to scheme described in group I.G with subsequent detection of level of rabies antibodies and non-specific immunological parameters.

Blood sampling

Blood samples were collected from dogs by *v. cephalica* puncture and subjected to immunological analysis. The 0-sampling confirmed the assumed immunosuppression in dogs. Additional

Table 1. Non-specific immunological parameters and rabies antiobody titres before and after vaccination

PARAMETER GROUP I. G Vaccination + glucan	SAMPLING 0 Primovaccination (CDV, CPV, $CAV_{1,2}$, CP_i)	SAMPLING 1 (day 14) Revaccination (+Leptospirosis)	SAMPLING 2 (day 28) Vaccination Rabies	SAMPLING 3 (day 42)	SAMPLING 4 (day 56)	SAMPLING 5 (day 70)
FA Le %	X 32 ns	35.8 ns	33. 75 ns	36.75ns	42.83 ns	54.46ns
	SD 7.2	15.1	13.36	10.37	14.35	3.59
FI Le	X 5.25ns	6.21ns	5.23 *P < 0.05	6.543333 ns	7.72ns *P < 0.05	8.88 ns
	SD 0.9	1.65	1.12	0.89	1.51	1.83
IMA	X 1.25ns	1.25ns	1.39ns	1.512 ns	1.56ns	1.97ns
	SD 0.13	0.12	0.22	0.248	0.254	0.375
ChI	X 1.43 ns	1.796 *P < 0.05	1.41ns	1.432 ns	1.164ns	1.25ns
	SD 0.47	0.238	0.54	0.227	0.460	0.16
SIP	X 1.4 ns	3.62 ***P < 0.0001	2.125ns	1.2 ns	1.7ns	1.13ns
	SD 0.5	1.17	0.521	0.258199	0.62	0.497
Ab Rabies UE.ml^{-1}	X 0.508 ns	0.66 ns	1.028 *P < 0.05	1.523 ***P < 0.0001	1.77 ***P < 0.0001	1.568 ***P < 0.0001
	SD 0.308	0.432	0.217	0.304	0.158	0.350
PARAMETER GROUP II. K **Vaccination**	**SAMPLING 0** **Primovaccination** **(CDV, CPV, $CAV_{1,2}$, CP_i)**	**SAMPLING 1** **(day 14)** **Revaccination (+Leptospirosis sp.)**	**SAMPLING 2** **(day 28)** **Vaccination Rabies**	**SAMPLING 3** **(day 42)**	**SAMPLING 4** **(day 56)**	**SAMPLING 5** **(day 70)**
FA Le %	X 29.3 ns	30.67 ns	32.166 ns	36.83333 ns	36.54ns	57.83333 ns
	SD 6.7	23.37	8.45	13.303	19.551	16.036
FI Le	X 5.31 ns	8.02 ns	7.708ns	7.66ns	5.57 ns	9.346667 ns
	SD 1.5	3.14	2.22	2.34	1.545	2.602
IMA	X 1.16 ns	1.285 ns	1.25ns	1.56ns	1.584ns	2.24ns
	SD 0.13	0.123	0.18	0.147	0.228	0.531
ChI	X 1.29 ns	1.41ns	1.366 ns	1.42ns	1.15ns	1.11 ns
	SD 0.15	0.148	0.182	0.212	0.288	0.109
SIP	X 1.4 ns	2.02 ns	2.183333 ns	0.9 ns	1.57ns	1.4 ns
	SD 0.8	0.9	0.756	0.283	0.89	0.389
Ab Rabies UE.ml^{-1}	X 0. 451 ns	0.459 ns	0.637ns	0.669ns	0.640 ns	0.488ns
	SD 0.070	0.039	0.310	0.346	0.302	0.277

FALe – phagocytic activity of leukocytes, FILe – phagocytic index of leukocytes, IMA – index of metabolic activity of phagocytes, ChI – index of chemotactic activity, SIP – stimulation index of lymphocytes by means of PHA
Ab Rabies – rabies antibodies Ab UE.ml^{-1}, X – mean, SD – standard deviation,
Statistical comparison sk. I. G vs II. K: *** – P < 0.0001, ** – P < 0.01, * – P < 0.05, ns – non-significant

Table 2. Statistical comparison of sampling 0 and remaining samplings in individual groups before and after vaccination

	COMPARISON	FA LE	FI LE	IMA	CHI	SIP	AB RABIES
Group I.G	Day 0 vs day14	ns	ns	ns	ns	***P < 0.0001	ns
	Day 0 vs day 28	ns	ns	ns	ns	*P < 0.05	***P < 0.0001
	Day 0 vs day 42	ns	ns	ns	ns	ns	***P < 0.0001
	Day 0 vs day 56	ns	***P< 0.0001	*P < 0.05	ns	ns	***P < 0.0001
	Day 0 vs day 70	***P < 0.0001	***P < 0.0001	***P < 0.0001	ns	ns	***P < 0.0001
Gropup II.K	Day 0 vs day 14	ns	*P < 0.05	ns	ns	ns	ns
	Day 0 vs day 28	ns	ns	ns	ns	ns	ns
	Day 0 vs day 42	ns	ns	**P < 0.01	ns	ns	ns
	Day 0 vs day 56	ns	ns	**P < 0.01	ns	ns	ns
	Day 0 vs day 70	***P < 0.0001	***P < 0.0001	***P < 0.0001	ns	ns	ns

Legend: see Table 1

samples were taken on days 14, 28, 42, 56 and 70 after administration of glucan and vaccination.

Immunological analysis

Blastogenic response of blood lymphocytes to mitogens was evaluated by ELISA BrdU (colorimetric) test, using 20 μg.ml^{-1} phytohaemagglutinin PHA – P (Sigma, USA). Its level was expressed as a stimulation index (SI).

Phagocytic ability of blood leukocytes were determined by the method of Větvička *et al.* (10). The phagocytic activity of leukocytes was expressed as per cent of leukocytes phagocytising 3 and more MSHP particles and the phagocytic index as a ratio of the number of phagocytised MSHP and the number of all potential phagocytes.

Chemotactic activity was determined using the method of chemotaxis of polymorphonuclears (PMNL) under agarose according to Mareček and Procházková (4). The chemotactic index (CHI) was determined as a ratio of length of chemotactic and spontaneous migration paths.

Iodonitroterazolim (INT) test was carried out using modification according to Mareček and Procházková (4). An important characteristics of functional ability of phagocytes is the ratio of spontaneous activity and activity after stimulation, the so-called index of metabolic activity (IMA).

Level of specific anti-rabies antibodies was determined by ELISA test. Anti-dog IgG /Px conjugate was used to detect and quantify rabies antibodies (1, 6). Antibody titres were expressed in UE.ml^{-1} (units equivalent to international units) with titres above 1.0 UE.ml^{-1} considered as positive.

Statistical analysis was carried out by ANOVA test ("Bonferroni's Multiple Comparison Test").

RESULTS AND DISCUSSION

Table 1 presents results obtained in groups I.G and II.K and their statistical evaluation. Table 2 shows comparison of 0-sampling and other samplings in both groups before and after vaccination.

FA Le % increased in comparison with 0-sampling with highest significance (***P < 0.0001) at the last sampling in both groups, I.G and II.K. **FI Le** in group I.G. showed a significant difference compared to II.K already on days 28 (*P < 0.05) and 56 (*P < 0.05) after administration of glucan. In the control the difference compared to 0-sampling was significant (*P < 0.05) on days 14 and 70 post-vaccination. In dogs administered glucan, FI Le reached significant values on days 56 and 70 compared to 0-sampling (***P < 0.0001). Differences in **IMA** levels between vaccinated animals with glucan and control dogs were insignificant, however, compared to 0-sampling, they were significantly higher on day 56 (*P < 0.05) and 70 (**P < 0.01) in both groups. **CHI** changed significantly in group II.K already at samplings 3 and 4 (**P < 0.01) with the highest significance (***P < 0.0001) on day 70 (last sampling). The difference between the groups was significant (*P < 0.05) only at 2nd sampling. In the I.G. group **SIP** increased significantly l (***P < 0.0001) already on day 14 compared to II.K. and the most significant increase (***P < 0.0001) was observed in puppies from I.G group between days 0 and 14. Significant difference in this group was observed also between days 0 and 28 (*P < 0.05).

AB Rabies UE.ml^{-1}: in the I.G group a protective level of rabies antibodies (> 1 UE.ml^{-1}) was detected at each sampling starting from the onset of immunoprophylaxis while in the II.K group the dogs failed to reach this level at all samplings. The highest level of Ab, 1.77 UE.ml^{-1}, in dogs treated with glucan was reached 28days after administration of anti-rabies vaccine (sampling 4) compared to the control (***P < 0.0001). From this day onwards significantly different results (***P < 0.0001) were obtained on days 42, 56 and 70 compared to group II.K and day 0 in group I.G. Contrary to the previous studies (9, 3) which failed to detect significant difference in antibody response after vaccination in immunosupressed and immunocompetent animals, our results showed that production of rabies antibodies in dogs with altered immune parameters differed significantly. According to WHO, the titre of rabies antibodies measured by ELISA test is considered protective (1) at the level > 1.0 EU.ml^{-1} (2). Such a titre was not detected in immunosuppressed dogs without immunostimulative action of glucan in comparison with dogs the immunity of which was supported with β(1,3/1,6) D-glucan which affected positively the altered specific and non-specific immunological parameters in the respective individuals.

CONCLUSION

Our study proved that β(1,3/1,6)D-glucan exhibits important immunostimulative properties in dogs with altered immunity and may be used as an immunostimulative preparation in small animal clinical practice. In case of confirmed immunosuppression it is recommended to repeat anti-rabies vaccination because primo-vaccination itself is unable to provide sufficient protection.

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THE EFFECT OF SAGE EXTRACT AND BACTERIOCIN-PRODUCING STRAIN *ENTEROCOCCUS FAECIUM* EF55 ON NON-SPECIFIC IMMUNITY OF CHICKENS INFECTED WITH *SALMONELLA* ENTERITIDIS PT4

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ABSTRACT

The study investigated the influence of sage and *E. faecium* EF55 strain on non-specific (phagocytic activity and index of metabolic activity) immune response in chickens infected with *Salmonella* Enteritidis PT4. The experiment was carried out on 160 chickens of ISA Brown hybrid, divided to 8 groups, 4 of them infected with *S.* Enteritidis (SE, EFSE, EŠS, ŠSE) and 4 free of *S.* Enteritidis (C, EF, Š, EFŠ). The chickens were administered *E. faecium* starting from day 2 of age for 21 days in the form of lyophilisate and sage extract was administered to then in the same way. On day 4 of the experiment the respective chickens were administered *S.* Enteritidis PT4 *per os* at a dose of 10^8 CFU in 0.2 ml PBS. On day 8 of the experiment we observed a significant increase in phagocytic activity of leukocytes (FA Le) in the group SE ($P < 0.05$) and a significant increase in the index of metabolic activity (IMA) in groups EFSE ($P < 0.01$) and EŠS ($P < 0.001$) compared to the control K. On day 22 of the experiment Fa Le was increased significantly in group EFŠ ($P < 0.05$) and Fi Le and Fi He in group Š ($P < 0.05$). Our results indicate that the combination of sage extract and *E. faecium* lyophilisate appeared to be an optimum option affecting immune response of chickens suffering from salmonellosis.

Key words: chickens; *Enterococcus faecium EF55*; index of metabolic activity; phagocytic activity; sage; *Salmonella* Enteritidis PT4

INTRODUCTION

Salmonella Enteritidis causes mostly subclinical infection but in one-day-old chickens may be associated with increased morbidity and mortality (7). Probiotics and bacteriocin-producing strains with probiotic action were suggested as one of the alternatives for prevention and therapy of animal gastroenteritis (4). *Enterococcus faecium* EF55 is a bacteriocin-producing strain with probiotic action. It is a fowl isolate showing good adherence to intestinal mucosa and containing genes for production of enterocins A and P. It produces bacteriocin-enterocin EF55 active against Gram-positive and Gram-negative bacteria (11, 12). *Salvia officinalis* L. (Lamiaceae) is known for its content of polysaccharides and polyphenols that exhibit good immunomodulation properties (10). This is the reason why the present study investigated the effect of sage and strain *E. faecium* EF55 on non-specific (phagocytic activity and index of metabolic activity) immune response in chickens infected with *Salmonella Enteritidis* PT4.

MATERIAL AND METHODS

The experiment was conducted on 160 chickens of ISA Brown hybrid (Parovské Háje). They were divided to 8 groups, 4 groups were infected with salmonella and/or were administered bacteriocin-producing probiotic strain *E. faecium* EF55 (13); the following groups were included in the experiment: S. Enteritidis (SE); *E. faecium* EF55 + *S. Enteritidis* (EFSE); *E. faecium* EF55 + *Salvia officinalis* (EŠS), *Salvia officinalis* +

S. Enteritidis (ŠSE) and 4 additional groups were not infected and served as a control (K): *E. faecium* EF55 (EF); Salvia officinalis (Š, Calendula, Nová Ľubovňa, s. r. o.); *E. faecium* EF55 + *Salvia officinalis* (EFŠ). Starting from the age of 2 days, the chickens were administered *E. faecium* EF55 in the form of lyophilisate at a dose of 3 g per day for 21 days and dry sage extract at a dose of 9600 ppm. On day 4 of the experiment the respective chickens were infected per os with *S. Enteritidis* PT4 at a dose of 10^8 CFU.ml^{-1} in 0.2 ml PBS.

Preparation of lyophilised strain EF55 according to Štrompfová et al. (12).

Blood sampling. Chicken blood was collected after decapitation on days 8 and 22 of the experiment.

Immunological analysis. Phagocytic ability of leukocytes was determined by ingestion of 2-hydroxyethyl metacrylate particles (MSHP, diameter 1.2 μm, ARTIM Prague, CR) (13).

Metabolic activity of phagocytes (INT test) – 2-(4-iodophenyl)-5-fenyltetrazolium chloride – INT (Lachema Brno) – was used as an indicator of metabolic processes occurring in phagocyting leukocytes (production of microbicidal substances, particularly H_2O and O_2). Zymozan (fy Sigma, USA) served as a stimulating factor. The INT test was carried out on microscale according to Mareček and Procházková (9).

Statistical analysis – one-way ANOVA, Bonferoni test (GraphPad InStat).

RESULTS

On day 8 of sampling, the FA Le results showed a significant increase ($P < 0.05$) in the salmonella group (SE) compared to the control but on day 22 an increase ($P < 0.05$) was observed in the group EFŠ in relation to control chicks. Evaluation of FI Le revealed the highest level on day 8 in groups receiving combinations sage + salmonella (ŠSE) and E. faecium + sage + salmonellaa (EŠS) compared to the control. On day 22 of the experiment there was a significant increase ($P < 0.05$) in the sage group (Š) in comparison with the control. On day 8 of the experiment the phagocytic activity of heterophils (FA He) was most increased in groups EŠS and EFŠ compared to the control while on day 22 the highest levels were detected in groups EF and EFŠ. Evaluation of FI He revealed that on day 8 the levels were increased in all groups compared to C with the highest level in the group EŠS, while on day 22 there was a significant increase ($P < 0.05$) in sage group (Š) compared to K. Changes in IMA were observed on day 8 of the experiment with a significant increase in the groups EFSE ($P < 0.01$) and EŠS ($P < 0.001$) compared to the control.

Table 1. Day 8 of sampling

Parameters	FA (Le)	FI (Le)	FA (He)	FI (He)	IMA
K	34.80 ± 21.63	4.64 ± 2.31	83.3 ± 12.38	4.86 ± 2.30	3.11 ± 0.98
SE	58.88 ± 13.82*	4.85 ± 1.71	71.5 ± 20.8	4.89 ± 1.70	5.14 ± 2.26
EFSE	39.75 ± 11.67	5.18 ± 2.37	72 ± 20.94	5.21 ± 2.37	8.13 ± 1.04***
EŠS	18.50 ± 13.10	7.41 ± 3.25	92.6 ± 15.01	7.77 ± 3.44	8.11 ± 1.14****
ŠSE	40.0 ± 15.45	7.41 ± 2.61	89.9 ± 10.57	7.44 ± 2.64	6.21 ± 3.19
Š	36.25 ± 15.44	5.34 ±1.65	78.6 ± 13.50	5.35 ± 1.65	4.56 ± 1.20
EF	53.80 ± 8.07	5.23 ± 2.06	87.5 ± 2.75	5.24 ± 2.05	3.61 ± 1.22
EFŠ	41.80 ± 13.18	6.20 ± 1.17	92.44 ± 7.70	6.34 ± 1.20	6.09 ± 1.50

Legend: significance at the levels
* – $P < 0.05$; *** – $P < 0.01$; **** – $P < 0.001$

Table 2. Day 22 of sampling

Parameters	FA (Le)	FI (Le)	FA (He)	FI (He)	IMA
K	25.60 ± 11.42	4.53 ± 0.89	91.88 ± 8.27	4.68 ± 0.81	1.69 ± 0.32
SE	30.0 ± 8.57	4.05 ± 1.41	78.4 ± 14.3	4.05 ± 1.42	4.46 ± 1.09
EFSE	33.20 ± 11.43	4.54 ± 1.15	80.82 ± 5.23	4.62 ± 1.1	2.77 ± 0.55
EŠS	36.40 ± 10.55	5.98 ± 0.46	88.04 ± 7.01	6.02 ± 0.44	3.72 ± 1.57
ŠSE	29.20 ± 7.29	4.92 ± 0.68	84.3 ± 10.26	5.03 ± 0.74	3.00 ± 1.38
Š	30.29 ± 7.39	6.63 ± 1.75 *	97.31 ± 2.14	6.95 ± 1.08 *	3.72 ± 1.57
EF	32.80 ± 4.15	6.02 ± 0.55	99.44 ± 1.25	6.07 ± 0.57	1.45 ± 0.36
EFŠ	44.20 ± 5.85 *	6.14 ± 1.01	98.04 ± 4.38	6.23 ± 1.05	2.03 ± 0.91

Legend: significance at the level * – $P < 0.05$

DISCUSSION

According to our results, administration of SE stimulated leukocytes to considerable phagocytic activity by day 8 post infection. The combination of *E. faecium* EF55 (6, 8) and SE resulted in an insignificant increase in FA Le at both samplings compared to the control. Ghadban (3) stated that probiotics stimulate the immune system

and increase the defence against pathogenic bacteria. The most pronounced stimulation was observed after combined administration EFŠ and at ŠSE combination in both samplings. Sage itself (Š) failed to stimulate FA Le, however, it potentiated ingestion capacity of leukocytes (FI). Significant differences were observed on day 22 of the experiment. Insignificantly higher FI Le was found in groups EŠS a ŠSE on day 8 post infection in comparison with the control. The levels of FI Le proved that supplementation of a probiotic strain in combination with sage or supplementation of sage alone had good immunomodulative effects in salmonella infected chicks which was confirmed also by Kolodziej (5), Čapek and Hríbalová (1) and Nosáľ *et al.* (10). Evaluation of heterophils showed an upgoing activity in groups EŠS and ŠSE on day 8 post infection. By day 22 this activity in the mentioned groups decreased. Also FI of heterophils was higher in comparison with the control. Groups EFSE ($P < 0.01$), EŠS ($P < 0.001$) and ŠSE exhibited the highest index of metabolic activity on day 8 post infection. Demeterováv *et al.* (2) observed non-specific immune response in chickens fed diet supplemented with *E. faecium* DSM 7134 and recorded an increased index of metabolic activity as it was observed also in our experiment in groups EFSE and EŠS.

CONCLUSION

The present experiment confirmed a positive influence on non-specific immune response in terms of increased levels of all investigated parameters compared to the control particularly in groups supplemented with combinations sage + *E. faecium*. Evaluation of FI Le and FI He indicated that sage alone is a suitable amendment which affects positively the non-specific immune response of chicks infected with salmonella.

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THE INFLUENCE OF SALMONELLA INFECTION AND SAGE EXTRACT ON PRODUCTION OF MUCIN IN THE INTESTINE OF CHICKENS

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ABSTRACT

We investigated the influence of salmonella infection and sage extract on mucin secretion in duodenum, jejunum, ileum and caecum of chickens. The content of mucin was evaluated by ELISA method. The experiment was carried out on 20 chickens, hybrid ISO Brown, divided to 4 groups as follows: control (C), *S. Enteritidis* (SE), sage + *S. Enteritidis* (SSE) and sage (S). When evaluating the individual effects of sage (S) and infection (SE) on mucin content on day 8 of the experiment, we observed an increased content of mucin in duodenum and jejunum in the group (SE) in comparison with the control. The secretion of mucin in this group (SE) was increased also in comparison with SSE. A significant decrease in mucin secretion was observed in jejunum after supplementation of sage (S) and also in the caecum in comparison with groups (C) and (SE). On day 22 of the experiment the SE group showed an increased content of mucin in duodenum, jejunum and ileum in comparison with the control. Sage supplementation decreased content of mucin in group S. Sage supplementation resulted in a significant decrease in the content of mucin in group S in comparison with (SSE) in jejunum and caecum. A significant decrease in mucin secretion was recorded in ileum in group SSE compared to SE. Our results point to a positive influence of supplementation of sage extract on mucin secretion in individual sections of the intestine and to its anti-inflammatory properties.

Key words: chicks; mucin; sage extract; *Salmonella Enteritidis;* small intestine

INTRODUCTION

Gastrointestinal tract of poultry is heavily populated with micro-organisms (commensal and pathogenic) of intensive metabolic activity. The first defence line that prevents penetration of bacteria through intestinal epithelium is a gel layer covering the mucous membrane. The mucous gel consists of polymeric mucous glycoproteins produced by goblet cells (8). These glycoproteins compete with bacteria for adherence to the intestine with the help of their heterogenous oligosaccharide chains and prevent the contact of harmful agents with the epithelial cells. At the same time these glycoproteins provide a suitable environment for multiplication of specific microflora. The type and quantity of mucus are basically the essential requirements for generation of intestinal barrier. Both the morphology and content of mucus change along the intestine. The mucus layer in the large intestine is thicker compared to the small one and the thickness increases gradually in cranio-posterial direction (4).

Extract of sage is known for its protective effects. It inhibits oxidative processes of amino acid intermediary metabolism, suppresses growth of bacteria and fungi in the digestive tract and stabilizes intestinal microflora (6).

Salmonella infection of chicks is characterised by invasion of intestinal mucosa and systemic spreading mediated by the ability of micro-organisms persist in macrophages. Salmonellosis is mostly subclinical but in one-day-old chickens may cause increased morbidity and mortality (10).

The aim of the study was to investigate the influence of sage and salmonella infection on quantity of mucin secreted in individual sections of the intestine in chicks.

MATERIAL AND METHODS

The experiment was carried out on 40 one-day-old chickens of hybrid Iso Brown (Párovské Háje). The experimental chickens were divided to 4 groups, 10 chickens in each, as follows: control (C), sage (S), *S. Enteritidis* (SE), sage + *S. Enteritidis* (SSE). The mixed feed of chickens in the group S was supplemented with sage extract (*Salvia officinalis* L.) at a dose of 9 600 ppm.kg^{-1} throughout the experiment (22 days). The feed was offered to chickens *ad libitum*. On day 4 the group SE was given *S. Enteritidis per os* at a dose of 10^8 CFU in 0.2 ml PBS. Samples were taken on days 8 and 22 of chicken age. Samples of the intestine were taken from the central segment of duodenum, section between the entry of biliary ducts and Meckel's diverticulum (jejunum) and midway between Meckel's diverticulum and ileocaecal junction (ileum) (11). The samples were processed as follows: extraction for 2 hours with 0.1 % solution of alcian blue which was dissolved in 0.16 mol saccharose with 0.05 mol sodium acetate and adjusted with 36–38 % HCl to pH 5.8; washed with 0.25 mol saccharose for 15 min and 45 min. Finally the tissues were immersed in 10 g.l^{-1} solution of docusate sodium overnight at room temperature, centrifuged at 1 800 r. p. m. and evaluated spectrophotometrically at wavelength 630 nm (Elisa Reader).

RESULTS

1st sampling – day 8 of the experiment: Evaluation of the influence of salmonella infection and sage extract on the level of mucin in individual intestinal segments showed a significant increase in mucin content in duodenum and jejunum of the infected group (SE) in comparison with the control and the group SSE. A significant decrease in mucin level was observed in jejunum of chickens supplemented with sage extract and in the caecum of this group (S) the level of mucin was decreased significantly in comparison with groups C and SE (Tab. 1).

Table 1. The influence of salmonella infection (*Salmonela Enteritidis* PT4) (SE) and sage extract (*Salvia officinalis* L.) (S) on mucin level in GIT of 8 days old chickens

Intestinal segment	Mucin level (μg AB.cm^{-2})			
	Control (C)	SE	S	SSE
Duodenum	28.68 ± 1.66^{a}	68.30 ± 4.63^{abc}	18.8 ± 1.54^{ac}	45.40 ± 4.10^{b}
Jejunum	52.00 ± 3.61^{ab}	71.30 ± 2.73^{ac}	18.2 ± 2.23^{bc}	53.80 ± 3.71
Ileum	41.20 ± 3.74	48.80 ± 1.96^{a}	25.70 ±2.42^{a}	56.40 ± 5.49
Caecum	81.40 ± 2.53^{abc}	161.5 ± 3.81^{adc}	21.80 ±1.23^{bc}	48.50 ± 5.14^{cd}

Significant differences are marked with identical letters, P < 0.05; mean ± SD, n = 5

2nd sampling – day 22 of the experiment: A significant increase in mucin level in duodenum, jejunum and ileum was observed in the group SE in comparison with the control. Supplementation of sage extract resulted in significant decrease in mucin content in jejunum and caecum in group S in comparison with SSE. A significant decrease in mucin level in ileum was observed also in SSE group in comparison with SE (Tab. 2).

Table 2. The influence of salmonella infection (*Salmonella Enteritidis* PT4) and sage extract (*Salvia officinalis* L.) (S) on mucin level adhered to GIT of 22 days old chicks

Intestinal segment	Mucin level (μg AB.cm^{-2})			
	Control (C)	SE	S	SSE
Duodenum	40,80 ± 3,17^{a}	62,70 ± 4,85^{ab}	32,20 ± 2,56^{b}	49,80 ± 2,04
Jejunum	28,60 ± 1,60^{a}	49,10 ± 1,79^{a}	35,60 ± 2,09^{b}	56,10 ± 2,96^{b}
Ileum	33,80 ± 3,14^{a}	70,60 ± 2,68^{abc}	43,30 ± 2,95^{b}	51,20 ± 1,45^{c}
Caecum	51,90 ± 5,54	42,50 ± 1,61	31,80 ± 2,09^{a}	63,40 ± 3,72^{a}

Significant differences are marked with identical letters, P < 0.05; mean ± SD, n = 5

DISCUSSION

Our results showed decreased level of adhered mucus in individual intestinal segments in older chickens. We assume that this phenomenon was caused by gradual postnatal maturing of the immune system in individual intestinal sections. Increased level of mucins during early postnatal life is an important innate barrier as the acquired immune system is not fully functional in the neonate intestine and thus is more prone to infections (4). The increased layer of mucus protects maturing cells of the intestinal epithelium against potential diseases in the early postnatal stage.

The level of mucin after supplementation of sage extract was in obvious contrast with the level of mucus after infection with *S. Enteritidis*. We assume that the increased amount of mucus is related to the response of goblet cells to the infection of intestinal epithelium. Perorally infecting *S. Enteritidis* invaded intestinal epithelium and induced changes in immunocompetent cells including cellular and humoral immune response (2). The increased secretion of mucus is one of the basic responses to acute catarrhal inflammation (9). Perorally infected intestinal epithelium induced increased secretion of mucus to protect the damaged epithelium and induce reparative processes in the intestine. Increased secretion of mucus at salmonella infection was observed also by Arnold *et al.* (1). Similar observations on changes

in mucus content were described at internal inflammatory diseases (3, 5).

Decreased content of mucus in some intestinal segments after supplementation of chicken feed with sage extract points to anti-inflammatory activity of sage. The anti-inflammatory activity was demonstrated also by Howes *et al.* (7). Decreased level of mucus in chickens fed sage-supplemented feed in our study confirmed the anti-inflammatory effect of this additive.

Our results indicated positive influence of sage extract on secretion of mucin in individual segments of the intestine and anti-inflammatory properties of this extract. On the other hand, increased secretion of mucin at salmonella infection was related to the inflammatory effect of *S. Enteritidis*.

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INFLUENCE OF DEOXYNIVALENOL AND LIGNIN ON LYMPHOCYTE SUBPOPULATIONS IN BLOOD AND INTESTINAL MUCOSA IN CHICKENS

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ABSTRACT

The aim of the study was to investigate the influence of dietary lignin supplementation (0.5 %) with deoxynivalenol (DON, 2.95 mg.kg^{-1}) on granulocyte function and immunocompetent cells in blood and intestines of broiler chickens. Dietary supplementation of lignin alone showed beneficial effect on the number of peripheral blood leukocytes and lymphocytes. The function of granulocytes was lower in comparison with the negative control. Supplementation of lignin together with DON resulted in a decrease in immunocompetent cells in the peripheral blood and increase in the intestinal mucosa.

Key words: DON; immunity; intestine; lignin; subpopulations

INTRODUCTION

Fusarium graminearum and related species – important pathogens of grains and maize, produce economically important and structurally different toxins called trichothecenes. The most common mycotoxin, deoxynivalenol (DON), is produced by genera *Fusarium* and *Stachybotrys* (9). Trichothecenes are cytotoxic and inhibit protein synthesis (1). They damage parenchymatous organs as well as the digestive, nervous and immune systems (8).

Mycotoxin-induced immunosuppression results from continuous damage to proliferation and differentiation of cells that participate in immune-mediated activities and control the complex communication network between cellular and humoral components. Immunosuppression may be manifested by a decrease in the activity of T and B lymphocytes, disturbed production of antibodies and damage to effector functions of neutrophils and macrophages (4, 6). The natural mixed occurrence of mycotoxins and damage caused by them due to their additive or synergistic action is also very important. Observation of total immunity is well elaborated but according to Oswald *et al.* (13) it is highly probable that mycotoxins affect predominantly the mucosal lymphoid tissue even before they are absorbed and subsequently metabolised.

Presently the use of mycotoxins-binding adsorbents is the most frequently used method of protection of animals against adverse effects of contaminated feed acting directly in the gastrointestinal tract (GIT). Lignin belongs among such organic adsorbents. Lignin is relatively hydrophobic and aromatic, capable of cross-binding the polysccharides in plants and undergoes no digestion by enzymes of mammals and other animals. It was observed that lignin can bind 60 % of chromium from water solutions and it could play an important role in removal of deoxynivalenol.

The aim of the present study was to investigate the influence of supplementation of lignin to DON-containing diet on immunocompetent cells in blood and intestines of broiler chickens.

MATERIAL AND METHODS

Broiler chickens of Ross 308 hybrid of both sexes were divided to 4 groups (n = 10): negative control (NC), lignin (L), DON and DON+L. All chickens were fed for 2 weeks mixed feed HYD 01 BR1, containing 0.1 mg.kg^{-1} DON and 0.005 mg.kg^{-1} zearalenon

(ZEA). After two weeks the diet of chickens in the group NC remained unchanged but in the group L was supplemented with 0.5 % of lignin. The diet of chickens of the DON group contained higher level of mycotoxins, namely 2.95 mg.kg^{-1} DON and 1.59 mg.kg^{-1} ZEA, and the diet of chickens from the group DON+L contained the highest concentration (0.5 %) of lignin. The described diets were fed to the respective chickens for 2 weeks. Contaminated maize was obtained by cultivation of maize with *Fusarium graminearum* for 4 weeks (SPU, Nitra).

At the age of 4 weeks the broilers were euthanized under anaesthesia by intracardiac heart puncture. Blood samples were collected into heparin and the dissected intestines into PBS solution.

White blood cells were evaluated by routine laboratory method and phagocyte function by flow cytometry. Phenotyping of blood lymphocytes was carried out according to Levkut *et al.* (12). Duodenal lymphocytes were examined immunohistochemically in frozen sections by means of unmarked CD3, CD4, CD8 and IgM murine anti-chicken antibodies (SouthernBiotech, USA). Commercial kit ELITE PK-6102 Mouse IgG VECTASTAIN ABC (Vector, USA) and diaminobenzidine were employed for binding of antibodies and visualisation of cells. The results were evaluated statistically using Tukey test.

RESULTS

Examination of the total number of leukocytes (G.l^{-1}) showed that the highest values were obtained in group L (23.7) and lowest in group DON+L (15.7), the difference being significant L ($P < 0.05$). Similar situation was observed with absolute number of leukocytes. The absolute number of heterophils was lowest in NC and DON+L groups and the highest (insignificant difference) in group L.

Determination of phagocytic activity (PhA) of granulocytes in peripheral blood in per cent showed an insignificant decrease in experimental groups in comparison with NC. The highest PhA values were found in group DON.

Determination of CD3 and CD4 lymphocytes in blood showed an insignificant decrease (G.l^{-1}) in DON chickens (6.2 and 5.5) compared to NC (6.8 and 5.6). The highest absolute numbers of these subpopulations were detected in group L (8.2 and 7.7) and the lowest in DON+L (4.5 and 4.4), the difference being significant ($P < 0.05$). Subpopulation of CD8 lymphocytes was the most numerous in NC (3.2) and decrease in this parameter recorded again in group DON+L (1.7) was significant compared to L (2.9, $P < 0.05$). The number of IgM cells was the lowest in NC group (1.1) and significantly increased in experimental group DON (2.1, $P < 0.05$) and DON+L (2.2, $P < 0.001$). The DON+L group exhibited significantly higher number also in comparison with L ($P < 0.01$).

Immunohistochemical examination of the intestine showed the highest numbers of CD4 lymphocytes in L (57, $P < 0.001$) and the lowest in DON group (33, $P < 0.001$) in comparison with NC (41). The number of CD8 cells was significantly higher in groups L (61, $P < 0.001$) and DON+L (54, $P < 0.05$) compared to the control (39). However a significant decrease was observed in DON group (43) in comparison with L ($P < 0.01$). The IgM level was the highest in NC group (14) and the increase was significant in comparison with DON (5, $P < 0.001$) and DON+L (10, $P < 0.05$). Significant differences were observed between higher levels in L (11) and lower in DON group (5, $P < 0.001$).

DISCUSSION

Contamination of chicken diet with DON at a dose of 2.95 mg.kg^{-1} failed to induce marked changes in blood parameters in DON group, however, the DON+L group showed decrease in total number of leukocytes and absolute number of lymphocytes and heterophils. Supplementation of the diet with lignin alone in 0.5 % concentration resulted in increase in the level of leukocytes, lymphocytes and heterophils.

The studies dealing with host resistance, antibody response and cell-mediated immunity showed that trichothecenes stimulated or suppressed immune functions in dependence on dose, exposure frequency and timing of functional immunity tests (17). *In vivo* observations proved that these toxins showed stimulative action in some leukocyte models but in other they had inhibitory effect and, paradoxically, both types of action occurred even together.

The dose of 2.95 mg.kg^{-1} of DON activated % PhA, which was reflected in its higher index in DON+L group in comparison with group L. Trichothecenes caused decreased chemotaxis and phagocytosis in neutrophils and macrophages in various species (5, 6). The mechanisms that may contribute to this might be based on superinduction of genes encoding IL-2 and IL-1 in lymphocytes and macrophages (10).

The number of T cells in peripheral blood was the highest in group L but of IgM cells in DON+L. Many toxins were associated with disturbances to humoral immunity (4). Peculiar is the effect of DON on antibody synthesis. Low doses of DON stimulated increase in IGA level but also impaired cell-mediated and humoral immunity in some animal species (7).

The beneficial effect of lignin in the intestine was manifested by increased number of T and B cells which resulted not only in their highest number in group L but also in increased level in group DON+L which may be explained by adding lignin to 0.5 % concentration as the level of the mentioned cells in group DON was the lowest. An important aspect of DON toxicity is damage to GIT. DON also affects morphology of chicken intestine, particularly duodenum and ileum, which was confirmed by shorter and thinner villi (2). On the other hand, the study of Baurhoo *et al.* (3) described a favourable effect of lignin on jejunum, reflected in higher villi and higher number of goblet cells.

The favourable effect of lignin supplemented to diet of broiler chickens on the number of immunocompetent cells in their intestine may be explained by antibacterial properties of lignin and partially also by absorption of DON by the lignin (11).

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COMPARISON OF THE LEVEL OF LACTIC ACID, PHOSPHORIC ACID AND pH IN BREAST AND THIGH MUSCLES OF MALLARDS *(Anas Platyrhynchos)*

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ABSTRACT

The goal of our study was to analyse physico-chemical parameters of the quality of mallard meat (lactic acid, phosphoric acid and pH) at the beginning of the ripening process. Samples of breast and thigh muscles of 10 mallards were collected within 24 hours after shooting and analysed using an electrophoretic analyzer and pH-meter. The mean level of lactic acid (0.868 ± 0.337 g.100g^{-1} of sample) and phosphoric acid (0.828 ± 0.245 g.100g^{-1}) in breast muscles was higher than that in the thigh (0.535 ± 0.093 and 0.668 ± 0.290 g.100g^{-1}, respectively). The pH in breast and thigh muscles (6.066 ± 0.203 and 6.126 ± 0.186, respectively) did not exceed 6.6, the level indicating potential health and hygiene problems with the respective game.

Key words: lactic acid; mallard; pH; phosphoric acid

INTRODUCTION

Mallard (Anas Platyrhynchos) is the only species of wild ducks that can be hunted in Slovakia. The period of hunting lasts from September 16 to January 15 (10). Mallard meat is a valuable food characterised by high content of easily digestible proteins, low content of fat and connective tissue, delicate fibrous structure, pleasant odour and taste which are species specific (11). The meat contains 19–23 % proteins, 2–3 % fat and 0.3–0.5 % carbohydrates (12). After killing it undergoes complex biochemical changes. One of the most important post-mortem changes in meat involves decrease in pH as a result of production of lactic acid (4). The principal metabolic substrate responsible for production of lactic acid and thus also decrease in pH is glycogen which, after killing, is subjected to anaerobic decomposition – glycogenolysis (3, 5). Glycogenolysis progresses until glycogen deposits are depleted or the enzymatic process is stopped as a result of low pH (6).

The pH of meat is not affected only by the level of lactic acid but also by phosphoric acid and other acids (7, 8). Phosphoric acid is formed during energy metabolism when ATP is degraded to ADP by the enzyme ATPase and inorganic phosphate and energy is released. Phosphorylation of ADP results in regeneration of ATP. Creatine phosphate is needed for this regeneration. After depletion of creatine phosphate, ADP is degraded to AMP which is converted irreversibly to IMP by means of the enzyme AMP-deaminase (9).

After killing the animal, pH of its muscles decreases from the neutral zone to about 5.4. Many micro-organisms do not survive at low temperatures or their multiplication slows down significantly. Moreover, the slightly acidic taste of lactic acid together with the products of metabolism of energy- rich phosphates contribute to the typical aroma of meat (11).

The aim of our study was to observe the levels of lactic acid, phosphoric acid, and pH in breast and thigh muscles of mallards at the beginning of the ripening process.

MATERIAL AND METHODS

Ten free-ranging mallards (*Anas platyrhynchos*) were shot in the region Lemešany, SR. Within 24 hours after killing, samples of breast and thigh muscles were collected and homogenised. The analytes examined were obtained by extraction from a water

extract. After measuring pH (pH-meter, InoLab WTW 720) the extract was diluted 100-fold and applied to an electrophoretic analyzer EA102 (Villa Labeco, SR) with conductivity detection. 10 mM HCL, β-alanine and 0.1 % mHEC were used as a leading electrolyte and 5mM caproic acid and 5mM TRIS as a terminating electrolyte. The results were evaluated using software ITPPpro 32 and were analysed statistically by means of Student *t*-test, correlation coefficient and other statistical characteristics using Microsoft Office Excel, 2007.

RESULTS AND DISCUSSION

Samples of mallard muscles examined within 24 hours after killing showed differences in the level of lactic acid (Tab. 1). The mean level of lactic acid in breast muscles reached 0.868 ± 0.337 g.100g^{-1} of sample while in thigh muscles it was lower and reached 0.668 ± 0.290 g.100g^{-1}. The value of the correlation coefficient was –0.402. Higher level and more pronounced dynamics of lactic acid formation in breast muscles compared to thigh muscles can be explained by higher level of glycogen in breast muscles (1).

Table 1. Comparison of the level of lactic acid, phosphoric acid (g.100g^{-1} of sample) and pH in breast and thigh muscles of mallards

Statistical parameters	Lactic acid		Phosphoric acid		pH	
	A	B	A	B	A	B
X	0.868	0.668	0.828**	0.535	6.066	6.126
SD	0.337	0.290	0.245	0.093	0.203	0.186
V (%)	38.824	43.413	29.589	17.383	3.346	3.036

A – breast muscles, B – thigh muscles; n = 10; P ≤ 0.01**

The level of phosphoric acid (Tab. 1) in breast muscles was significantly higher (P ≤ 0.01; 0.828 ± 0.245) than that in the thighs (0.535 ± 0.093). The correlation coefficient was 0.484. Phosphoric acid in the muscles arises from energy-rich phosphates by degradation of glycogen to lactic acid (11). As phosphoric acid arise during glycogenolysis just as lactic acid one may assume that its level is affected by the content of glycogen in the muscles.

In addition to the above mentioned acids, trace amounts of malic acid (from 0.046 g.100g^{-1} in thigh up to 0.104 g.100g^{-1} in breast muscles) and citric acid (from 0.032 g.100g^{-1} in thigh up to 0.144 g.100g^{-1} in breast muscles) were found in examined samples.

The presence of the mentioned acids was reflected also in pH (Tab. 1). No significant differences were recorded between the muscles observed. The mean pH in breast and thigh muscles reached 6.066 ± 0.203 and 6.126 ± 0.186, respectively. The correlation coefficient was 0.333. The pH level was affected by physiological condition at the time of killing and to a great extent also by buffering capacity of meat (2, 7). Thus the difference in pH in both types of muscles could be ascribed not only to different levels of the above mentioned acids but also to differences in their buffering capacity.

CONCLUSION

Our results indicated differences in the levels of the acids observed as well as in pH in breast and thigh muscles of mallards. On the basis of this knowledge one may expect differences in the course of the ripening process which can be reflected in sensory characteristics and water retaining capacity of muscles.

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BIOLOGICAL HAZARD ASSOCIATED WITH WASTEWATER TREATMENT

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ABSTRACT

The study investigated bioaerosol levels in various sections of municipal wastewater treatment plant with focus on total count of bacteria (TCB), total coliforms (TC), moulds and yeasts using a sampler MAS-100 Eco and Petri dishes with corresponding nutrient agars. During wastewater treatment bioaerosols are released due to mixing, spattering, aeration, splashing and other processes and may affect personnel particularly in enclosed and poorly ventilated spaces.

The level of observed micro-organisms was higher in the first stage of treatment (coarse and fine mechanical treatment) compared to biological stage (activation) and treatment of liquid sludge. The results obtained were also affected by environmental conditions (temperature, relative humidity) and season.

Key words: bioaerosol; relative humidity; temperature; wastewater treatment

INTRODUCTION

Bioaerosol is an airborne dispersion of biotic and abiotic particles of diameter 20 nm to 100 μm. Biotic components include bacteria, viruses, fungi and protozoa, most of them persisting in the atmosphere only for short time. Abiotic particles are endotoxins, mycotoxins, inorganic dust, plants, animal proteins, dead biotic components and others. Environmental conditions affect significantly the level of bioaerosols in the air. Temperature, relative humidity, season, time of day, sunlight, and particle size are all thought to affect bioaerosol survival (2, 3). In general, dustiness also increases the number of micro-organisms in the air and supports agglomeration of micro-organisms which are thus protected against adverse environmental conditions (7).

In dependence on the source, dispersion mechanisms in the air and prevailing environmental conditions, the composition of bioaerosol, size of particles and concentration of microbial components may change considerably. Wastewater treatment processes involve aeration, spattering, splashing, formation and breaking of bubbles and other processes that may lead to release of aerosols (1). Higher risk of bioaerosols was observed in small rooms with inadequate ventilation where the environment supports growth and survival of micro-organisms in bioaerosols (9). Increased incidence of various diseases was observed in personnel in wastewater treatment plants (5). Bioaerosols can persist in the outer environment and spread to considerable distances presenting risk to public health. Recently, with increased use of antibiotics and increased antibiotic-resistance of micro-organisms, bioaerosols may present serious risk (6).

The aim of the present study was to determine the counts of selected micro-organisms in various stages of wastewater treatment in relation with various environmental conditions.

MATERIAL AND METHODS

Bioaerosol samples were collected from various sites of a municipal wastewater treatment plant (MWTP) treating wastewaters produced by approx. 250 000 inhabitants by means of a sampler MAS-100 Eco. It is based on the principle of Anderson aeroscope and is capable of aspirating various pre-set

volumes of air through a perforated disc. The stream of air impinges on the surface of respective nutrient media (Endo agar, meat-peptone agar, Sabouraud agar) in a standard Petri dish. Micro-organisms were collected from air volumes 5, 10 and 20 l. Measurements were conducted in winter and spring and air temperature and relative humidity (RH) was recorded in parallel. After exposure the plates were incubated at 37 °C for 24 h with the exception of plates with Sabouraud agar which were incubated at room temperature for 72 hours. After the incubation the CFU were counted, adjusted by means of correction tables supplied with the sampler, recalculated per 1 m^3 of air and presented as CFU.m^{-3}.

RESULTS AND DISCUSSION

The results obtained are presented in Tables 1 and 2 as means of 3 samplings conducted in different time of the day.

The counts of micro-organisms in the influent area reflect pollution of wastewaters in the respective location and vary throughout the day. They are affected considerably by wind direction and distance of sampling site from water surface. The highest concentrations were detected in the first stage of treatment with increased RH (aeration, mixing, splashing) and abundance of organic substances in treated water which is important for survival of micro-organisms in bioaerosols. Close to the activation tank and tanks for treated sludge the levels of micro-organisms in bioaerosols were a little lower, which was probably related to treatment processes and cleaner water, but the means still reached the same order of magnitude. In the section of sludge dewatering there was increased temperature and decreased RH but almost the same level of micro-organisms as close to the activation tank.

In winter we observed a decreased level of moulds but increased level of TCB and TC. In both seasons the counts in the secondary (biological) treatment section were a little lower compared to the primary (mechanical) treatment.

Our results agree with those of other authors (4, 8) that pretreatment and primary treatment are the stages with the highest emission of bioaerosols and thus also higher risk to occupationally exposed personnel.

Table 1. Mean bioaerosol levels in winter (CFU.m^{-3})

Place of sampling	Influent	Coarse treatment (out)	Coarse treatment (in)	Coarse debris screen	Fine mech. treatment (out)	Sediment. Tank	Activation tank	Dewatered sludge
Air temp.	0.9 °C	0.3 °C	3.5 °C	1.0 °C	0.3 °C	1.6 °C	1.6 °C	3.5 °C
RH	68.2 %	71.5 %	73.7 %	71.9 %	80.5 %	69.7 %	73.9 %	76.9 %
TCB	0.72×10^3	1.4×10^3	7.7×10^3	3.6×10^3	12.3×10^3	0.65×10^3	0.3×10^3	1.2×10^3
TC	0.23×10^3	0	2.3×10^3	3.3×10^3	7.1×10^3	0.1×10^3	0	0.1×10^3
Moulds	0.3×10^3	0.1×10^3	0	0.2×10^3	0	0.2×10^3	0	0.2×10^3
Yeasts	0.3×10^3	0.2×10^3	0.86×10^3	0.6×10^3	1.45×10^3	0.1×10^3	0.5×10^3	0

Table 2. Mean bioaerosol levels in spring (CFU.m^{-3})

Place of sampling	Influent	Coarse treatment (out)	Coarse debris screen	Fine mech. treatment (in)	Fine mech. treatment (out)	Activation tank	Liquid sludge	Dewatered sludge
Air temp.	11.5 °C	11 °C	14 °C	13.8 °C	11 °C	11.6 °C	15.4 °C	14.6 °C
RH	54 %	56.9 %	47.7 %	57.4 %	61.7 %	56.7 %	50.5 %	49 %
TCB	2.15×10^3	2.6×10^3	5.15×10^3	3.4×10^3	3.9×10^3	1.0×10^3	4.0×10^3	0.9×10^3
TC	1.4×10^3	0.5×10^3	6.6×10^3	1.4×10^3	9.6×10^3	0	0	0
Moulds	0.55×10^3	0.8×10^3	1.9×10^3	4.0×10^3	2.9×10^3	0.8×10^3	1.5×10^3	0.7×10^3
Yeasts	23×10^3	0.65×10^3	3.8×10^3	9.8×10^3	8.2×10^3	0.6×10^3	0.4×10^3	1.1×10^3

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ETHICAL ASPECTS RELATED TO INVOLVEMENT OF ANIMALS IN ANIMAL ASSISTED THERAPY

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ABSTRACT

Therapy with involvement of animals presently attracts considerable attention as a new modern method facilitating and supporting treatment of a wide scale of diseases. In contrast to increased interest in investigating and proving positive effects of animal therapy on people only little attention is paid to the animals involved. Stress affects the well-being, health and performance of all live beings. It is inevitable to have sufficient knowledge of physiological forms of animal behaviour, understand the animal's body language and be able to recognize symptoms of stress, discomfort, fear and fatigue in order to prevent the risk of potential compromising of animal welfare. Suitable methods of selection, training, care and control of animals together with appropriate professional education of therapeutists can increase considerably the quality of therapeutic programmes, identify animals unsuitable for such activities and look for alternative ways of their management and other forms of their involvement.

Key words: animal therapy; ethics; therapeutic animals; welfare

INTRODUCTION

Utilisation of animals by man has long-lasting history. Animals provided people with food, materials, workforce, sport self-realization, friendship and serve them in many different ways. However, animals have their intrinsic interests and pursue their own needs and aims by means of species-specific behavioural patterns. The man-animal relationship acquires morally problematic character when the aims of the two subjects collide, when people cause pain, fear, stress, injure the animals or interfere with their need to satisfy physiological requirements (10).

Some groups of people are unable to become involved in the man-animal relationship. This includes old and sick people or people with some limitations, incapable of taking care of animals or living temporarily or permanently in the facilities providing health or social care. (1). These are most frequently the target groups of therapy with animal involvement which includes two basic components: animal assisted activities (AAA) and animal assisted therapy (AAT) (7).

Animals allow people to escape from everyday reality, offer friendship and love and to older people also sense of safety and needfulness, help to teach children to assume responsibility, alleviate pain and depression, stimulate, calm down, decrease blood pressure and reduce stress, fear, loneliness, are nice to look at and pleasant to touch, help to take pleasure of life and lengthen the life of their owners. We recognize many other positive effects of animals on humans. Many studies were devoted to the issue what animals can do for people and how people can benefit from them (8). On the other hand, there is only very limited knowledge about the effect of the therapeutic activities on animals themselves. Animals are exposed to patient's moodiness and stress which can influence their physical and psychical health. According to Goldstein (3) diseases may develop due to action of infectious germs, genetics or insufficient functioning of the immune system based also on negative emotions and feelings. In the past 30 years the interest in animal therapy increased considerably. Many organizations and associations have been established with the

aim to become involved in the field of therapy with the help of animals. Despite that we still lack standards for selection, training and evaluation of both animals and therapeutists that would help to reveal or avoid to potential risks resulting from this type of activities (5).

Potential sources of problems related to welfare of animals included in AAA/AAT:

Individual animal species differ in their social and behavioural needs. Frustrated or dispirited animal is more prone to the development of ethopathies (stereotypies, automutilation and similar). Each animal should be provided a safe place within its working environment into which it can retire when it feels exhausted or stressed. Animals used in AAA/AAT need some rest away from the patient without constant contact with him. Many animals are selected for therapeutic purposes just because of strong motivation to seek interactions with other species and ability to form strong bonds with their human partners. These animals must endure changing owners and clients with varying characteristics, experience and motivation to own an animal, which can be rather stressful to the animal itself. The majority of AAA/AAT animals are "imprisoned" in systems in which they have little self-control over their social life and are unable to avoid to unwanted social environment (9).

Domesticated animals show higher degree of tolerance to stressful situations and stimuli in comparison with non-domesticated species even if they had been kept in captivity. Non-domesticated species are more difficult to train and require constant strengthening stimuli. This makes them less suitable for the use in AAA/AAT. Experiments were carried out to train a capuchin monkey (*Cebus capucinus*) to become an AAT animal. In the majority of cases the respective animals had to be castrated and their eyeteeth removed to be able to use them safely in therapeutic programmes. These primates had to wear remote electric collars or harnesses in order to ensure control over their potentially aggressive and unpredictable behaviour. The necessity of use of such extreme invasive measures raises doubts about practical value of similar programmes and respecting welfare of animals involved in them. An inevitable aspect of AAT is education of therapeutists and their appropriate knowledge level. The field of animal ethology is frequently neglected. Many therapeutists are unable to judge whether the animal behaviour is normal or non-physiological. An animal manifests its internal frame of mind by its posture – body language. In the course of animal therapy the animal may cope with the stressful situation by means of the so-called calming behaviour. The calming signals, most frequently observed in dogs, include nose licking, yawning, increased respiration rate, staring at or turning away from the stressful stimulus or sniffing the ground. If the therapeutist knows the animal's body language, he/she can understand the animal, communicate with it and prevent refusal of its collaboration. Otherwise he is unable to recognize the symptoms of stress, discomfort or fear of the animal, to tell when the animal is no more capable of tolerating the respective situation and take appropriate steps, which may endanger the animal, client and even himself. Wrong interpretation of animal behaviour in such situations may result in insufficient calming down of the animal, failure to provide the animal some rest or terminate the therapy and eventually lead to permanent consequences to its psychics (4).

Not all patients have sufficient experience with keeping and care of the animals. The animals then may receive unsuitable or ill-timed commands and be punished for failure to perform tasks which they did not understand. Thus it is in the interest of organization performing animal therapy as well as the clients themselves to teach them to handle the animal properly, take care of it and, if needed, look for solutions to potential problems. As far as the process of keeping and training the animal is concerned, switching to individual consecutive owners should occur in such a way which causes minimal stress to the respective animal due to breaking of existing social bonds and which allows the animal to maintain its performance and ability to cooperate (6).

In therapy of HIV positive clients, the immunodeficient patients, the health state of animals is of ultimate importance. When cats are involved in the treatment, there is a risk of scratching which is sometimes resolved by non-ethical interventions, such as surgical removal of claws, their extreme shortening or wrapping the paws with protective covers. More suitable approach is the use of animals older than 9 months with appropriate temperament and regular proportionate shortening of claws (2).

CONCLUSION

The involvement of animals in AAA and AAT is very demanding and requires their cooperation in stressful and constrained environment. One should always ensure central position of the animal within the animal-intervention programme. If the animal feels uncomfortable or suffers, so suffers the entire programme. Besides preparing the animal for therapy one must ensure also its adequate protection against potential negative influences. According to the resolution of the European Parliament, regarding the action plan of the Society for animal protection and welfare for the period 2006–2010, all activities aimed at protection and good living conditions of animals should keep in mind that animals are sentient beings with specific needs that should be taken into consideration.

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