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# HYGIENIC AND ECOLOGICAL PROBLEMS IN RELATION TO VETERINARY MEDICINE

**International scientific conference**
**held to commemorate the 90th anniversary of birth of**
**Prof. MVDr. Ján Rosocha, CSc.**
**September 22—23, 20011, UVMP Košice, The Slovak Republic**

**A selection of papers written on topics presented at the conference**
Selected by Prof. MVDr. Miloslav Ondrašovič, PhD.

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# FOREWORD

*The International scientific conference ECOLOGY AND VETERINARY MEDICINE VIII was held to commemorate the 90th anniversary of birth of Prof. MVDr. Ján Rosocha, CSc. In this way we would like to honour the memory of this excellent specialist and extraordinary person who despite passing away in 1993 will always be a permanent part of our university and us all.*

*Prof. MVDr. Ján Rosocha, CSc. was born on September 9, 1921, in Negrovec, Carpatho-Ukraine. In 1930 the family moved to Blatné Remety. He graduated from the Russian Secondary grammar school in Uzhhorod in 1943 and in 1945 entered the Veterinary College in Brno. After graduation, he joined the staff of the newly established Veterinary College in Košice, Institute of general hygiene, bacteriology and animal hygiene. In 1961he defended his habilitation thesis and in 1965 was appointed Professor for scientific branch epizootology and animal hygiene. Throughout his professional life, until retirement, he was the member of staff of the Department of Microbiology and Animal Hygiene, in the period from 1980 till 1987 as its head. This was the department to which he devoted his entire professional career.*

*The first friendly contacts and professional communication of animal hygiene specialists from western and eastern European states date back to the sixties of the past century. The main stimulus for development of this branch of science was the rapidly developing animal production, its intensification and the associated health risks. The efforts in improvement of information exchange and scientific collaboration resulted in the statutory meeting of the International Society for Animal Hygiene (ISAH) in Budapest, Hungary. This meeting was initiated by Prof. Dr. Ferenc Kovács from the University of Veterinary Sciences in Budapest in close collaboration with Dr.h.c. Prof. Johann Kalich from Ludwig Maximillian University in Munich and Prof. Dr. Ján Rosocha from Veterinary College in Košice. The following animal hygienists also participated in this statutory meeting: Assoc. Prof. Dr. J. Hojovec from Brno, Prof. Dr.h.c. Prof. Hermann Willinger from Vienna, Prof. Dr.h.c. Dieter Strauch from Stuttgart, Prof. Dr. M. Cena from Wroclaw and many others. The aim of the Society was to accelerate the spreading of scientific information, to assist in the formation of uniform understanding of animal hygiene as one of the branches of veterinary science, to help to implement new scientific achievements for the benefit of animal production and to serve to both economic production of food of animal origin and improvement of quality and safety of the products.*

*Through all his activities, Prof. Rosocha contributed significantly to development of animal hygiene in Slovakia and in other European countries. He was part of the generation which laid the foundation-stone of animal hygiene as a scientific branch on international level. Prof. Rosocha served as the President of ISAH*

*for the period of 1980–1982 and organized the IVth International Congress of the Society at Štrbské Pleso, the High Tatras, in former Czechoslovakia. On the basis of his long-term scientific and organizational contributions, in 1991 Prof. Rosocha was elected the Honorary member of the Society.*

*The lifetime efforts of Prof. Rosocha will be honoured the best by preservation of independence of animal hygiene branch and its further development in accordance with the current animal production conditions. In this respect one should not forget to stress the environmental protection as safe food can be produced only by healthy animals living in healthy environment. The motto of Prof. Rosocha was: "It is easier to prevent diseases than to treat them".*

*Besides his teaching activities, Prof. Rosocha held several important posts and contributed considerably to formation and orientation of scientific research at the University, He was a vice-dean between 1959 and 1964 and a vice-rector between 1969 and 1976*

*He established intensive contacts with a number of European veterinary universities and acted as the Chairman of veterinary section of the Slovak Society for Food, Agricultural and Forestry sciences at the Slovak Academy of Science. In appreciation to his contributions he was awarded a gold medal.*

*Prof. Rosocha was strict but fair with students and thus loved by them. He willingly and unselfishly helped to his colleagues at the department and in practice. His professional achievements and positions did not prevent him from showing the deeply caring and friendly facets of his personality through which he could inspire joy, optimism and well-being with elegance of his own.*

*He has recorded deeply in the annals of our University, history of animal hygiene and the hearts of those who had the honour to know him or work with him. This conference should remind all his achievements and at the same time to prove that we continue the work to which he devoted his professional life.*

*Prof. Ing. Oľga Ondrašovičová, PhD.*
*The vice-rector of UVMP in Košice*

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# CONTAMINATION OF FOOD CHAIN BY DIOXINS AND *E. COLI* – PROBLEM-SOLVING AND DECISION-MAKING IN THE EU

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## ABSTRACT

**The intention of submitted paper is to analyze possible feed and food hazards in Germany due to the contamination of feedstuffs by dioxins originating from technical fats and foodstuffs by pathogenic strains of *E. coli* originating from vegetable. A part of the work deals with risk assessment and risk management and the measures that should be taken on the national and EU level to prevent such food hazards in future.**

**Key words: contamination: dioxins; *E. coli;* food chain**

## INTRODUCTION

German feed dioxins incident reported in 2010–2011, that emerged after the incorporation of the mixed fatty acids intended for technical purposes and containing dioxins into compound feeds for food-producing animals revealed how effectively the control of feed and food in protection of individual points of food chain against food risks is working in both the country being affected and other EU countries. Since early May 2011, an increased incidence of haemolytic uraemic syndrome (hereinafter referred to as the "HUS") and bloody diarrhoea related to Shiga toxin-producing *Escherichia coli* (hereinafter referred to as the "STEC") infections has again been observed in Germany, with most cases occurring in the northern parts of the country. Cases reported from other European countries were in individuals who had travelled to this area. The initial results of a case-control study conducted in Hamburg had suggested a link between illness and the consumption of raw tomatoes, fresh cucumbers and leaf salad.

## RESULTS AND DISCUSSION

### Dioxins

One of Germany's compound feed operators (Lower Saxony) had carried out his own control to detect contaminating substances in feed. A laboratory analysis carried out on 21 December 2010 detected dioxins over the limit in the compound feed (1.56 ng.kg$^{-1}$ PCDD/F-WHO TEQ). The sample with over-limit dioxins was collected at the end of November 2010 and the compound feed contained contaminated feed fat. On the next day, December 22, 2010, the operator telephonically notified the competent supervisory authority of the finding and results. Tracing back revealed that in November and December 2010 a biodiesel manufacturer (PETROTEC, Emden, Lower Saxony) delivered 7 consignments of technical mixed fatty acids (about 180 tons) to a feed fat producer (Harles & Jentzsch, Uetersen, Schleswig-Holstein) *via* a Dutch trader.

It is assumed that a total of 2256 tons of contaminated mixed feed fat were produced from technical mixed fatty acids and mixed into compound feed for laying hens, fattening poultry, cattle, pigs and rabbits at a mixture ratio of between 2 and 10% and thereafter delivered to 25 compound feed manufacturers in several German Federal States (Saxony-Anhalt, Lower Saxony, North Rhine-Westphalia and Hamburg). The official samples of compound feed intended for laying hens, fattening poultry, dairy cattle, pigs and rabbits were collected from all 25 compound feed holdings being affected. Altogether 4760 subjects were identified.

Based on this dioxin incident and to avoid similar situations in future, the competent authorities in Germany are planning to adopt the following measures and procedures:

• **obligation of feed establishments to be approved** – the manufacturers of feed fats shall be approved in the future. The procedure for approval shall be subject to a strict evaluation of background documents. The establishments shall be obliged to submit results from official sample analyses as well as reports of official controls declaring that the limits for critical substances being analyzed were not exceeded. The manufacturing of feed fats will not be possible in the same establishment where both feed compounds for animals and foodstuffs intended for human consumption are manufactured. The management of feed establishments should be professionally qualified;

• **the strict physical separation of technical fat production activities from the feed fat production should be required;**

• **expansion of legal norms for feed control** – obligation of feed business operators to carry out stringent controls into their products. There should be an obligation to analyze feed materials for toxic substances and report the results to the competent authority;

• **obligation of private laboratories to report the results of analyses;**

• **insurance of guarantee against risks** – feed business operators shall be obliged to insure their holdings/establishments and production;

• **revision of penalty framework** – obligation to check the amount of penalty in case of a violation of the law relating to foodstuffs and feedstuffs;

• **establishment of Rapid Alert System for Dioxins** – all data on dioxins in food, feed and environment shall be accumulated and evaluated in one aggregated data point, including the accessibility and input of results being from in-house checks. The establishment of the rapid alert system shall help to recognize problems early and to introduce swifter measures;

• **evaluation of supervision quality over food and feed** – the quality of supervision over food and feed shall be improved. It is important to have a transparent system of organization and practical realization of official controls, including their independent assessment;

• **transparency for consumers** – competent authorities in each German Federal State shall be obliged to publish all the over-limit results of undesirable substances found within the scope of official food control. In this respect, Germany is currently preparing an amendment of the law on consumer awareness.

In connection with the Germany's action plan, the European Commission has also begun a process for taking the following measures:

• obligatory approval of establishments that manufacture, process and trade in fats and fatty acids;

• the proposal for the separation of technical fat production activities from the feed fat production shall be examined in details. In this context, the following issues should be settled – the product intended for feeding shall be produced and stored in the plants/premises being specifically designed, resp. transported by special vehicles. There is a need for a system that shall guarantee the separation of feed from the products intended for technical purposes. This challenge could be reached through taking the measures directed towards technical equipment, dying or labelling of the products;

• strict plans for sampling and analyses of critical materials in the establishments for fat production;

• extension of obligations for private laboratories in respect of reporting positive results found under the monitoring programme for dioxins;

• the dioxin incident in Germany has underlined the need for full enforcement of Food and Feed Law in the Member States;

• in such case, when the Member State is not taking sufficient national measures, third countries may decide on imposing restrictive measures and after all there is a real risk that Germany will not be the only affected country but it could be expected broader negative effects on all Member States.

***Escherichia coli***

The large number of people suddenly affected, the geographical and demographic distribution as well as the first results of epidemiological studies in patients had suggested STEC-contaminated food by verotoxin-producing *E. coli* (STEC/VTEC). Animal food products like raw milk and raw meat, which were identified as vehicles in former STEC outbreaks, did not confirm any access of human illness in this case (1). Preliminary results of a case-control study conducted by the Robert Koch-Institut (RKI) and the Hamburg health authorities demonstrated a significant association between illness and the consumption of raw tomatoes, fresh cucumbers and leafy salads originating from Spain. Haemolytic and uraemic syndrome (HUS) is a serious and sometimes deadly complication that can occur in bacterial intestinal infections by Shiga toxin-producing *Escherichia coli*, also called verotoxin-producing *E. coli* (STEC/VTEC) (2). The complete clinical picture of HUS is characterized by acute renal failure, haemolytic anaemia and thrombocytopenia. Typically it is preceded by diarrhoea, often bloody. The outbreak strain isolated from several patients in Germany is an *E. coli* strain of serotype O104:H4 producing Shiga-toxin 2. The category of enterohaemorrhagic *E. coli* (EHEC) is differentiated on the basis of pathogenic features which can result in symptoms ranging from watery diarrhea or hemorrhagic colitis to Haemolytic and Uremic Syndrome (HUS). These strains can be classified into six groups or pathotypes:

• enteropathogenic *E. coli* (EPEC),

• attaching and effacing *E. coli* – colonizing strains, producing proteins responsible for attachment and interaction between the bacteria and the epithelial cells *E. coli* (A/EHS),

• enterotoxigenic *E. coli* (ETEC),

• enteroinvasive *E. coli* (EIEC),

• enterohemoragic *E. coli* (EHEC),

• enteroaggregative *E. coli* (EAggEC).

*E. coli* is a bacterium that very easily and frequently exchanges the genetic information with *E. coli* like bacteria as are *Salmonella* spp., *Shigella* spp. and other strains of *E. coli*, by mechanisms of horizontal gene transfer. Therefore, bacte-

rium *E. coli* may show the characteristics that were obtained from other pathogenic sources that are of broad range. Deadly *E. coli* that were diagnosed in Germany are not supposed as completely new bacteria but as a new "hybrid" strain. Based on the results obtained from genotyping of *E. coli* 0104 : H4, we received information that the bacterium has a circular chromosome 5 278 kbp in length (1 kbp is equal to 1 000 base pairs of DNA) and three additional plasmids 88 kbp, 75 kbp and 1,5 kbp in size, respectively. The chromosome contains around 5 000 predicted coding sequences covering 87 % of the genome. The biggest plasmid carries additional multidrug resistance genes. For haemolytic and uraemic syndrome (HUS) in humans, often with a fatal course, is responsible a gene cluster that encode Shiga toxin production.

Investigations at the National Reference Centre for *Salmonella* and other bacterial enteric pathogens at the Robert Koch-Institut in Wernigerode of isolates from patients from Hesse and Bremerhaven suggest that the outbreak is due to an *E. coli* strain of serotype O104 : H4 with the following characteristics: Shiga toxin 2 (vtx2a)-producing, intimin (eae)-negative and enterohaemolysin (hly)-negative. This strain shows high resistance to third generation cephalosporins and a broad antimicrobial resistance to other antibiotics including trimethoprim/sulphonamide and tetracycline. O104 : H4 could be classified as an atypical EHEC based on PCR result for stx (stx-positive) and eae (eae-negative). The stx genes are absolutely required to induce the symptom of HUS but other factors, involved in colonization and persistence in the host, are also necessary. So, stx is necessary but not sufficient to characterize a pathogenic *E. coli* strain. Further investigations at the Robert Koch-Institut have shown that the O104 : H4 strain carries aatA, aggR and aap genes in particular as associated virulence factors.

Regardless of the epidemic context, thorough cooking of food destroys these pathogenic bacteria and standard recommendations for domestic hygiene remain applicable. All measures that may prevent cross-contamination are essential. It is recommended for consumers to observe effective personal hand hygiene in general and when handling food. Fruits and vegetables should be washed thoroughly and, if necessary, peeled, and hands, knives, chopping boards and other kitchen utensils carefully washed and disinfected before preparing dishes. The same applies for hand hygiene after using the bathroom or changing diapers. In the current circumstances, considered a serious outbreak, the Federal Institute for Risk Assessment in Germany (BfR) recommends for precautionary reasons, in addition to these hygiene measures, to cease the consumption of uncooked vegetables, suspected to be the cause of the outbreak, until the exact cause of the outbreak has been identified. For individuals with diarrhoea, the importance of thorough hand hygiene is stressed in order to avoid transfer of bacteria from person to person. Individuals with bloody diarrhoea should seek medical assistance immediately.

## CONCLUSIONS

The work deals with the dioxin incident in Germany that emerged after the incorporation of technical fats into compound feeds for food-producing animals. The paper is also focused on another risk for consumers appeared in association with hemolytic uremic syndrome caused by Shiga toxin-producing *Escherichia coli* (STEC) after consumption of uncooked vegetables. An important part of this specialized publication applies to practical experiences, risk analysis and risk management and to the measures targeted at the protection of food chain and consumers against dioxins and *E. coli*.

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# CHANGES IN THE DISTRIBUTION OF *IXODES RICINUS* TICK IN SLOVAKIA IN THE PAST THREE DECADES AND THE ASSESSMENT OF ITS CAUSES

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## ABSTRACT

**The study deals with changes in altitudinal distribution of *Ixodes ricinus* tick in Slovakia. While in 1950–1980 the estimated upper limit of the tick distribution was 600–800 m above sea level (ASL), the border is currently above 1 000 m ASL, i. e. the elevation shift of hundreds of meters. Relative densities of ticks were significantly higher in the habitats at altitudes of 600–800 m than were the densities at lower altitudes. This is probably due to the climate changes, particularly an increase in the average air temperature in mountainous areas of the northern Slovakia, increased precipitation, as well as changes in landscape management and utilisation in the last decades.**

**Key words: climate changes; extension area; *Ixodes ricinus;* montainous areas; Slovakia**

## INTRODUCTION

In recent decades, human pressure on the individual components of the environment develops into a problem that affects more and more aspects of our lives. One of the problems here in Slovakia is changes in the geographical distribution of tick-borne diseases. The pressure of human society, which is being manifested as global warming, changes in land utilisation, and increased interests in outdoor activities, exposes humans to infections. Their geographical distribution is often hard to define and so is the search for the measures to eliminate them completely. The most widespread and medically most important tick species is the common ectoparasite *Ixodes ricinus* (L.), the most important vector of zoonotic diseases in Europe. Geographical and temporal changes in the distribution of ticks and tick-borne diseases are now increasingly associated with climate change (2, 8). The easiest observable effect of the increase in average annual temperature, in terms of the ecology of ticks, is their shift to higher altitudes, since it is the temperature and relative humidity that are the limiting factors in extending the upper limit of ticks. The aim of this study was to determine whether the area of *I. ricinus* tick has been extended in Slovakia. The shift of tick-borne encephalitis to higher altitudes in Slovakia has already been highlighted (4).

## MATERIALS AND METHODS

*I. ricinus* ticks were collected from vegetation by flagging with a light cotton fabric, 1 × 1 m, at selected sites in different habitats in the Inner West and East Carpathians in 2005–2011, including 2 elevation transects, to the altitude of 1 600 m. Model sites were positioned along the Inner Carpathians, vertical transects were selected in Strážov Hills and Great Fatra Mountains. Totally 6 273 *I. ricinus* ticks (3 619 adults and 2 654 nymphs) were collected.

## RESULTS AND DISCUSSION

*I. ricinus* was collected at almost all model sites to an elevation of 1250 m ASL (Choč Mountains) in typical habitats of its occurrence. In Strážov Hills ticks were present up to 950 m ASL, in the village of Great Fatra, Vlkolínec, up to an altitude of 1053 m. Ticks were commonly collected from vegetation in the Liptov basin in the range of 480–640 m ASL.

Ticks were found in the Belianske Tatras in the surroundings of Ždiar (981 m ASL) and Tatranská Javorina (999 m ASL), in the High Tatras during the summer tourist season in July 2011 normally located near hiking trails and local parks in Stará Lesná (812 m ASL), Tatranská Lomnica (862 m ASL) and Starý Smokovec (1037 m ASL), along the main road through the Ždiar Tatra Basin to Vyšné Hágy (1 100 m ASL). At Štrbské Pleso (1 350 m ASL), during a three-hour sampling, the ticks were not recorded on vegetation. In the East Carpathians, the model sites were located in the Vihorlat Mountains where ticks were collected up to the 969 m ASL.

The relative density of ticks was evaluated in Strážov Hills (400–1050 m ASL), with the highest value recorded in oak-hornbeam habitats at an altitude of 595–620 m. In these sites the relative density was higher than 100 ticks per 100 $m^2$. With an increasing and decreasing altitude the relative density of ticks was reduced. At 400–430 m ASL less than 20 ticks and, at 760–775 m ASL less than 40 ticks per 100 $m^2$ were collected. Nymphs were occasionally collected up to 950 m ASL in Veľká Fatra (665–1063 m ASL), with predominance of maple-beech forests, the highest relative density was recorded at 850–860 m ASL where more than 50 ticks per 100 $m^2$ were found and ticks were quite common to the upper boundary, 1 050 m ASL. Relative density at altitudes close to 700 m and 900 m was usually less than 20 ticks per 100 $m^2$, at 1 050 m ASL less than 10 ticks per 100 $m^2$ were found.

An altitude, a significant factor in *I. ricinus* distribution, is basically a set of changes in different microclimatic environmental conditions that define the habitat suitability for the development of ticks (5). Daniel *et al*. (1) defined the maximum limit where environmental conditions allow *I. ricinus* to complete the life cycle at around 600 m ASL. Rosický (7) stated that *I. ricinus* species is very rare at altitudes between 600–1000 m and at altitudes above 1 000 m it does not occur. Since the 1980s, an increase in the upper limit of tick occurrence has been observed in several European countries, including neighbouring Czech Republic, together with a gradual increase in the incidence of tick-borne diseases. Many authors attributed this fact to changes in temperature, especially increasing the average winter temperature (2, 3, 7) and different socio-economic factors (6).

## CONCLUSIONS

The study showed that the upper limit of *I. ricinus* tick in Slovakia has moved to higher altitudes, up to 1250 m ASL, i.e. the shift about 400–450 altitudinal metres since the 1980s. The areas that were once the upper limit of tick expansion are now densely populated by *I. ricinus*. During the course of the study, the average annual temperature in Slovakia has risen by 1.5 °C. Given that temperature is a limiting factor for the survival of ticks at the upper limit (except for the presence of suitable hosts), increasing average annual temperature in Slovakia is most likely responsible for the expanding of this tick species within mountain areas, that are very attractive tourist destinations. As long-term climate models have predicted a continual increase in average temperature, it may result in further distribution of ticks to even higher altitudes. The role of the ever-changing economic factors in such phenomenon should not be forgotten.

## ACKNOWLEDGEMENT

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# EXPLOITATION OF CUBICLES LITTERED WITH SEPARATED SLURRY SLUDGE BY DAIRY COWS

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## ABSTRACT

**We evaluated cubicles for housing of dairy cows littered with separated digested sludge of slurry. Evaluation included 86 cubicles. The cows were milked in morning and evening with 12 hour period. Exploitation of cubicles was evaluated on the basis of daily behaviour of cows by direct observation. In stable a 10 minute interval of monitoring was practiced to evaluate the cubicle exploitation according to daily behaviour of animals by the same observers. They observed the following behaviour: normal lying with cow's head situated near the brisket locator (L), opposite lying with cow's head situated near the rear curb (Lc), standing with two front legs in a cubicle (Sf) and standing with 4 legs in acubicle (S). Furthermore, we evaluated lying in passages (Lp), standing in passages (Sp), movement in passages (M), feeding (F) and drinking (D). In the stable cows rested in cubicles in natural position (L) for 33.13 % time out of 12 hours of investigation per day. The total time, which the cows spent in cubicles by lying or standing (L + Lo + S + Sf) reached 40.98 % of total time. But cows used to lie down in wet passages, too.**

**Key words: bedding; cubicle; dairy cows; lying; separated slurry**

## INTRODUCTION

Behaviour of animals is a welfare indicator. The animals respond sensitively to parameters of housing environment which influence their comfort through individual behavioural manifestations, such as lying, standing, movement, drinking, *etc.* Mainly the time spent by lying, frequency of lying down and lying length are considered as sensitive indices of the housing comfort (4). Lying as a welfare parameter has a higher priority than feeding and social contacts when the possibilities of expressing normal behaviour are limited (5). Time spent by lying and lying frequency during 24 hours is used as an indicator of animal comfort. Animal behaviour related to resting in lying area in loose housing system is influenced by construction factors of the stable and its operating mode. The most important factors are surface properties of floor and quality of bedding (5), stable orientation and internal dispositional layout (8), as well as the size of housing area per animal.

The aim of this paper was to evaluate the exploitation of cubicles in houses for dairy cows littered with separated slurry. This modified bedding material is a progressive substitute of straw bedding, which is more advantageous from the operational and cost view.

## MATERIAL AND METHODS

In a house for dairy cows a separated digested sludge from biogas station was used as bedding. We evaluated 86 dairy cows housed in deepened cubicles arranged in two rows, with 3.2 m wide dunging passage and external feeding. Cubicles were 1 200 mm wide and 2 400 mm long, with 300 mm height of the rear curb, and a concrete brisket board delimited the area for lying in a position 1 900 mm from the entry edge of the cubicle, with superelevation of a passage for 400 mm. The diagonal distance from the rear curb edge to neck rail was 1 985 mm. Milking process was organised in a dovetail milking parlour (2 × 12) at 7.00 a. m. and at 7.00 p. m. During milking (absence of dairy cows) the rear cubicle section was inspected and soiled bedding (with urine and excrements) was removed manually into the dunging passage. Additional bedding material was added

up to the curb level, if necessary. The passages were cleaned by automatic spade in two-hour intervals. Heat stress was eliminated in summer by 8 outlets of diameter 910 mm and ensuring air exchange of 21 200 $m^3.h^{-1}$.

Cubicle exploitation was evaluated on the basis of behavioural monitoring of dairy cattle with direct observation in 10 minute intervals during the day. The following behaviour was recorded: normal lying with cow's head situated near the brisket locator (L), opposite lying with cow's head situated near the rear curb (Lc), standing with two front legs in a cubicle (Sf) and standing with 4 legs in a cubicle (S). Furthermore, we evaluated lying in passages (Lp), standing in passages (Sp), movement in passages (M), feeding (F) and drinking (D). The cows were observed during the day between two milkings (12 hour period). Total time of animal behaviour manifestation, partial manifestations in one hour intervals and the exploitation of cubicles by dairy cows were evaluated from the obtained ethograms.

## RESULTS AND DISCUSSION

In the evaluated stable cows rested in cubicles in natural position (L) for 33.13% of the total evaluation time (12 hours). The total time which the cows spent in cubicles by lying or standing (L + Lc + S + Sp) reached 40.97%. However, opposite lying with cow's head situated near the rear curb (Lc) made the smallest part of the investigated period. Greater part was spent by standing with two front legs (Sf) in a cubicle (6.27%) or standing (S) with 4 legs in a cubicle (1.38%). Total lying time took 33.13%. Opposite lying (Lc), resulting in considerable soiling of the cubicle, was observed for 0.19% of time and it happened only in three cubicles wider than 1 200 mm (from 1 350 to 1 400 mm). Lying in passages (Lp), related to considerable soiling of udder and belly, took only 0.53% of time. Standing with two front legs (Sf) and standing with four legs in a cubicle was observed for nearly 20% of total cubicle occupation time (out of 40.97% – together lying, opposite lying, standing and standing by two front legs). This is time when the animal's claws are not with direct contact with the wet soiled floor. Probably, the cross ventilation producing direct intensive airflow around cows in standing position, played an important role.

The analysis showed that the cubicles located at the house entrance near the watering place were used only minimally (Fig. 1). It is necessary to mention that the drinker was not protected by any barrier and the lying places became wet on many occasions, or were occupied by cows in an effort to gain access to the drinker.

It seemed that the animals perceived these disturbances searched for calmer places. Lying cubicles situated opposite the drinkers were regularly occupied. The most preferred cubicles were occupied from 380 to 440 minutes. These were cubicles 13, 14 and 15 in the left row and 20, 21 and 22 in the right row in front half of the object. In the back half of the stable there were cubicles 33–38 in the left row and 26, 29, 30 and 36 in the right row. The marginal 6–8 cubicles were generally exploited for less than 50% of time, some cubicles at the end were not used at all during the day. The length of lying can be influenced not only by the quality of the resting area but also by better harmonization of daily operations with the regimen of animals. Reserves occurred, for example, in late littering and cleaning of cubicles after return of animals from the milking parlour and their feeding, or if the feeding machine came to the group of cows too late before milking. This is a time when the animals would probably like to rest, but the noise produced by feeding machine is an impulse for leaving the resting area and move to the feeding manger, which shortens the lying time. Monitoring of the cows' behaviour showed that animals were lying in cubicles for 34.07% of the 12 hour observation period. The time of milking had a minor effect. The total time spent in the cubicle (L + Lc + S + Sf) reached together 61.33% of the net animal stay in stable between milkings. In this stable we also

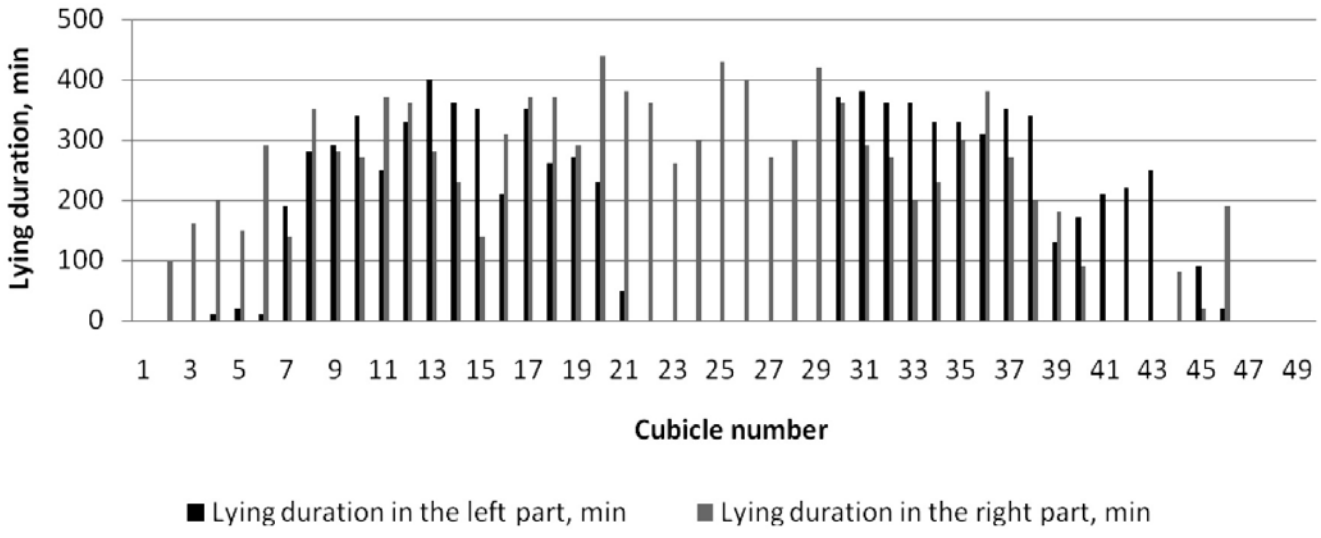


**Fig. 1. Utilization of individual cubicles for natural lying (L)**

observed late deposition of bedding and feeding during resting time. Lying time could be affected by evaporative cooling and vents. In one group the marginal cubicle row was sun-lit up to the noon, in the second group in the afternoon, but moderate increasing of local air circulation traditionally in the afternoon made it possible to remove excess heat from the area of animals.

Feeding, drinking and milking together represented a smaller part but a great part of feeding was performed before the morning milking. The area near the drinkers was often attended, but cows remained in this place not always for the purpose of drinking. This was a free area with often increased airflow and the animals just preferred to stand there. Separated sludge offers bedding material in the form of "plastic bedding" comparable to straw. There was observed only little soiling of animals on small body areas, increased only by 6% in comparison with straw bedding (6). Studies of occupation of cubicles bedded with separated sludge showed that one should consider also other factors. Drissler *et al.* (2) found that lying time declined by about 11 minutes per day for every 10 mm decrease in bedding. Therefore it is necessary to add periodically the bedding and ensure its sufficient level. Incorrect position of neck rail can cause problems with lying down process – mainly to big animals (1, 4). The time of lying is shortened and the time of standing is prolonged in this way, having unfavourable impact on leg health. Also a very high brisket board, or its very close location to the rear curb, rise concern about lying down of dairy cows.

## CONCLUSION

Monitoring of behaviour of dairy cows housed in stables with cubicles bedded with separated digested sludge showed no negative impact on any of the observed parameters compared with the conventional bedding material, the straw. The results were satisfactory as far as the somatic cell counts, milk yield and overall health of animals were concerned. Detailed monitoring of cubicles showed that there were other factors which, in addition to the bedding material, influenced evidently the total lying time. The most striking factor was disharmony in working operations (food provision and adding of bedding at night when the animals prefer to rest). The bedding material itself was well-accepted by the animals and subsequent analyses of odour level and concentration of emissions did reach only one third of the critical values.

## ACKNOWLEDGEMENT

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FOLIA VETERINARIA, 55, Supplementum I: 17–19, 2011

# HEATING OF PIGLETS BY WARM-WATER PANELS IN FARROWING PENS

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## ABSTRACT

**The aim of this paper was evaluation of piglets heating by warm-water panels in strawless farrowing pens with crate on the basis of their surfaces temperatures. Two warm-water panels with total area of 0.48 m$^2$ were installed in each pen. Surface temperature of 96 panels in 4 sections was measured by infrared thermometer (AMIR 7811-50B) in February 2011. Outdoor average air temperature was 3.5 °C and indoor temperature in the farrowing sections was 18.7 °C during the measuring day. Warm-water panels of 2nd pens had the highest average surface temperature 40.65 ± 1.10 °C; the panels of 8th pens (i. e. last in the row) had the lowest temperature 37.91 ± 2.29 °C (P < 0.001). As far as the average surface temperature is concerned, almost 92.7 % warm-water panels had suitable and 57.3 % optimum temperature.**

**Key words: farrowing pen; heating; piglets; warm-water panel**

## INTRODUCTION

Piglets do not have the ability of thermoregulation at birth (3). Piglet upon its birth experiences a sudden 15–20 °C decrease in ambient temperature (6). At birth, the new born pig leaves a draft free environment (38.9 °C) which is the sow's womb. Even a temperature of 35 °C is cold to the piglet in its first few hours. The piglet needs heat immediately to survive and grow. Temperatures less than 37.8 °C will result in the piglet using the sow milk to warm itself (2). For new born pigs in its resting place the temperature should be from 32 °C (5, 8) to 35 °C (10), which is gradually decreased till 22–24 °C when weaned (6).

In Slovakia is recommended the air temperature for piglets 22–32 °C as optimum and for sows 16–22 °C at relative humidity 50–75 % (4, 9). To keep minimum ambient temperature of 20 °C till piglets' age of 1 month it is necessary to use local heating of their area (4, 9). Electric or warm-water heated panels for bottom local heating are used. Warmth transmission to piglets occurs by their body contact with surface of heated panel. From the viewpoint of comfort zone for piglets (2), correct regulation of temperature, either optimum or advantageous surface temperatures of heated panels are important factors.

## MATERIAL AND METHODS

The objective of this work was to evaluate surface temperatures of warm-water panels for heating of piglets in strawless farrowing pens with crate and a plastic slotted floor. In farrowing house were 5 sections with 3 rows and 8 pens in each row. Pens were situated across the alley in sections. System of warm-water panels was used for heating of piglets. Panels in first pens in all rows were situated at the enclosure wall adjacent to marginal lengthwise passage in stable and panels of last (8th) pens at external enclosure wall. Piglets had at disposal tipping covers with plastic sheets. Two panels with total area of 0.48 m$^2$ were installed in each pen. Surface temperature of 96 warm-water panels in 4 sections was measured by infrared thermometer (AMIR 7811-50B) in February 2011. At data evaluation we analyzed average surface temperature of panels first till eighth pens in rows (using by descriptive statistics) including frequency distribution of temperatures (in the range from 34 °C to 43 °C with scale of 1 °C). To determine the homogeneity of measured surface temperatures Tukey HSD test (at $\alpha = 0.05$) was used within the

ANOVA. Temperature and relative humidity of air was registered in sections and in external environment during the day.

## RESULTS AND DISCUSSION

Average indoor temperature of air in the farrowing sections was 18.7 ± 0.49 °C during the measuring day and outdoor average air temperature was 3.5 ± 0.93 °C (Tab. 1). Average indoor relative humidity of air was 56.2 ± 3.19 % and in external environment 77.1 ± 4.85 %. Measured internal temperatures of air were lower than the bottom limit of the presented optimum for piglets. The values of indoor relative humidity come under the required optimum (4, 9, 10). Warm-water panels of 2nd pens had the highest average surface temperature 40.65 ± 1.10 °C (Tab. 2) adjacent to lengthwise passage in stable. The lowest temperature 37.91 ± 2.29 °C ($P < 0.001$) had panels of 8th pens in rows, which were situated at external enclosure wall, and internal wall in the area of pen was not thermal insulated (by plastic plate) as in the first pens. Warm-water panels of 1st pens, which were closest to the regulation unit, had average surface temperature 40.14 ± 1.55 °C, i. e. by 0.51 °C lower than panels of 2nd pens. From frequency distribution of surface temperatures of warm-water panels results (Tab. 3) that average surface temperature 37–43 °C, i. e. advantageous temperature, had 92.71 % panels and temperature in the range of 39–41 °C, i. e. optimum temperature (1), had 57.29 % panels.

## CONCLUSION

The lowest temperature had panels of 8th (last) pens in rows, which were situated at external enclosure wall without thermal insulation. As far as the average surface temperature is concerned, almost 92.7 % warm-water panels had suitable and 57.3 % optimum temperature. It is possible to get improvement of temperature conditions by additional insu-

**Table 1. Temperature and relative humidity of air on the day of measurement**

| Parameter | Air temperature, °C | | Relative humidity, % | |
|---|---|---|---|---|
| | Indoor | Outdoor | Indoor | Outdoor |
| **Average ± SD** | 18.7 ± 0.49 | 3.5 ± 0.93 | 56.2 ± 3.19 | 77.1 ± 4.85 |
| **Minimum – Maximum** | 17.6–19.6 | 1.0–4.6 | 49.6–62.6 | 68.6–86.2 |

**Table 2. Surface temperatures of panels in pens according to their order in rows**

| Parameter | Pens order in a row in individual sections | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th |
| **Average*** | 40.14$^{a}$ | 40.65$^{a}$ | 40.23$^{a}$ | 40.22$^{a}$ | 40.55$^{a}$ | 39.95$^{a}$ | 39.22$^{ab}$ | 37.91$^{b}$ |
| **SD** | 1.55 | 1.10 | 1.24 | 1.37 | 1.02 | 0.90 | 1.49 | 2.29 |
| **Minimum** | 36.6 | 38.5 | 38.7 | 38.5 | 38.9 | 38.6 | 35.6 | 34.6 |
| **Maximum** | 42.1 | 42.3 | 42.2 | 42.8 | 42.5 | 41.6 | 40.7 | 41.1 |

* – Significance of differences at $P < 0.001$, $^{a-b}$ – Data with identical superscripts do not differ significantly r (Tukey HSD test at $\alpha = 0.05$)

**Table 3. Frequency distribution of surface temperatures of warm-water panels**

| Para-meter | Range of surface temperatures of heated panels in °C | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 34–35* | 35–36 | 36–37 | 37–38 | 38–39 | 39–40 | 40–41 | 41–42 | 42–43 |
| **Number** | 1 | 3 | 3 | 2 | 11 | 24 | 31 | 14 | 7 |
| **%** | 1.04 | 3.13 | 3.13 | 2.08 | 11.46 | 25.00 | 32.29 | 14.58 | 7.29 |

* – The lower limit of range includes the value and the upper limit is less than the value

lation of the external enclosure wall in the area of pens as well as by optimal heat regulation of panels according to the position of lying piglets.

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# MECHANISMS OF EFFECT OF THERMAL STRESS ON FARM ANIMALS

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## ABSTRACT

**The present paper represents a review of original data concerning character and mechanisms of action of thermal stress – high temperatures on viability, reproductive functions and performance in farm animals (rabbits and pigs). It is demonstrated, that thermal stress can negatively affect consumption of nutrients, mortality, gravidity and daily gain of animals. High temperature does not affect release of corticosterone and ultrastructure of ovarian cells, but alters ovarian cell proliferation, apoptosis, synthesis and accumulation of heat shock proteins, release of reproductive steroid hormones and IGF-I, and the response of ovarian cells to physiological stimulators (IGF-I, leptin, FSH). Additions of hormones IGF-I, leptin and FSH can affects these parameters and to prevent the effects of hyperthermy on porcine ovarian cells. It is not to be excluded that IGF-I, leptin, FSH and progesterone can be used for neutralisation of heat stress on animal reproduction**.

**Key words: FSH; hormone; heat shock protein; IGF-I; leptin; ovaries; temperature**

## INTROD UCTION

Studies of effects of high temperatures and of their mechanisms can be important for requirements to resist the negative effects of global worming and to provide adequate conditions for farm animal production, care and management. It is possible, that high temperature could affect farm animal reproductive system via the hypothalamo-hypophysial-gonadal endocrine axis and related intracellular mediators of their action: apoptosis-, proliferation- related substances and heat shock proteins (HSPs).

The aim of our studies was to examine endocrine and intracellular mechanisms mediating effect of heat stress on farm animals (rabbits and pigs), as well as to use the obtained knowledge for both detection and neutralization of negative effect of this stress.

## MATERIAL AND METHODS

During in-vivo studies, we examined the effect of long (up to 6 months) exposure of adult and newborn rabbits to normal (room) and high (32–34 °C) temperature. In the collected blood samples we analysed steroid hormones and insulin-like growth factor I (IGF-I). In the *in vitro* experiments, we studied the secretory activity (release of steroid hormones and IGF-I) of ovarian granulose cells isolated from rabbits subjected to normal and high temperatures (3). In the next series of *in vitro* experiments, we have compared the functions of porcine ovarian granulosa cells (proliferation, apoptosis, steroidogenesis, expression of HSPs) cultured with and without serum and hormones in conditions of normal (37.5 °C) and high (41.5 °C) temperatures (2).

Levels of hormones in either blood plasma and ovarian cell culture medium were determined by RIA/IRMA/ELISA. Proliferation (expression of markers of proliferation PCNA, CDC2/p34 and cyklinu B), apoptosis (expression of apoptosis-related peptides TdT, bax, caspase 3) of cultured ovarian cells was assessed by immunocytochemitry, TUNEL and SDS PAGE-Western immunbolotting, while the expression of HSPs within the cells was evaluated by SDS PAGE-Western immunbolotting and RT-PCR (1, 2).

## RESULTS AND DISCUSSION

It was observed, that exposure of young rabbits from 35 to 56 days of age to thermal stress significantly inhibited daily gain and food consumption versus control. Furthermore, high temperatures dramatically increased mortality in adult (up to 20%) and especially in young animals (up to 85%).

Results of *in vivo* experiments showed, that thermal stress can reduce blood plasma level of progesterone, estradiol, corticosterone, insulin-like growth factors I (IGF-I) and increase plasma testosterone level in both young and adult males and females (Fig. 1).

Furthermore, *in vitro* experiments showed, that high temperatures affected the ability of isolated ovarian cells to produce these hormones. These observations suggested that thermal stress can suppress reproductive functions (release of sexual hormones), mechanisms of adaptation (corticosterone) and growth promoter (IGF-I).

Results of *in vitro* experiments on porcine ovarian granulose cells demonstrated, that release of steroid hormones and IGF-I by isolated cells can be promoted by FSH, and that thermal stress can suppress the release of progesterone and its response to FSH. Furthermore, thermal stress was able to alter the accumulation of markersof proliferation (PCNA, cyklin B1), apoptosis (bax, caspase 3) and HSPs (HSP70, HSP72 and HSP105) and/or their mRNAs. Moreover, it was observed that heat stress had substantial effect on the expression of these markers.

These changes were associated with reduction in accumulation of HSP70. Treatment with some hormones (IGF-I, FSH, leptin) were able to prevent the effects of high temperatures (2). The RT-PCR data demonstrated the stimulatory influence of thermal stress on the expression of mRNA for HSP70, HSP72 a HSP105 in porcine granulose cells. Addtions of IGF-I, leptin and FSH were able to prevent these effects (1), as well as the effects of thermal stress on ovarian hormones release (2).

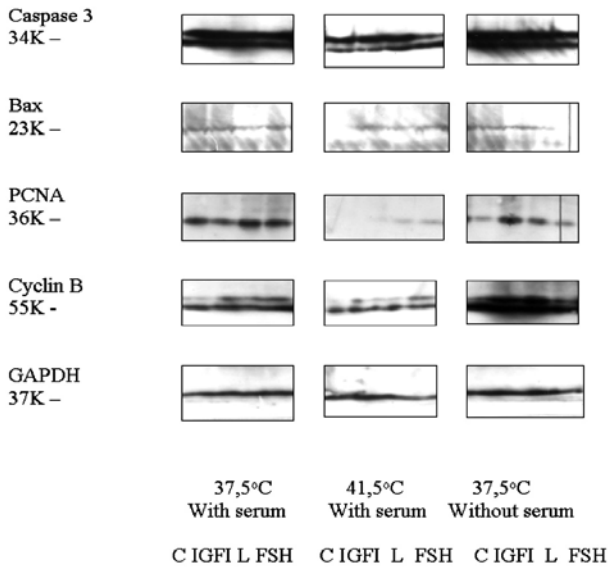


**Fig. 2. Thermal stress (41.5 °C) reduces the expression of markers of proliferation (PCNA, cyclin B1) and apoptosis (caspase 3) in ovarian granulose cells cultured with and without IGF-I, LH (L) and FSH (SDS PAGE-Western blotting) (2)**

## CONCLUSIONS

The results obtained allowed us to draw the following conclusions:

- high temperatures have negative effect on farm animal (rabbit) growth, gravidity and mortality. They influences ovarian cell proliferation, apoptosis, HSPs and trelease of ovarian steroid hormones and IGF-I, as well as the response of ovarian cells to physiological stimulators (IGF-I, leptin and FSH),
- hormones IGF-I, leptinu and FSH are able to prevent the effect of thermal stress on ovarian cells. High temperatures can suppress reproductive functions and growth through the production of IGF-I and steroid hormones and HSPs. It is possible that IGF-I, leptin, FSH, progesterone can

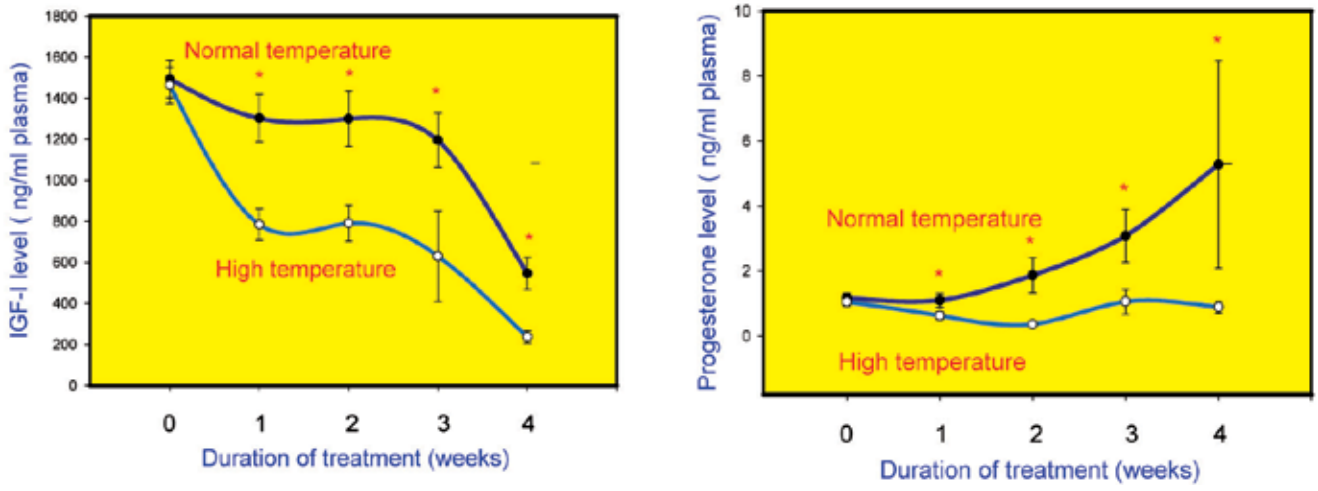


**Fig.1. Thermal stress reduces plasma level of IGF-I and progesterone in adult rabbits**

be useful for detection and neutralization of negative effects of thermal stress on farm animal reproductive processes.

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# THE INCIDENCE OF BOVINE SPONGIFORM ENCEFALOPATHY IN CATTLE SINCE 2001 IN RELATION TO A NEW VARIANT OF CREUTZFELDT-JAKOB DISEASE IN HUMANS IN SLOVAKIA

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## ABSTRACT

**From 2001 to 2010 a total of 610 225 animals were tested for Bovine spongiform encefalopathy (BSE). The target groups of investigated animals: dead animals, emergency slaughter, with clinical signs at *ante mortem* examination, healthy, slaughtered because of BSE eradication and suspicious. Overall, from 2001 to June 2011, we recorded in Slovakia 27 positive cases of BSE on 23 farms. Due to the eradication of BSE, 616 pieces of animals were included in cohort, one piece was positive. Age of positive animals ranged from 42 to 136 months, mean age was 73.22 months. According to the data from the European Creutzfeldt-Jakob Disease Surveillance Network (EUROCJD) from 1995 to March 2011, no case of a new variant Creutzfeldt-Jakob Disease (vCJD) has been reported in Slovakia.**

**Key words: bovine spongiform encefalopathy (BSE); cattle; monitoring; new variant of Creutzfeldt-Jakob Disease (vCJD)**

## INTRODUCTION

BSE is fatal nervous disease of adult cattle detected in 1986 in United Kingdom (UK). Pathology, epizootology and the mode of transmission of the disease suggest that BSE is one of the spongiform encefalopathies caused by prions. Cattle are infected by meat and bone meal made from sheep suffering from scrapie. The clinical course is varied, the disease can occur up to several years. Suspected BSE occurs in ruminants (cattle, goats, sheep and other wild game), that was a positive rapid test specific for (the presence of pathological prion protein) BSE (4, 5, 6).

A new variant of Creutzfeldt-Jakob Disease (vCJD) was described in 1996 in humans. It is a sporadically occurring, fatal neurodegenerative disease that was probably connected to the transmission of BSE to humans. Clinical manifestations differ from the sporadic form of CJD: typical ataxia, reading failures and early psychiatric symptoms (depression, anxiety, behavioural changes, sometimes aggressiveness, psychotic symptoms, gradually developing dementia). The new variant of Creutzfeldt-Jakob Disease affects mostly younger individuals, the course is slower, the incubation period is about 10 or more years, mean disease duration is 14 months (2, 3).

The aim of the study was to monitor BSE in cattle in the Slovak Republic for the period from July 2001 to June 2011 and compare the results with occurrence of vCJD in human population in Slovakia since 1996.

## MATERIAL AND METHODS

The samples of the brainstem with intact area *OBEX* together with part of *medulla oblongata* from bovine animals slaughtered normally for human consumption over the age of 30 months, emergency slaughtered animals over the age of 24 months, in animals displaying a neurological disorder over the age of 24 months, in

dead animals not slaughtered for human consumption, that died on the farm or during transport over the age of 24 months were investigated in the National reference laboratory – State Veterinary Institute in Zvolen and in the Institute of Neuroimmunology of the Slovak Academy of Sciences in Bratislava by one of the approved rapid tests for detection of pathological prion protein in cattle (Western-Blot technique, ELISA methods, immunoassay method). In the case of positive or doubtful result of rapid test the samples were tested repeatedly. In case of repeated positive or doubtful result, the samples were investigated by approved confirmation test (histopathological examination, immunohistochemical examination, confirmation technique Western-Blot).

The incidence of vCJD was determined on the basis of data from EUROCJD from 1995 to March 2011 (1).

## RESULTS AND DISCUSSION

The first ever officially confirmed case of BSE in Slovakia was recorded in 2001 on the farm Horná Ždaňa in the district Žiar nad Hronom. According to the data from the State Veterinary Institute in Zvolen and annual reports of the State Veterinary and Food Administration of the Slovak Republic, from 2001 to 2010 testing for BSE was carried out on total of 610 225 animals. Target groups of animals were as follows: dead animals, emergency slaughter, with clinical signs at *ante mortem* examinations, healthy, killed due to the eradication of BSE and suspect. Overall, the Slovak Republic from 2001 to June 2011 recorded 27 positive cases of BSE on 23 farms. There have been no positive cases of BSE in Slovakia in 2006 and 2009. The last positive case of BSE was in May 2010 on the farm Zboj in district Snina. Due to the eradication of BSE, from 2001 the cohort included 616 animals and only 1 was positive. The age of positive animals ranged from 42 to 136 months, mean age was 73,22 months.

According to the data from EUROCJD no case of vCJD was recorded in Slovakia since 1995. Most of the cases from 1995 to March 2011 were reported in United Kingdom (175 cases) and France (25) cases. The majority of cases of vCJD was recorded in United Kingdom in 2000 (28 cases) (1).

## CONCLUSION

Total number of positive cases of BSE recorded in Slovakia was 27. According to data from EUROCJD, no case of vCJD has been reported in Slovakia from 1995 to March 2011.

By implementation of Commission Decision of 17th June, 2011, the Slovak Republic was included in the group of certain Member States that revised their annual BSE monitoring programmes. According to this Decision the Slovak Republic increased the age for mandatory investigations of cattle for BSE to older than 72 months of age for healthy animals slaughtered for human consumption and to older than 48 months of age for dead animals and emergency slaughter cattle (7).

Currently there has been a decrease in positive cases of BSE in Slovakia (7) and in EU countries as well as in vCJD cases, particularly in the United Kingdom (1).

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# ANTIBIOTIC RESISTANCE IN *STAPHYLOCOCCUS* SPP. ISOLATED FROM THE COW'S MILK

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## ABSTRACT

**Bacteria *Staphylococcus* spp. (n = 85), isolated from 482 cow milk samples, collected from a herd of 120 dairy cows during one year, showed high frequency of resistance to penicilin (88.2 %), erytromycin and novobiocin (35.3 %), cloxacilin (31.8 %), tetracyclin (29.4 %), streptomycin (28.2 %), neomycin (24.7 %) and other antibiotics. The resistance of all thirteen isolated *Staphyloccocus* spp. to 14 tested antibiotics differed. Increased resistance as one of the factors of virulence of strains in relation to acute or subacute mastitis forms was confirmed primarily for bacteria *S. xylosus* (25.7 % or 38.9 %, resp.). Similar trend was observed with *S. aureus* (20.0 % and 15.5 %, resp.) and *S. haemolyticus* (14.3 % and 11.1 %, resp.) while bacteria *S. chromogenes* (65.0 %) were isolated from latent and subclinical mastitis.**

**Key words: antibiotics; cows; mastitis; resistance; *Staphylococus* spp.**

## INTRODUCTION

The observations in our country and abroad indicated that not even implementation of approved antimastitis methods can prevent fully the acute and subacute forms of udder inflammation. *Staphylococcus aureus* and *Streptococcus agalactiae* have been reported as the main infectious agents of mastitis in dairy cows. Intramammary infections and clinical inflammations of the mammary gland induced by this the bacteria are frequently. Today it is well known that not only these bacteria, but also coagulase-negative staphylococci play an important role in incidence of other forms of mastitis. The necessity of intensive, more exact approach to this problem was well stressed in the past (6). This should lead to better knowledge of pathogenesis and virulence factors of *Staphylococcus* spp. (4). Vasiľ *et al.* (7) pointed to the increasing occurrence of subclinical and latent forms of mastitis caused by resistant coagulase-negative staphylococci. This is the reason why determination of antibiotic resistance of bacterial mastitis pathogens should be the starting point resulting in effective suppression of mastitis (1, 8).

The aim of our study was to determine antimicrobial resistance of *Staphylococcus* spp. isolated from milk samples of dairy cows.

## MATERIALS AND METHODS

The study was performed on 120 (Slovak Spotted) dairy cows kept in loose houses with elevated boxes under standard animal hygiene conditions. The cows were milked twice a day in a tandem milking parlour.

The measures focused on udder health of cows during one year examination included the following: clinical examination of e mammary gland, NK test reaction (2, 3), bacteriologcal examination of collected milk samples (quarter samples). Cultivation and identification of bacteria was performed on 5 % blood agar, Medium No. 110. Gram staining, determination of catalase activity, coagulation of rabbit's plasma, haemolysis and pigments production were also carried out. The isolated bacteria were examined by a commercial set STAPHYtest 24, using identification software TNW 7.0 (PLIVA-Lachema, Brno, the Czech Republic).

All isolated bacteria of *Staphylococci* spp. were tested *in vitro* for antimicrobial susceptibility, using 14 antibiotics, Mueller-Hinton agar and the disc method (5). Concentration of antibiotics in the discs: Ampicilin (10 µg); Amoxycilin (25 µg); Cloxacilin (5 µg); Cefaperazone (30 µg) Erythromycin (10 µg); Linkomycin (15 µg); Neomycin

(10 µg); Novobiocin (5 µg); Penicillin (10 IU); Streptomycin (10 µg); Ampicilin/Sulbactam (10 µg); Oxacilin (5 µg); Cephalotin (30 µg) Tetracyklin (10 µg) (Oxoid, Anglicko). Staphylococci were regarded as resistant or sensitive according to the reference zones (5).

## RESULTS AND DISCUSSION

Examination of 482 milk samples allowed us to identify *Staphylococcus* spp. in 85 cases. The ratio of resistant *Staphylococcus* spp. bacteria is shown in Table 1. All isolated bacteria (n = 85) showed highest resistance to penicillin (88.2 %),

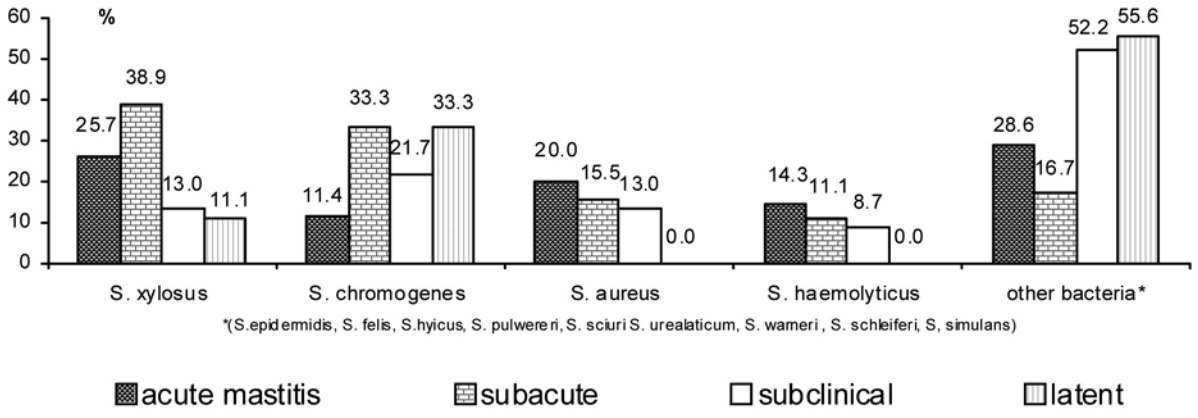


**Fig. 1. Most frequently isolated *Staphylococcus* spp. resistant to antibiotics and their proportion in individual forms of mastitis in dairy cows**

**Table 1. Antibiotic resistance of *Staphylococcus* spp. bacteria isolated from 85 cases of mastitis of dairy cows**

| Taxonomy | n | Antibiotic resistance of Staphylococcus spp. (%) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| ***S. aureus*** | 11 | 36.4 | 9.1 | 18.2 | 9.1 | 18.2 | – | 9.1 | 9.1 | 90.9 | 27.3 | – | – | – | 18.2 |
| ***S. epidermidis*** | 2 | – | – | 50.0 | – | 50,0 | – | – | 50,0 | 100 | – | – | – | – | 100 |
| ***S. felis*** | 8 | – | – | 12.5 | – | 12.5 | – | 12.5 | – | 62.5 | 12.5 | – | 12.5 | – | – |
| ***S. haemolyticus*** | 9 | 22.2 | 33.3 | 33.3 | 11.1 | 55.5 | 22.2 | 11.1 | 55.5 | 44.4 | 33.3 | 11.1 | 22.2 | 22.2 | 44.4 |
| ***S. hyicus*** | 5 | – | 20.0 | 20.0 | 20.0 | – | – | – | 20.0 | 100 | – | – | – | – | 40.0 |
| ***S. chromogenes*** | 18 | 38.9 | 16.7 | 22.2 | 11.1 | 27.8 | – | 22.2 | 5.6 | 94.4 | 22.2 | – | – | – | 11.1 |
| ***S. pulvereri*** | 4 | – | 100 | 50.0 | 25.0 | – | – | 100 | – | 100 | – | – | – | – | 50,0 |
| ***S. sciuri*** | 1 | – | – | 100 | – | – | – | 100 | 100 | 100 | 100 | – | – | – | 100 |
| ***S. urealyticum*** | 2 | – | 50.0 | 50.0 | 50.0 | 50.0 | – | 50.0 | 100 | 100 | 100 | – | 50.0 | 50.0 | 50.0 |
| ***S. warneri*** | 3 | – | 33.3 | 33.3 | 33.3 | – | – | 33.3 | – | 100 | – | – | – | – | 66.7 |
| ***S. xylosus*** | 20 | 10.0 | 40.0 | 45.0 | 5.0 | 75.0 | 65.0 | 40.0 | 85.0 | 100 | 40.0 | – | 5.0 | 5.0 | 30.0 |
| ***S. schleiferi*** | 1 | 100 | – | – | – | – | – | – | – | 100 | 100 | – | – | – | – |
| ***S. simulans*** | 1 | – | – | – | – | – | 100 | – | 100 | 100 | 100 | – | 100 | – | 100 |
| **Summary** | 85 | 18.8 | 24.7 | 31.8 | 12.9 | 35.3 | 18.8 | 24.7 | 35.3 | 88.2 | 28.2 | 1.2 | 7.1 | 4.7 | 29.4 |

1 – Ampicilin 10 µg; 2 – Amoxycilin 25 µg; 3 – Cloxacilin 5 µg; 4 – Cefoperazone 30 µg; 5 – Erytromycin 15 µg; 6 – Linkomycin 15 µg; 7 – Neomycin µg; 8 – Novobiocin 5 µg; 9 – Penicillin 10 IU; 10 – Streptomycin 10 µg; 11 – Amp/Sulb. 10 µg; 12 – Oxacillin 5 µg; 13 – Cephalothin 30 µg; 14 – Tetracyclin 10 µg

erythromycin and novobiocin (35.3%), cloxaciline (31.8%), tetracycline (29.4%), streptomycin (28.2%) and neomycin (24.7%). Individual species of bacteria differed in their resistance to the tested antibiotics.

The most frequent resistance to antibiotics was observed in four species of staphylococci: *S xylosus*, *S chromogenes*, *S. aureus* and *S. haemolyticus*. It was 64.7% (55/85) of all tested bacteria. This is important particularly with regard to their involvement in acute (71.4%), subacute (83.3%), subclinical (47.8 %) and latent (44.4%) forms of mastitis (Fig. 1).

Increased resistance as a factor of virulence of strains in relation to acute or subacute forms of mastitis was confirmed in *S. xylosus* (25.7% and 38.9%, resp.). Similar tendency was observed with *S. aureus* (20.0% and 15.5%, resp.), *S. haemolyticus* (14.3% and 11.1%, resp.), while *S. chromogenes* participated in up to 65.0% proportion of latent and subclinical forms of mastitis.

## CONCLUSIONS

We evaluated frequency of resistance to 14 different antibiotics in 85 bacteria of *Staphylococcus spp.*, isolated from 482 milk samples originating from 120 dairy cows, during one year. The highest resistance was observed in the following species: *S. xylosus* 5.0 – 100.0%, *S. aureus* 9.1 – 90.9%, *S. chromogenes* 11.1 – 94.4% and *S. haemolyticus* 11.1 – 55.5%. The resistance was important particularly with regard to proportion of these bacteria involved in acute (71.4%) and subacute mastitis (83.3%) in experimental dairy cows.

## ACKNOWLEDGEMENTS

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# NUTRITIONAL POSSIBILITIES TO REDUCE THE NITROGEN EXCRETION OF PIGS

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## ABSTRACT

**Our investigations of the influence of diets with different protein on biochemical and fermentation process in digestive system 16 weaned piglets revealed the following: the pigs fed the diets 19.5 % crude protein (CP) and 16.2 % CP received lysine (Lys), threonine (Tre) and methionine (Met) The decrease in the diet CP content was manifested by significant ($P < 0.01$) decrease in concentration of blood urea (2.48 mmol.l$^{-1}$, and 4.84 mmol.l$^{-1}$) which corresponded to the increase in biological value of the feed. The decrease in diet crude protein content was manifested by decreased amount of volatile fat acids (VFA) (44.18 g.kg$^{-1}$ DM, 38.53 g.kg$^{-1}$ DM), crude protein (238.9 g.kg$^{-1}$ DM and 198.9 g.kg$^{-1}$ DM; $P < 0.01$) and $NH_3$ 436 mg.kg$^{-1}$ DM and, 383 mg.kg$^{-1}$ DM ($P < 0.01$).**

**Key words: amino acids; ammonia excretion; nitrogen; nutrition; urea; volatile fat acids; weaned piglets**

## INTRODUCTION

Protein source is a very important factor for nursery piglets growth because poor amino acid and protein nutrition have a profound effect on physiology, health and growth of these animals. Diets with high crude protein (CP) content are commonly supplied to early-weaned piglets. This kind of diet can improve growth performance of the animals, but is always associated with incidence of diarrhoea (5). The primary factors affecting bioavailability are the efficiencies of protein digestion and amino acid absorption and the efficiency of using amino acids at the tissue level after absorption. One cause of reduced digestibility of high protein diets for weaned piglets is their high buffering capacity (2). Reducing dietary crude protein level balanced with amino acids (AA) has become an alternative approach to reduce the incidence of diarrhoea and maintain performance in weaned piglets (7). Commonly, only lysine, methionine, threonine, or tryptophan, are available in commercial and economic quantities and can be added to piglet diets in order to maintain the ideal protein profile as dietary CP decreases.

The aim of the present experiments was to determine the effects of reducing the dietary CP content from 19.5 to 16.2 % on serum biochemical parameters and fermentation process in the digestive system of weaned piglets.

## MATERIALS AND METHODS

The experiment was conducted on 16 crossbred piglets (Slovakian White x Landrace); with an initial mean body weight (BW) 11.1 ± 0.59 and 11.0 ± 0.62 kg. At weaning, piglets were divided into two groups (8 and 8). The groups contained equal number of females and castrated males. The experimental diets were formulated with 2 levels of CP (19.5 and 16.2 %). The low CP diet was supplemented with lysine (Lys), methionine (Met) and threonine (Tre). The experiment was carried out in the facilities of the Institute of Animal Nutrition and Dietetics at the UVMP in Košice in compliance with the EU regulations concerning the protection of experimental animals. The diets were analyzed for dry mater (DM), crude protein (CP), crude fibre (CF), neutral detergent fibre (NDF),

**Table 1. Chemical composition of experimental diets**

| Parameters | Control diet [g.kg$^{-1}$] | | Experimental diet [g.kg$^{-1}$] | |
|---|---|---|---|---|
| **Dry mater** | 888.40 | 1000 | 887.50 | 1000 |
| **CP** | 195.00 | 219.50 | 161.70 | 182.20 |
| **EE** | 21.50 | 24.20 | 20.40 | 23.00 |
| **CF** | 33.10 | 37.30 | 32.90 | 37.10 |
| **NDF** | 198.70 | 223.70 | 162.80 | 183.40 |
| **Ash** | 57.00 | 64.20 | 54.60 | 61.50 |
| **NFE** | 581.80 | 654.80 | 617.90 | 696.20 |
| **Lys** | 14.00 | 15.70 | 13.50 | 15.20 |
| **Tre** | 9.00 | 10.10 | 8.60 | 9.70 |
| **Met + Cys** | 7.70 | 8.60 | 7.90 | 8.90 |

tal protein, albumin, and glucose did not differ significantly within the groups. However, throughout the experiment, serum urea nitrogen was significantly ($P < 0.01$) lower in pigs fed low CP diet supplemented with lysine, methionine, threonine, compared to piglets consuming higher CP diet. Serum or plasma urea nitrogen can be used in various animal species to quantify N utilization and excretion rates. Lower blood urea nitrogen indicated higher availability of dietary nitrogen (3).

High dietary CP concentrations in the diets supplied to early-weaned pigletss increase microbial fermentation in the gastrointestinal tract. Bacterial fermentation of undigested protein produces VFA and potentially toxic substances, such as ammonia and amines (3).

In conclusion, our study showed that decrease in the dietary CP concentration from 19.5 to16.2 % caused a significant ($P < 0.01$) decrease in concentration of blood urea which means that an increase in biological value of the mixed feed mixture and reduction of fermentation processes in the digestive system decreased the level of volatile fatty acids by 13 % and also of crude protein ($P < 0.01$) and $NH_3$ ($P < 0.01$).

**Table 2. Effect of dietary CP on biochemical parameters of piglets**

| | Control diet (19. 5 % CP) | | | | Experimental diet (16. 2% CP) | | | |
|---|---|---|---|---|---|---|---|---|
| **Week** | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| **Total protein g.kg$^{-1}$** | 52.10 ± 2.75 | 45.2 ± 2.91 | 56.30 ± 2.80 | 44.80 ± 3.20 | 54.20 ± 2.89 | 51.20 ± 3.69 | 54.30 ± 2.82 | 46.60 ± 2.33 |
| **Urea mmol.l$^{-1}$** | 4.11$^a$ ± 0.10 | 4.85$^a$ ± 0.42 | 4.68$^a$ ± 0.22 | 5.72$^a$ ± 0.48 | 2·70$^b$ ± 0.30 | 2.11$^b$ ± 0.29 | 2.63$^b$ ± 0.38 | 2.51$^b$ ± 0.39 |
| **Albumin g.l$^{-1}$** | 35.40 ± 1.29 | 34.40 ± 1.99 | 33.50 ± 1.53 | 33.20 ± 2.10 | 35.17 ± 2.13 | 32.45 ± 1.38 | 32.66 ± 2.21 | 30.32 ± 1.94 |
| **Glucose mmol.l$^{-1}$** | 5.72 ± 0.41 | 5.85 ± 0.33 | 5.81 ± 0.41 | 5.28 ± 0.69 | 5.63 ± 0.86 | 5.68 ± 0.77 | 5.63 ± 0.92 | 4.45 ± 0.92 |

a, b – in the superscript indicates significant differences ($P < 0.01$)

ether extract (EE) and ash by the AOAC methods (1). Biochemical parameters: total proteins, albumin, urea and glucose in blood serum were determined spectrophotometrically using commercial Bio-La-Tests (Pliva-LaChema, Brno Ltd; the Czech Republic). Volatile fatty acids (VFA) were determined by isotachophoresis. Differences between the groups were evaluated by paired t-test.

## RESULTS AND DISCUSION

The nutrient content of diets used in experimental periods is shown in Table 1 and the metabolic variables in blood serum, determined in the study are presented in Table 2.

The biochemical parameters in blood serum of weaned piglets varied within relatively wide ranges of physiological values for pigs, presented by Kraft and Durr (4) and Vrzgula (6). The mean values of biochemical parameters as to-

**Table 3. Parameters of fermentation processes in the digestive system**

| Parameters | Control diet | Experimental diet |
|---|---|---|
| Acetic acid g.kg$^{-1}$ | 21.70 ± 2.21 | 18.17 ± 2.27 |
| Propionic acid g.kg$^{-1}$ | 15.12 ± 2.24 | 14.53 ± 1.83 |
| Butyric acid g.kg$^{-1}$ | 7.36 ± 0.49 | 5.83 ± 0.77 |
| Total VFA g.kg$^{-1}$ | 44.18 | 38.53 |
| pH | 6.46 | 6.72 |
| Crude protein g.kg$^{-1}$ | 238.90 ± 12.90$^a$ | 198.90 ± 18.60$^b$ |
| $NH_3$ mg.kg$^{-1}$ | 436.00 ± 13.00$^a$ | 383.00 ± 15.00$^b$ |

a, b in the superscript indicates significant differences ($P < 0.01$)

## ACKNOWLEDGEMENTS

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# β-1.3/1.6-D GLUCAN FROM *PLEUROTUS OSTREATUS* IN THE DIETS OF BROILER CHICKS

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**ABSTRACT**

**The effect of β-glucan (added to the diet at 0.02 g.kg$^{-1}$ – group A; 0.04 g.kg$^{-1}$ – group B, 0.02/0.04 g.kg$^{-1}$ – group C) on performance, nutrient utilization and excretion of nitrogen in broiler chicks was studied in the experiment. The supplementation of β-glucan did not influence significantly the body weight of chicks but improved feed conversion ratio (P < 0.001), protein conversion ratio (P < 0.01; P < 0.001), increased content of dry matter (P < 0.001) and decreased concentration of crude protein (P < 0.05; P < 0.001) in droppings (with the strongest expression in group B).**

**Key words: excretion; prebiotics; poultry; production**

## INTRODUCTION

Prebiotics are defined as non-digestible food ingredients which beneficially affect the host by selectively stimulating the growth and/or the activity of bacteria in the colon (5). Prebiotics are principally oligosaccharides but simple saccharides, disaccharides and polysaccharides could be used as well (6, 9). β-Glucans are polymers of glucose, which are major structural components of the cell walls of cereals, yeast, fungi and some bacteria (10). Many authors in their studies observed that these substances posses immunostimulatory (3), anticancer (8) and antimicrobial properties (7).

The objective of the present study was to investigate the effect of supplementation of diets with ·glucan on performance, nutrient utilization and excretion of nitrogen in broiler chicks.

## MATERIAL AND METHODS

In this research, a total of 200 unsexed one day old broiler chicks (Ross 308) were used. The hicks were weighed, randomly divided into four groups (n = 50) and housed on deep bedding in agreement with the technological instruction for Ross 308 chicks. They were fed with complete mixture mash according to the growth phases, ad libitum. The treatment group diets were supplemented by β-glucan (93% ± 2%; NATURES s. r. o., Trnava, SR) isolated from *Pleurotus ostreatus* in following concentrations: group A – 0.02 g.kg$^{-1}$ diet, group B – 0.04 g.kg$^{-1}$ diet, throughout the trial (42 days), group C – 0.04 g.kg$^{-1}$ diet in the first phase (weeks 1–2) and 0.02 g.kg$^{-1}$ diet in the second (weeks 3–5) and third (week 6) phases. Neither antibiotic growth promoters nor anticoccidials were added to the diets. The birds were individually weighed and feed consumption was recorded weekly. Samples of droppings were collected during weeks 2–5 of the trial and were analyzed for dry mater and crude protein by the AOAC methods (2). One-way ANOVA (Tukey's multiple comparison test; software GraphPad Prism 5) were used for statistical evaluation.

## RESULTS AND DISCUSSION

Throughout the trial the mean weight of chickens from groups A (2524.0 g) and B (2557.6 g) was insignificantly higher compared to the control group (2469.1) and group C (2444.9 g). The most intensive growth was noted in the group B. Low influence on the weight of chickens was observed by Chen *et al.* (3), who discovered the effect of β-1.3/1.6-glukan isolated from *Schizophyllum commune* in concentrations of 0.025, 0.05 and 0.1 %.

**Table 1. Dry matter (DM) content and crude protein (CP) in chicken droppings (x ± SEM)**

| | Control | | | A | | | B | | | C | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Week 2** | | | | | | | | | | | | |
| **Dry mater (g)** | 171.4 | ± | 0.76$^{a}$ | 178.4 | ± | 0.62$^{b}$ | 179.0 | ± | 0.58$^{b}$ | 176.8 | ± | 0.31$^{b}$ |
| **CP (g.kg$^{-1}$ DM)** | 306.6 | ± | 1.28$^{a}$ | 299.7 | ± | 0.50$^{bA}$ | 289.0 | ± | 1.24$^{bB}$ | 294.4 | ± | 1.06$^{bC}$ |
| **Week 5** | | | | | | | | | | | | |
| **Dry mater (g)** | 177.7 | ± | 0.93$^{b}$ | 177.4 | ± | 0.52$^{b}$ | 183.6 | ± | 0.64$^{a}$ | 174.8 | ± | 1.01$^{b}$ |
| **CP (g.kg$^{-1}$ DM)** | 319.2 | ± | 0.50$^{bA}$ | 317.2 | ± | 0.75$^{bC}$ | 305.7 | ± | 0.17$^{a}$ | 319.9 | ± | 0.24$^{bA}$ |

$^{ab, AB}$ – $P < 0.001$; $^{BC}$ – $P < 0.01$; $^{AC}$ – $P < 0.05$

The feed conversion ratio (kg.kg$^{-1}$) under the influence of β-glukan supplementation was significantly lower in all treatment groups (A 1.63, B 1.60, C 1.55) compared to the control (1.73; $P < 0.001$). Similar results were obtained for protein conversion ratio (g.kg$^{-1}$) (A 348.9 g; $P < 0.01$, B 338.6 g and C 327.1 g; $P < 0.001$) compared to the control (367.8 g).

The content of dry matter and crude protein in chicken droppings is shown in Table 1. After two weeks of the trial, the dry matter content was significantly higher ($P < 0.001$) and crude protein content significantly lower ($P < 0.001$) in all treatment groups than in the control group. The dry matter content in the group B after five weeks of the trial was significantly higher than in the control group and other trial groups ($P < 0.001$). The crude protein content in droppings after five weeks of the trial was significantly lower in group A ($P < 0.05$) and group B ($P < 0.001$) when compared to the control group and group C.

Significant reduction in crude protein in chicken droppings indicates better utilisation of protein and decreases ammonia production by micro-organisms in the litter (1). Due to significantly higher dry matter content of chick excrements, the air humidity decreases which is directly related to the growth of nitrogen-fixing bacteria (4).

Our results show that β-glukan supplementation of feed in the concentrations used results in significantly better feed and protein conversion. Significantly higher dry matter and significantly lower crude protein content in chicken droppings in the trial groups indicates that β-glukan can support better environment. The strongest effect seems to result from supplementation of feed with the highest concentration of β-glukan throughout the trial.

## ACKNOWLEDGEMENTS

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# PETS AS A SOURCE OF PARASITIC SOIL CONTAMINATION IN THE SETTLEMENTS OF MARGINALISED GROUPS OF INHABITANTS

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## ABSTRACT

**Parasitic contamination of soil and presence of endoparasites in the dogs' faeces in selected settlements of marginalized inhabitants in Košice and Prešov regions were investigated. Altogether 106 soil samples and 42 dogs' faeces samples from Jarovnice, Sečovce, Zemplínska Teplica, Svinia and Košice-Luník IX were examined. Eggs of endoparasites were detected in up to 79.2 % of soil samples. *Toxocara* spp. (61.3 %), *Ascaris* spp. (58.5 %) and *Trichuris* spp. (50.9 %) occurred most frequently. Endoparasites were also detected in the dogs' faeces (73.8 %). *Toxocara canis* (45.2 %), *Toxascaris leonina* (40.5 %) and *Trichuris vulpis* (19.0 %) were the most frequently detected species. Frequent finding of parasites in the soil samples and dogs' faeces from the marginalised areas is an important epizootiological and epidemiological problem.**

**Key words: contamination; dogs' feces; endoparasites; soil**

## INTRODUCTION

Household pets (e. g. dog, cat) play an important role in the transmission of agents causing parasitic infections. Dogs are definitive hosts of many zoonotic species of protozoa and helminths (e. g. *Echinococcus* sp., Toxocara *canis*, *Ancylostoma* sp., *Giardia* sp.). In the socially and economically backward areas, where not enough attention is paid to environmental or personal hygiene, the risk of animal or human infection is high. In Slovakia, this situation prevails in the areas of marginalized Roma communities. Many of these settlements have the same characteristics – polluted environment, low standard of personal and communal hygiene, lack of sanitation, waste pits or landfills and persistent over-occupancy of small areas where residents share their household with pets. Infected animals can disseminate large number of endoparasites' eggs into the environment through the faeces. Many eggs and (oo)cysts of parasites are very resistant and are able to survive outdoors for months or years. Consequently, contaminated environment constitutes a health risk for both animals and humans.

In this study we investigated contamination of soil by parasitic agents and prevalence of intestinal parasites in faeces of dogs in marginalized settlements in Košice and Prešov regions.

## MATERIALS AND METHODS

A total of 106 soil samples and 42 dogs' faeces samples were collected from selected settlements of marginalized population in Košice and Prešov region and subjected to parasitological examination. Analysis of soil samples for the presence of parasitic agents was carried out according to Kazacos (5). Examination of faeces was carried out according to Jurášek *et al.* (4).

## RESULTS AND DISCUSSION

The main source of soil contamination with parasites are faeces of infected household animals, especially dogs and cats (3), which may be a reservoir of parasites with zoonotic potential (e.g. *Toxocara* spp. or *Trichuris vulpis*). We can predict the level of contamination by analyzing samples of faeces from public areas but only examination of soil samples can determine the real direct-contact risk of zoonoses (1). Especially in areas where the animals have free movement and do not get adequate veterinary care, the faecal contamination of the environment and the risk of subsequent infection of animals and humans are high. Therefore we monitored the occurrence of endoparasites' eggs in soil and faeces in selected areas with low hygienic standards.

Altogether 106 soil samples from settlements in Jarovnice, Sečovce, Zemplínska Teplica, Svinia and Košice-Lunik IX were examined. Developmental stages of parasites were detected in up to 79.2% of soil samples. Eggs of *Ascaris* spp., *Toxocara* spp. and *Trichuris* spp. were present in soil samples from all studied areas, even strongyloid eggs were present frequently. The majority of positive samples originated from Jarovnice (83.4%) (Table 1).

Samples of faeces were collected from the same locations. We examined 42 samples of dog excrements by coprological methods in order to determine the prevalence of endoparasites. Intestinal parasites were present in 73.8% of samples. The most frequently detected were eggs of *T. canis* (45.2%), *T. leonina* (40.5%) and *T. vulpis* (19.0%). In samples from Jarovnice eggs of *Capillaria* spp. (11.2%) and eggs from the family Ancylostomatidae (44.5%) were also present (Table 2). In this area the number of positive parasitological findings in the soil was the highest.

Similar results were published by Mandarino-Pereira *et al.* (6), who carried out the survey on prevalence of intestinal parasites in the faeces of dogs and soil in an underdeveloped part of Brazil. The prevalence of endoparasites in the faeces was very high (92.6%) with predominance of zoonotic species. In 22.4% of soil samples endoparasites' eggs of *Ancylostoma* spp. *Toxocara* spp. *Trichuris* spp. *Ascaris* spp. and larvae of *Strongyloides* spp. were detected. Similarly Cassenote *et al.* (2) monitored soil contamination in Fernandopolis (Brazil). In positive samples the eggs of *Toxocara* spp. (79.3%), *Trichuris* spp. (13.8%) and eggs from the family Ancylostomatidae (6.9%) were detected most frequently. The authors indicated that high prevalence of zoonotic species of parasites posed a serious risk to public health. Martinez-Barbabosa *et al.* (7) carried out examination of dogs' faeces collected in fouled area of Mexico (Chiapas) to determine the frequency of contamination of the environment. A

**Table 1. Species of endoparasites in soil samples**

| | Jarovnice (%) | Sečovce (%) | Zemplínska Teplica (%) | Svinia (%) | Košice-Luník IX (%) |
|---|---|---|---|---|---|
| ***Ascaris* spp.** | 79.2 | 60.0 | 59.4 | 61.5 | 23.5 |
| ***Toxocara* spp.** | 70.8 | 50.0 | 78.1 | 46.2 | 41.2 |
| ***Trichuris* spp.** | 45.8 | 50.0 | 68.8 | 30.8 | 41.2 |
| **Strongyloid** | 20.8 | 35.0 | 31.3 | 7.7 | 0.0 |
| ***Spirocerca lupi*** | 4.2 | 0.0 | 18.8 | 30.8 | 11.8 |
| ***Toxascaris leonina*** | 12.5 | 0.0 | 0.0 | 23.1 | 11.8 |
| **Coccidia oocysts** | 0.0 | 0.0 | 31.3 | 7.7 | 0.0 |

**Table 2. Species of endoparasites in dogs' faeces**

| | Jarovnice (%) | Sečovce (%) | Zemplínska Teplica (%) | Svinia (%) | Košice-Luník IX (%) |
|---|---|---|---|---|---|
| ***Toxocara* spp.** | 22.3 | 77.8 | 50.0 | 33.4 | 25.0 |
| ***Trichuris* spp.** | 33.4 | 33.4 | 25.0 | 0,0 | 0.0 |
| ***Toxascaris* spp.** | 44.5 | 44.5 | 25.0 | 83.4 | 40.0 |
| ***Capillaria* spp.** | 11.2 | 0.0 | 0.0 | 0.0 | 0.0 |
| **Ancylostomatidae** | 44.5 | 0.0 | 0.0 | 0.0 | 0.0 |

total of 200 faeces samples were examined, of which 37.0% had a positive parasitological finding. Oocysts of *Isospora canis* (2.5%) and helminth eggs of *T. canis* (19.0%) and *Ancylostoma caninum* (18.5%) were detected.

## CONCLUSIONS

Our findings suggest that infected animals can contaminate the environment through the faeces, as confirmed by examination of soil samples collected in the selected areas. Contaminated soil may thus represent a permanent risk of infection, which constitutes a significant problem for both veterinary and human medicine.

## ACKNOWLEDGEMENTS

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# SMALL MAMMALS – RESERVOIR HOSTS OF BLOOD PATHOGENS IN URBAN SURROUNDINGS

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## ABSTRACT

**Small mammals of the order *Eulipotyphla* and *Rodentia* were examined for the presence of blood parasites. The examined mammals were caught in autumn 2010, within the Košice area (127 individuals) and in autumns of 2008, 2009, 2010, within the České Budějovice area (40 individuals). The following groups of hemoparasites were detected in the blood smears: bacteria of the genus *Bartonella* and protozoan parasites of the genera *Babesia* and *Hepatozoon*. Insectivores were infected with the *Bartonella* species only, while in rodent samples all three parasitic genera were found. The occurrence of mixed infections was rare. We registered it only in *Apodemus flavicollis*.**

**Key words: *Babesia; Bartonella;* blood parasites; *Eulipotyphla; Hepazozoon; Rodentia;* small mammals**

## INTRODUCTION

Small mammals are reservoirs of several natural-foci infections, transmissible to humans and domestic animals. They also serve as the hosts for a great number of parasitic arthropods, which can also play a significant role as vectors. In terms of epidemiology, especially significant are small mammals, living in the vicinity of densely-built urban areas that are frequently visited by humans and their pets, such as parks, gardening areas, suburban forests. Such areas present a risk of infection both for humans and their pets.

The aim of the pilot study was to assess the infection rate of *Bartonella*, *Babesia* and *Hepatozoon* genera in small mammals (insectivores and rodents) in urban areas of two towns.

## MATERIALS AND METHODS

Material of small mammals originated from the recreation areas of Košice and České Budějovice. The captures in Košice were carried out in September – December, 2010, at four locations: 1 – Nižné Kapustníky gardening area (south); 2 – Vyšná úvrať below the southern part of housing estate Dargovských hrdinov; 3 – Botanic Garden compound; 4 – recreation area Anička. A total of 127 small mammals were examined in Košice, with two identified species of the order *Eulipotyphla* (*Crocidura suaveolens*, *Sorex araneus)* and four species from the order *Rodentia* (*Myodes glareolus*, *Apodemus agrarius*, *Apodemus flavicollis* and *Mus musculus*). Captures in České Budějovice have yielded a total of 40 specimens belonging to two insectivore species (*Sorex minutus*, *S. araneus*) and five rodent species (*M. glareolus*, *Microtus agrestis*, *Apodemus sylvaticus*, *A. flavicollis* and *Micromys minutus*). All animals were captured in autumn of 2008, 2009, 2010, in a forest park near housing estate Máj.

Blood smears were prepared from the blood of captured small mammals. They were fixed in methanol and stained with May-Grümwald and Giemsa-Romanovsky stains. Blood parasites were detected microscopically.

## RESULTS AND DISCUSSION

Insectivores comprised only 8.4 % of the studied material, represented by three species. As for insectivores, Gram-negative bacteria of the genus *Bartonella* were detected only in *S. araneus* in more than a half of examined individuals (57.1 %) both from Košice and České Budějovice (Table 1). Other studied blood parasites were not detected in insectivores.

Members of the genus *Bartonella* were also found in the blood of four rodent species (*M. glareolus, A. Agrarius, A. flavicollis, A. sylvaticus*), but were absent in the blood of *M. agrestis, M. minutus* and *M. musculus*. It may have been due to a rather low number of examined animals of the relevant species. The highest positivity was found in *A. flavicollis* (62.0 %), and both locations (Košice and České Budějovice) has almost consistent rates. In Košice, *Bartonella* was present at all four locations.

Several *Bartonella* species can parasitize in small mammals, some of them pathogenic to humans (*Bartonella grahami*) or pets (*Bartonella vinsonii* subsp. *berkofii*) (1, 3). It seems that some fleas and ticks can serve as vectors.

Small mammals are also reservoir hosts of *Babesia microti*, which poses certain risk for humans with weakened immunity or after splenectomy. Ticks are vectors for the pathogen. *Babesia* was sporadically detected in the blood of two microtid rodents (*M. glareolus* and *M. agrestis*) and two mice species (*A. flavicollis* and *A. sylvaticus*) (Table 1). The parasites were detected in animals from Košice locations 1 and 2 and in animals from České Budějovice captured in the forest park near a housing estate. The highest positivity was recorded in the genus *Apodemus*: *A. flavicollis* 12.0 %, *A. sylvaticus* 14.3 %. Besides, the single examined *M. agrestis* specimen was also positive. Despite the fact that 66 *A. agrarius* individuals were subjected to examination (the most numer-

**Table 1. Blood parasite occurrence in small mammals originating from Košice (SR) and České Budějovice (CR)**

| Location / Species | No. of examined species | Host positivity | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Bartonella sp.* | | *Babesia sp.* | | *Hepatozoon sp.* | |
| | | n | P % | n | P % | n | P % |
| **Košice** | | | | | | | |
| *C. suaveolens* | 6 | – | – | – | – | – | – |
| *S. araneus* | 3 | 2 | *(66.7) | – | – | – | – |
| *M. glareolus* | 7 | 1 | (14.3) | 1 | (14.3) | – | – |
| *A. agrarius* | 66 | 6 | 9.1 | – | – | – | – |
| *A. flavicollis* | 42 | 26 | 61.9 | 4 | 9.5 | – | – |
| *M. musculus* | 3 | – | – | – | – | – | – |
| **∑** | **127** | **35** | **27.6** | **5** | **3.9** | – | – |
| **Č. Budějovice** | | | | | | | |
| *S. araneus* | 4 | 2 | (50.0) | – | – | – | – |
| *S. minutus* | 1 | – | – | – | – | – | – |
| *M. glareolus* | 18 | 1 | 5.6 | – | – | 3 | 16.7 |
| *M. agrestis* | 1 | – | – | 1 | (100) | – | – |
| *A. flavicollis* | 8 | 5 | (62.5) | 2 | (25.0) | 1 | (12.5) |
| *A. sylvaticus* | 7 | 2 | (28.6) | 1 | (14.3) | – | – |
| *M. minutus* | 1 | – | – | – | – | – | – |
| **∑** | **40** | **10** | **25.0** | **4** | **10.0** | **4** | **10.0** |

n – number of positive hosts; P % – prevalence;
* – data in brackets are based on a small number of examined individuals

ous species in our material), they were all *Babesia*-free, unlike the study by Karbowiak *et al.* (2) who reported *B. microti* in a striped field mouse captured in the Košice Basin and in an individual locality from East Slovakia lowland.

Protozoan parasites of the genus *Hepatozoon* were also detected in the material. The species parasitizing in small mammals are not pathogenic for humans and domestic animals. Members of the genus *Hepatozoon* were identified in samples of four rodents, belonging to two species. Prevalence infestation in *M. glareolus* reached 12.0% and in *A. flavicollis* 2.0%. The parasite was detected only in samples from České Budějovice.

Mixed infections, comprising two parasitic species, were detected only in three *A. flavicolis* individuals – two cases of *Bartonella* and *Babesia* mixed infection and a single case of *Bartonella* and *Hepatozoon* infection.

## CONCLUSIONS

Small mammals captured in Košice and České Budějovice harboured blood parasites of the following genera: *Bartonella*, *Babesia* and *Hepatozoon*. Except for the latter, they can pose a certain risk even for humans and domestic animals.


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# APPLICATION OF NON-INVASIVE METHODS OF SAMPLING AS AN ALTERNATIVE TO CONVENTIONAL BLOOD TAKING IN WELFARE ASSESSMENT OF HORSES

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## ABSTRACT

**Non-invasive methods for taking samples make it possible to minimalize animal's stress activated by excessive contact with humans. In our study we focused on behaviour assessment in horses during collecting of saliva samples for cortisol determination. We took samples of saliva from 32 horses with the use of a Salivette bleeder set and evaluated behaviour of horses using the method by Harewood (1). Saliva samples were took by one person, in 34.4% the horse had to be fixed by the owner. From the decrease in behavioural point score on the second day of testing by 8.4% we assumed that taking of saliva samples was not stressful to healthy and mentally balanced horses, and did not induce horse's sensitisation as often seen during blood sampling. Therefore it is possible to practise repeated, long-time monitoring of an individual under test without affecting or distorting the evaluated parameters.**

**Key words: blood; horse; non-invasive taking of samples; saliva; welfare**

## INTRODUCTION

With few exceptions, the act of studying an animal affects what is being studied and can thereby confound interpretation of the results. There is need to minimize study-induced disturbances. This can be achieved by both reducing the extent of contact and the invasiveness of hands-on studies and, where practicable, engaging in hands-off studies. Hands-on studies may be favoured in situations where no hands-off study methods are available and are a trade-off between getting no data at all or acquiring data exhibiting some variance. The non-invasive contact activities belong: yarding, shearing, dipping, and the invasive: branding, castration, dehorning, tail docking, clinical and experimental surgery, blood sampling, *etc.* Familiarizing animals with hands-on procedures before the study may be helpful, however, such training is problematic as it may alter the animal responses during the study (3). One of the potential welfare indicator is presence or absence of stress. Changes in the activity of the hypothalamic-pituitary-adrenal axis (HPA) are routinely used to quantify response to stress. The stress associated with handling and restraint of animals with traditional blood sampling techniques can in itself cause activation of the HPA axis, thereby confounding such measurements (2). This effect is further exacerbated in wild and semi-domesticated animals.

The aim of this study was to evaluate horses' welfare through behavioural assessment during the non-invasive way of the collection of samples for cortisol determination in saliva.

## MATERIAL AND METHODS

The present study focused on assessment of hormonal and behavioural indicators of welfare in horses involved in hippotherapy in Slovakia. Because of owner's unwillingness and variance of results associated with blood sampling, we used saliva sampling for determination of cortisol. Tests were carried out on 32 horses (16 involved

in hippotherapy and 16 control horses, used mostly as recreational riding horses). Saliva samples were taken with Salivette (Cortisol – Salivette, SARSTEDT). Sterile swabs were placed in horses's muzzle for maximum 3 min. During the sampling, we recorded horses' behaviour and their reactions by the method of Harewood (1). During the first testing day the horses were not involved in hippotherapy or in other work, they were on pasture or in a stable and relaxed. Every horse was tested for a period of 3 hours and we took 4–6 saliva samples for determination of basal levels of salivary cortisol. During both testing days, we took samples at the same time to avoid influence of circadian rhythm on cortisol levels. During hippotherapy and riding (2nd testing day), saliva samples were taken in 30 min intervals. During this day we took 3–10 saliva samples from every horse. All swabs were stored at –20 °C (to prevent mould formation) till salivary cortisol determination.

## RESULTS AND DISCUSSION

Saliva samples were taken from 65.6 % horses by one person, 34.4 % of horses had to be fixed by the owner. In 5.73 % cases it was impossible to touch the horse muzzle, so sterile swab was attached on snaffle and located through the use of bridle in horse muzzle for 3 min. Reasons for excessive fixation were: 1) young animals were not habituated to snaffle and manipulation around their head (5.73 %); 2) sexual (stallions) and mental immaturity (2.87 %); 3) Sensitivity in head and muzzle location (11.47 % for medical reasons, e. g. otitic scabies; 2.87 % for mental reasons, such as brutal deworming; 8.6 % because of previous negative experiences); 4) distrust of humans (11.47 %, of that 5.73 % abuse in the past, 2.87 % solitary horse and 2.87 % for unknown reasons); 5) new environment for the horse (2.87 %). No other forms of fixation (twitch, untying, limbs lifting, etc.) were necessary. On the first testing day, the mean behavioural score during saliva sampling was 2.842, on the second 2.604 and the total score was 2.733. 34.4 % of horses had score over 3 (mostly horses that needed excessive fixation by an owner). The decrease in score on the second day of testing by 8.4 % indicated that saliva sampling is not stressful to healthy and mentally balanced horse, does not induce horse sensitisation and is therefore an applicable method for frequent, even long-time monitoring of animals without affecting and distorting the evaluated parameters.

Glucocorticoid are secreted in pulsatile waves, therefore their concentration can differ within few minutes. Many other physiological parameters show daily, seasonal rhytmicity or other periodicity, reflected in concentration of hormones (4). Interpretation of results based only on hormonal parameters from blood or on small amount of sample can be misleading. Stress caused by blood sampling and limited quantity of samples taken from small species (birds, rodents) can also affect the results. Blood withdrawal from some ZOO animals is dangerous and sometimes impossible. Cortisol concentration in saliva reflects amount of free cortisol in plasma which is the only biologically active portion of the hormone thanks to its ability to bind to cell receptors. Saliva sampling presents potential alternative for evaluation of stress responses in animals and is a non-invasive, rapid method of sampling that is repeatable and therefore suitable for evaluation of dynamic levels of the tested parameters (5).

Human-animal interactions during sampling can be stressful to tested animals. Therefore, during evaluating of HPA axis, less invasive and hands-off techniques are instrumental in monitoring of animals during longer periods in their natural environment, providing less distorted results. Blood sampling associated with excessive fixation and consequential evocation either of pain or fear can conduce to horse sensitisation. This manifests mostly as a panic fear of a veterinarian who took the sample, eventually as aggressiveness towards him, which markedly complicates any manipulation and work with such animal in the future. Saliva sampling in horses is tolerated better by the owner and the animal than blood sampling, does not require accessory fixation of the animal by another person and allows repeated taking of samples in relatively short periods without influence on evaluated parameters through human-animal invasive intervention.

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# AIRBORNE MICROORGANISMS IN ANIMAL HOUSING FACILITIES

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## ABSTRACT

**Aerial micro-organisms were sampled by means of a MAS-100 Eco sampler from different houses on cattle and pig farm on respective nutrient media (Meat-peptone agar, Endo agar, Sabouraud agar, MacConkey agar), the plates were incubated at appropriate temperatures for required time and plate counts were recalculated per 1 m$^3$ of air. Antimicrobial susceptibility of the obtained bacterial colonies (minimum inhibitory concentration MIC) was determined by colorimetric broth micro-dilution method according to CLSI guidelines using ampicillin, ampicillin and sulbactam, ceftiofur, ceftriaxon, ceftazidime, ceftazidime and clavulanic acid, gentamicin, streptomycin, neomycin, spectinomycin, nalidixic acid, enrofloxacin, ciprofloxacin, chloramphenicol, florfenicol, tetracycline and cotrimoxazol. On the pig farm we detected extended-spectrum beta-lactamase (ESBL) *E. coli* isolates and high level of chinolone resistant *E. coli*. No multiresistent *E. coli* were detected on cattle farm.**

**Key words: antibiotic resistance; bioaerosol; cattle; pigs**

## INTRODUCTION

Microclimate in animal housings is one of important factors affecting productivity and health of animals. Adverse microclimate supports the so-called stable fatigue and development of multifactorial diseases and may result in circulation of avirulent but also virulent micro-organisms in the environment.

A bioaerosol is a suspension of airborne particles containing living organisms. Bioaerosols in farm animal housings contain mostly saprophytic micro-organisms but the presence of pathogens is not excluded, originating from sick animals, contaminated feed, bedding and excrements (4). Microbial contamination of the air is directly related to air dustiness. Organic particles originate from animals, feed, bedding and dried excrements and carry on their surface also molecules of gases and micro-organisms. In addition to that they cause irritation of skin, eyes and respiratory tract with all undesirable consequences (5).

Repeated exposure of bacteria to antimicrobial agents and access of bacteria to increasingly large pools of antimicrobial resistance genes in mixed bacterial populations are the primary driving forces for emerging antimicrobial resistance (7). Resistant airborne micro-organisms are dangerous to both animals and the stockmen due to risk of development of serious, long-lasting diseases and potential failure of conventional treatment (6).

The aim of our study was to investigate the level of airborne micro-organisms on cattle and pig farm and their potential antimicrobial resistance.

## MATERIAL AND METHODS

In the experiment, we sampled air from different sites on pig and cattle farm. The samples were collected by a MAS-100 Eco sampler onto Petri dishes with respective nutrient media and incubated at appropriate temperatures for prescribed time (Meat-peptone agar and Endo agar at 37 °C for 1 day, Sabouraud agar at 20 °C for 5 days). The plate counts (colony forming units, CFU) were recalculated per 1 m$^3$ of air.

Antimicrobial susceptibility of obtained bacterial colonies (minimum inhibitory concentration – MIC) was determined by colorimetric broth micro-dilution method according to CLSI guidelines

using ampicillin, ampicillin and sulbactam, ceftiofur, ceftriaxon, ceftazidime, ceftazidime and clavulanic acid, gentamicin, streptomycin, neomycin, spectinomycin, nalidixic acid, enrofloxacin, ciprofloxacin, chloramphenicol, florfenicol, tetracycline and cotrimoxazol.

## RESULTS AND DISCUSSION

Intensive animal production has its positives and negatives. High stocking density is related to increased risk of diseases, production and accumulation of wastes that may affect negatively the areas proximal to large scale farms (1). Also stockmen and other farm personnel are exposed to a wide range of harmful substances (organic and inorganic dust, bacteria, endotoxins, moulds, *etc.*) contributing to diseases, particularly those of the respiratory tract (infections, allergies). Massive multiplication of aerial micro-organisms in animal houses affects negatively the animals, depletes oxygen, causes infection and slow healing of wounds and affects quality of milk (5).

Our results of air sampling on cattle and pig farm, together with relevant microclimate parameters, are presented in Tables 1 and 2.

On pig farm, the highest levels of total counts of bacteria (TCB), coliform bacteria (CB) and moulds were detected in the farrowing house, in weaned piglets and in fattening. This was related to higher temperature, humidity and inadequate ventilation. In houses for pregnant sows 20-fold lower level of aerial micro-organisms was found after replacement of bedding compared to situation before removal. Conditions in the house for pregnant sows were evaluated as good with regard to both microclimate and animal welfare. The maximum acceptable level of aerial micro-organisms in farm animal housings is 250 000 CFU.m$^{-3}$ (5).

On cattle farm, the aerial micro-organisms ranged from $1.02 \times 10^3$ CFU.m$^{-3}$ (in calves housed in hutches) up to $> 10^6$ CFU.m$^{-3}$ (in heifers). Ventilation in the houses with higher level of bacteria and moulds in the air was inadequate, which was indicated by high relative humidity (> 85 %). The level of coliforms was low on both farms. Effective ventila-

**Table 1. Microclimate parameters and mean levels (CFU) | of aerial micro-organisms in houses on cattle farm**

| Place of sampling | T | RH % | TCB | CB | Moulds |
|---|---|---|---|---|---|
| **Calving, section 1** | 10 °C | 74.3 % | $5.2 \times 10^5$ | $2.2 \times 10^3$ | $21.5 \times 10^3$ |
| **Calving, section 2** | 8.6 °C | 75.3 % | $13.4 \times 10^3$ | $0.6 \times 10^3$ | $14.6 \times 10^3$ |
| **Dairy cows** | 7.4 °C | 87.3 % | $1.02 \times 10^3$ | $0.15 \times 10^3$ | $1 \times 10^3$ |
| **Calves 2–6 months old** | 9.7 °C | 68.9 % | $16.4 \times 10^3$ | $0.15 \times 10^3$ | $10.9 \times 10^3$ |
| **Calves in milk nutrition** | 6.3 °C | 89.5 % | $80.5 \times 10^3$ | $1.4 \times 10^3$ | $11.3 \times 10^3$ |
| **Heifers** | 7.6 °C | 86.7 % | $>10^6$ | $1.2 \times 10^3$ | $37.8 \times 10^3$ |

T – temperature; RH – relative humidity; TCB – total count of bacteria; CB – coliform bacteria

**Table 2. Microclimate parameters and mean levels (CFU) of aerial micro-organisms in houses on pig farm**

| Place of sampling | T | RH % | TCB | CB | Moulds |
|---|---|---|---|---|---|
| **Pregnant sows** | 18.3 | 75 | $9.3 \times 10^5$ | $9 \times 10^2$ | $36 \times 10^3$ |
| **Weaned piglets** | 17.1 | 71.1 | $1.5 \times 10^5$ | $0.45 \times 10^2$ | $23 \times 10^3$ |
| **Farrowing house** | 16.4 | 67.8 | $8 \times 10^5$ | $5.6 \times 10^3$ | $68 \times 10^3$ |
| **Pre-fattening** | 17.6 | 73 | $9.6 \times 10^4$ | $5.6 \times 10^3$ | $49 \times 10^3$ |
| **Fattening** | 17.5 | 71.5 | $9.4 \times 10^4$ | $2.3 \times 10^3$ | $14 \times 10^3$ |
| **Vicinity of the farm** | 20.1 | 65.3 | $5.6x10^3$ | $0.06x10^3$ | $0.72x10^2$ |

T – temperature; RH – relative humidity; TCB – total count of bacteria; CB – coliform bacteria

tion is essential for good microclimate in animal housings. It decreases the risk of diseases and prevents corrosion and physical damage to buildings and equipment (8).

The most frequently used antibiotic growth regulator included macrolides (tylosin and spiramycin), polypeptides (bacitracin), glycolipids (bambermycin), streptogramins (virginiamycin), glycopeptides (avoparcin), quioxalins (carbadox and olaquidox|, everninomycins (avilamycin) and ionophores (monensin and salinomycin) (2).

Although the use of antibiotics for promotion of growth is prohibited in EU, they are still used for prophylactic and therapeutic purposes no longer used Aj keď sa už antibiotiká na podporu rastu v EU nepoužívajú sú v chove zvierat stále vo veľkej miere používané na profylaktické a terapeutické účely. Kmeť and Kmeťová (3) observed high levels of multiresistant *E. coli* isolates in calves and poultry.

Antimicrobial susceptibility of the obtained bacterial colonies (minimum inhibitory concentration – MIC), determined by colorimetric broth micro-dilution method according to CLSI guidelines, allowed us to detect extended-spectrum beta-lactamase (ESBL) *E. coli* isolates and high level of chinolone resistant *E. coli* on pig farm. No multiresistant *E. coli* were detected on cattle farm.

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# STRESS AS AN INDICATOR OF WELFARE AND ITS POSSIBLE ASSESSMENT

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## ABSTRACT

**Animal welfare is of increasing importance and absence of chronic stress is one of its prerequisites. The front-line hormones to overcome stressful situations are the glucocorticoids and catecholamines. The concentration of glucocorticoids can be measured in various body fluids or excreta. Stress assessment measurements need to be non-invasive and sufficiently benign so as not to confound the assessment of stress levels. Recently, enzyme immunoassays (EIA) have been developed and successfully tested, to enable the measurement of groups of cortisol metabolites in animal faeces. The determination of these metabolites in faecal samples is a practical method to monitor glucocorticoid production.**

**Key words: animal; stress measurement; welfare, assessment**

## INTRODUCTION

Animal welfare is the combination of subjective and objective (qualitative and quantitative) aspects of the conditions of life for animals, including health and disease, behaviour, husbandry and management and is thus, a complex and abstract construct (4,15).

## ANIMAL WELFARE AND STRESS

The questions how to define animal welfare and how to measure it are still under debate. A potential indicator of animal welfare is the absence of stress, but there is no standard definition of stress and no single biochemical assay system to measure stress (7). Thus, there is a need for additional biochemical or endocrine parameters for detection of disturbances.

### Sampling of materials for determining glucocorticoids (or their metabolites)

Some researchers have made use of special remote blood sampling devices (1). Alternatively, several authors have investigated non-invasive sampling procedures such as a corticoid (metabolite) determination in milk (16). Above all, faecal samples offer the advantage that they can be easily collected without stressing the animals.

### Metabolism and excretion of glucocorticoids

The metabolism of cortisol in the various species and the further conversions of these substances have not been fully investigated. Using HPLC and enzyme immunoassays (EIA), Palme and Möstl (12) showed that almost no authentic cortisol was excreted in sheep, even after intravenous infusion of 1 g of cortisol. A similar situation was described in ponies and pigs (10).

### Immunoassays

For measurement of faecal cortisol metabolites, Wasser *et al.* (17) investigated three different commercially available radioimmunoassays for cortisol and one assay for corticosterone in a variety of wildlife mammals. A corticosterone antibody (ICN Biomedicals, Costa Mesa, CA) gave best results. The assay system described by Palme and Möstl (9) used an antibody against 11-oxoetiocholanolone coupled at position C-3.

**Faecal sampling regimen**

It must be remembered that the concentration of cortisol metabolites in a faecal sample reflects the cortisol production after a species specific time period (e.g., ruminants: 10–12 h). Palme *et al.* (13) collected samples at every spontaneous defecation after a short term stress (transportation for 2 h) and showed that elevated levels of cortisol metabolites were present only in less than three to four consecutive faecal samples. Therefore, with a less strict sampling regime, peaks of cortisol metabolites might not be detected in faeces.

**Measurement of faecal cortisol metabolites in various farm animals**

Palme *et al.* (14) injected ACTH and dexamethasone into sheep and cattle. In cattle, a good correlation ($r = 0.77$) was found between the injected dose of ACTH and the increase of faecal cortisol metabolites, but no correlation was seen between dose and the increase of plasma cortisol concentrations. Therefore, faecal concentrations of cortisol metabolites reflect the total amount of excreted and, therefore, produce better cortisol estimates than a single blood concentration, which changes quickly. High inter-animal variations, occur for both plasma cortisol and faecal cortisol metabolites' concentrations.

## CONCLUSIONS

Measuring faecal cortisol metabolites as an indicator of adrenocortical activity in animals offers the advantage of a simple sampling technique that will not interfere with the results of the study and enables even long term, longitudinal studies. Thus, such methods will be a valuable tool in a variety of research fields such as animal welfare (handling, housing and transportation) but also in ethological and environmental studies (11). In addition, animal health needs also to be taken on board as the fulfilment of behavioural needs can impact on health (8) and pathologies can lead to modifications of behaviour (3). The use of less invasive stress measurements (urinary and faecal steroid monitoring) in combination with other measures (blood metabolites, behaviour, immune system function, pathology) has potential for monitoring stress of dairy cows on farm (9).

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# EVALUATION OF WATER QUALITY AND DRINKERS CLEANNESS ON ORGANIC FARMSING

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## ABSTRACT

**The aim of our study was to evaluate the quality of water supplied to animals on organic farms. Ten samples were collected from three farms. We focused on indicators of faecal contamination which may be related to serious health problems. Microbiological and chemical analysis showed that all samples exceeded the relevant limits at least in one parameter (particularly total coliform level). This indicates the need for increasing the level of hygiene (cleanness of drinkers) on investigated farms.**

**Key words: cattle; drinking water; faecal contamination, water quality**

## INTRODUCTION

Water plays an important role in animal nutrition. Its absence reduces the yield and degrades the animal health (1). Therefore it is important to monitor its quality at regular intervals. Neglecting this obligation may have negative impact on health because water can be a source of various pathogens, including bacteria, viruses and parasites. This obligation is results from current legislation. The Act No. 322/2003 about protection of animals kept for farming purposes, Annex 2, paragraph 16 (6), states that all animals must have access to a suitable water source or their demands on water should be satisfied in other adequate way. In 2007, the Government Regulation No. 368/2007 (5) came into force, amending the Government Regulation of the Slovak Republic No. 322/2003 about protection of animals kept for farming purposes (8). The aforementioned paragraph 16 inserts the phrase “drinking water” which means more stringent water quality requirements for all animals kept for farming purposes. Every source used for watering of animals must satisfy the strict requirements on quality of drinking water.

Therefore the aim of our study was to evaluate the water quality in drinkers. From the parameters used for minimum analysis of drinking water we selected those which indicate faecal contamination and thus also the highest risk to health.

## MATERIALS AND METHODS

In May and June 2011 we collected 10 samples of drinking water on three organic farms, some of them directly from drinkers. On Farm 1, which housed dairy cows, we collected 4 samples: 1 from the calving section, 1 from section for bull fattening, 1 from stable housing cows without market output of milk (CWMOM) and 1 from the water source (brook) supplying water to grazing animals.

Farm 2 was a dairy sheep farm which processed sheep milk into lump sheep cheese and aged sheep cheese “bryndza”. The farm also kept a small herd of CWMOM. On this farm we also collected 4 samples: from the drinker supplying water to CWMOM, from the drinker used by a breeding bull, from drinker for sheep and from a natural source which was used by animals during grazing (spring).

Two samples were collected from Farm 3, which kept CWMOM and sheep, namely stables used by CWMOM and from a natural source used by grazing animals (spring).

We carried out qualitative chemical examination to detect presence of ammonium ions ($NH_4^+$), nitrites ($NO_2$) and nitrates ($NO_3$) and positive samples were examined quantitatively by an Ino Lab pH/ION (WTW, GmbH, Germany) for ammonium ions and by Spectrophotometer DR 2800 (HACH LANGE) for nitrites and nitrates.

Microbiological examination included determination of total coliforms (TC) by filtration of 100 ml of sample through a prescribed sterile filter and cultivation on Endo agar at 37 ° C for 24–48 h and bacteria cultivated (BC) at 22 °C and 37 °C by pour-plate method with following cultivation on meat peptone agar at respective temperatures for 5 days and 24 hours, resp.

## RESULTS AND DISCUSSION

Results of chemical and microbiological examination are presented in Tables 1 and 2. They were compared with the Government Regulation No. 354/2006 (6), establishing requirements on water intended for human consumption and control of water quality intended for human consumption. The water supplied to the animals should correspond to these requirements. The above regulation states that if any limit value is exceeded, the drinking water loses suitable quality.

The above mentioned regulation sets the limit for ammonium ions to 0.5 $mg.l^{-1}$, for nitrites to 0.1 $mg.l^{-1}$ and for nitrates to 50 $mg.l^{-1}$. The results obtained showed that the limit for nitrates was not exceeded. The limit for nitrites, which indicate fresh faecal pollution, was slightly exceeded in samples 3, 4, 5 and 7 and ammonium ions were elevated considerably in sample 7. However, when considering the bacteriological examination we should state that all the samples exceeded the limit for total coliforms (0 CFU.100 $ml^{-1}$), the indicator of potential faecal pollution, even the sample taken from water springs. With regard to BC at 22 °C and 37 °C only samples 8 and 9 were acceptable.

*E. coli* infections in calves can be manifested as diarrhoea or septicaemia that can lead, subsequently, to arthritis, meningitis and pneumonia (4). Adult animals may not show clinical symptoms but can become the source of infection for other animals. Therefore cleanness and disinfection, particularly at calving, is important for prevention of infections (2). With regard to farm animals the owner is directly responsible for this on the basis of the Act No. 39/2009 on veterinary care (7). The farmers should remember that prevention of diseases is less expensive than the subsequent treatment (3). Such prevention is achieved by regular cleaning and disinfecting of drinkers and adequate hygiene of houses.

All examined samples exceeded the limit at least in one indicator which points to the lack of care on drinkers and shortcomings in the overall housing hygiene.

**Table1. Results of chemical examination of water samples**

| Place of sampling | | Sample No. | $NH_4^+$ $mg.l^{-1}$ | $NO_2^-$ $mg.l^{-1}$ | $NO_3^-$ $mg.l^{-1}$ |
|---|---|---|---|---|---|
| Farm No.1 | Calving | 1 | – | traces | 9.79 |
| | Fattening bulls | 2 | – | 0.07 | Traces |
| | CWMOM | 3 | – | 0.11 | Traces |
| | Brook | 4 | | 0.12 | 8.56 |
| Farm No. 2 | CWMOM | 5 | – | 0.13 | 3.86 |
| | Breeding bull | 6 | – | – | 9.53 |
| | Sheep | 7 | 1.84 | 0.13 | Traces |
| | Spring | 8 | – | – | – |
| Farm No. 3 | CWMOM | 9 | – | – | 1.97 |
| | Spring | 10 | – | traces | 1.11 |

**Table 2. Results of bacteriological examination of water**

| Place of sampling | | Sample No. | CB | BC 22 °C | BC 37 °C |
|---|---|---|---|---|---|
| | | | CFU.100 $ml^{-1}$ | CFU.$ml^{-1}$ | |
| Farm No.1 | Calving | 1 | >300 | >300 | >300 |
| | Fattening bulls | 2 | >300 | >300 | >300 |
| | CWMOM | 3 | >300 | >300 | >300 |
| | Brook | 4 | >300 | >300 | >300 |
| Farm No. 2 | CWMOM | 5 | >300 | >300 | >300 |
| | Breeding bull | 6 | 30 | 55 | >300 |
| | Sheep | 7 | >300 | >300 | >300 |
| | Spring | 8 | 61 | 0 | 0 |
| Farm No. 3 | CWMOM | 9 | >300 | 0 | 14 |
| | Spring | 10 | >300 | 0 | >300 |

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# THE ETHICAL ASPECTS OF THE HUMAN-ANIMAL INTERACTIONS RELATED TO ANIMAL WELFARE

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## ABSTRACT

**How people perceive and respond to animals varies greatly. The relationship that can exist between humans and animals can be strongly influenced by religious and cultural attitudes. In recent years a more positive approach has begun to emerge, with emphasis on the promotion of good welfare in the animal.**

**Key words: human-animal interactions; welfare**

## INTRODUCTION

The close relationship that can exist between humans and animals has long been recognised. Over the past 200 years, there has been a growth of interest in the welfare of animals. What is animal welfare is not easy to define and this presents impediments in legal and other hearings. One scientific explanation that is widely used is that “the welfare of an individual animal is its state as regards its attempt to cope including the functioning of body repair systems, immunological defences, the physiological stress response and a variety of behavioural responses” (1).

### State of The human – animal interaction CONCEPT

How people perceive and respond to animals varies greatly. Kellert (4) summarised as a spectrum of attitudes that people have to animals as follows: humanistic, naturalistic moralistic, ecologistic, aesthetic, scientific, utilitarian, dominonistic, negativistic and neutralistic. Although these terms appear self-explanatory, the distinction between them is often imprecise. Attitudes shown towards animals can be more simply divided into three main categories (3).

**Protectionist**

Animals are seen as fellow beings, often “companion”, worthy of full protection under the law. They may only be kept in captivity under very special circumstances and should only rarely be killed or exploited. Welfare is all-important.

**Stewardship**

Animals are seen as an integral part of biodiversity worthy of a degree of protection under the law. However, they may be killed, taken into captivity or used under controlled (humane) circumstances. Humans have a responsibility for them.

**Utilisation**

Animals are seen as just one component of the world, not warranting special protection. They are freely available to be used, taken into captivity or killed in the wild. Welfare is of little or no importance.

Awareness of these three broader categories can help those involved in a legal case to understand the different ways in which members of the public may perceive and treat animals. The distinction are not clear-cut. Within each of these three groups there is much variation and views may be influenced by peer pressure and by the media.

The relationship that can exist between humans and animals is not confined to wealthier countries and can be strongly influenced by religious and cultural attitudes. Animals have long played a part in religious ceremony and tradition (2).

There is much debate about the value of animals. The value of animals can be looked at in three ways (3):

1. Their worth in humans terms because of the pleasure or companionship that they provide, their emotional value and related psychological health benefits
2. Their compensatory value, if for instant, the animal is stolen or killed.
3. Their intrinsic worth, as living, sentient, creatures.

The welfare of animals can be an emotive subject and these likely to be involved in legal or other actions relating to animal welfare need to be aware of this.

Sentience is the key to current thinking. If an organism can be demonstrated to feel and be affected by adverse stimuli, then its welfare is important.

In recent years a more positive approach has begun to emerge, with emphasis on the promotion of good welfare in the animal. Often this has used as its framework concepts known as "The Five Freedoms".

**The Five Freedoms, and how they can best be ensured**

1. Freedom from hunger and thirst – by ready access to fresh water and a diet to maintain full health and vigour.
2. Freedom from discomfort – by providing an adequate environment including shelter and a comfortable resting area.
3. Freedom from pain, injury or disease – by prevention or rapid diagnosis and treatment.
4. Freedom to express normal behaviour – by providing sufficient space, proper facilities and, where appropriate, company of the animal's own kind
5. Freedom from fear and distress – by ensuring conditioning and treatment to avoid mental suffering.

## CONCLUSION

In practice, the Freedoms usually prove to be constructive and functional in terms of promoting better standards of husbandry but are often not easily applied to the judicial process.

The veterinary profession has traditionally played an important part in animal welfare and is still assumed by many to provide the best opinion on whether or not an animal suffers.

## ACKNOWLEDGEMENTS

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FOLIA VETERINARIA, 55, Supplementum I: 51–53, 2011

# TEMPERATURE AS AN IMPORTANT FACTOR IN COMPOSTING OF POULTRY MANURE

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## ABSTRACT

**We investigated the influence of temperature on changes in plate counts of micro-organisms in the course of composting poultry litter mixed with straw (1 : 1.63 by volume) in relation to its potential contamination with zoonotic pathogens, such as *Salmonella* spp., *Escherichia coli* and others. The core temperature peaked (62 °C) on day 9 of composting and then persisted above 60 °C for the following 6 days. The inoculated *Salmonella senftenberg* was not recovered after day 114 of composting. By the end of the observation period (190 days) psychrophilic and mesophilic bacteria decreased by one order, coliforms and faecal coliforms by two orders and faecal streptococci by one order of magnitude.**

**Key words: bacteria; composting; poultry manure; *S. senftenberg*; temperature**

## INTRODUCTION

Poultry manure is a good source of organic substances (nitrogen, phosphorus) and trace elements suitable for manuring of agricultural soil that contains presently low proportion of organic matter and low level of nutrients. On the other hand, the nutrients in poultry manure present considerable risk to the environment, particularly due to ammonia emissions, leaching of soluble forms of nitrogen and phosphorus to surface and ground water which may contribute to eutrophication (2).

Pathogens originating from poultry flocks, many of zoonotic character such as *Salmonella* spp., *Eschericia coli*, *Campylobacter* spp., *Clostridium, Cryptosporidium, Giardia, etc*., may be spread through poorly treated poultry manure (8).

Composting or controlled aerobic biological decomposition is the principal method of stabilization of poultry manure prior to application to soil (4, 7). By observation of all conditions of composting, one can obtain a product safe from the microbiological and nutrient point of view (9). Mature compost is obtained only after passing the thermophilic phase, i. e. heating of the substrate to at least 50–65 °C (1). Maintaining temperatures higher than 55 °C for minimum of 21 days is inevitable if the presence of pathogens is suspected. However, too high temperatures may devitalize also beneficial microbes. According to Stentiford *et al.* (6) temperature in composted substrate determines the rates of biological processes. The temperatures above 55 °C support hygienization processes, temperatures between 45 and 55 °C affect favourably biodegradation rates and those ranging between 35 and 40 °C contribute to maximum microbial diversity of composting processes.

The aim of the study was to record core and surface temperatures in composted poultry manure in relation to survival of inoculated strain *Salmonella senftenberg* as well as of other groups of bacteria reflecting processes in composted manure and its safety.

## MATERIAL AND METHODS

Poutry droppings were mixed with straw in the ratio of 1 : 1.63 (by volume). The total initial volume of composted substrate (3 $m^3$) was placed to an open composting box protected by roof to exclude direct influence of winter weather. For 190 days we recorded temperature in the core of the substrate (T2) and 0.1 m below its surface

(T1). The substrate was turned three times, on days 9, 21 and 94. Samples were collected to determine psychrophilic and mesophilic bacterial counts (cultivation on meat peptone agar at 20 °C and 37 °C, resp.) and total and faecal coliforms (Endo agar, 37 °C and 43 °C, resp.) and survival of inoculated strain *Salmonella senftenberg* (SK 14/39, SZU, Prague, the Czech Republic). Chemical parameters were also determined and the results were published elsewhere.

## RESULTS AND DISCUSSION

Fig. 1 shows the course of temperatures in the core of the substrate (T2) and 0.1 m below its surface (T1). External temperature during the experiment (November) ranged between 1.4 °C and 5.2 °C. The initial core temperature after construction of the pile was T2 = 7.3 °C and T1 = 7.2 °C. Already on second day of composting T2 increased to 44.1 °C and T1 to 37.7 °C. By day 9 of composting, T2 peaked at 62 °C and remained above 60 °C for the following 6 days. This was most likely related to turning of the substrate on day 9 and increased access of oxygen which stimulated metabolism of micro-organisms responsible for degradation processes. T2 temperature persisted above 55 °C for 10 days, while increase in T1 was observed only for one day (day 9). The temperature sufficient for sanitation of composted substrate affected plate counts of all tested bacteria.

The temperature level at 0.1 m below substrate surface (T1) remained above 50 °C only for 4 days compared to 11 days in the core. The thorough turning on day 21 failed to affect markedly the temperatures in both sites as T1 was only 37 °C and T2 49.3 °C nor were they increased significantly after turning on day 94 of composting (T1 = 8.2 °C; T2 = 9.6 °C). Toward the end of the experiment, the substrate temperatures were affected by external temperature. Throughout the experiment the course of temperatures (T1 and T2) was in correlation and the difference between them was significant ($r = 0.9920$; $P < 0.0001$).

The inoculated *Salmonella senftenberg* strain was not recovered neither after 114 nor after 190 days of composting. By the end of the observation period (190 days) the counts of psychrophilic and mesophilic bacteria decreased by one order, those of coliforms and faecal coliforms by two orders and faecal streptococci decreased by one order of magnitude.

Besides other factors, microbial diversity of composted substrate plays an important role. Antagonism of high number of diverse microbes increases competition between substrate populations. Particularly fungi and actinomycetes produce substances that suppress other species of micro-organisms (1). Reduced water content in the composted substrate after the thermophilic phase or during maturation is unfavourable for re-population of the material with initial microbes. Because of that microbial diversity of the final product of composting is low and thus it may present a suitable environment for re-contamination with pathogens (5). Presence of *Salmonella* spp. is considered a problem related to hygiene quality of compost particularly due to the fact that these bacteria are almost ubiquitous and capable of rapid growth (3).

## CONCLUSION

Composting is a recommended way of treatment of animal excrement because it is advantageous from the environmental point of view as the thermophilic temperatures developing in composted substrates will inactivate potential pathogens and turn the substrate into material safe for application to agricultural soil. A problem may arise if not all pathogens are killed as some of them may survive in soil or on its surface for long time. Thorough and frequent turning of substrate, particularly in the initial period of composting, may decrease such risk.

## ACKNOWLEDGEMENT

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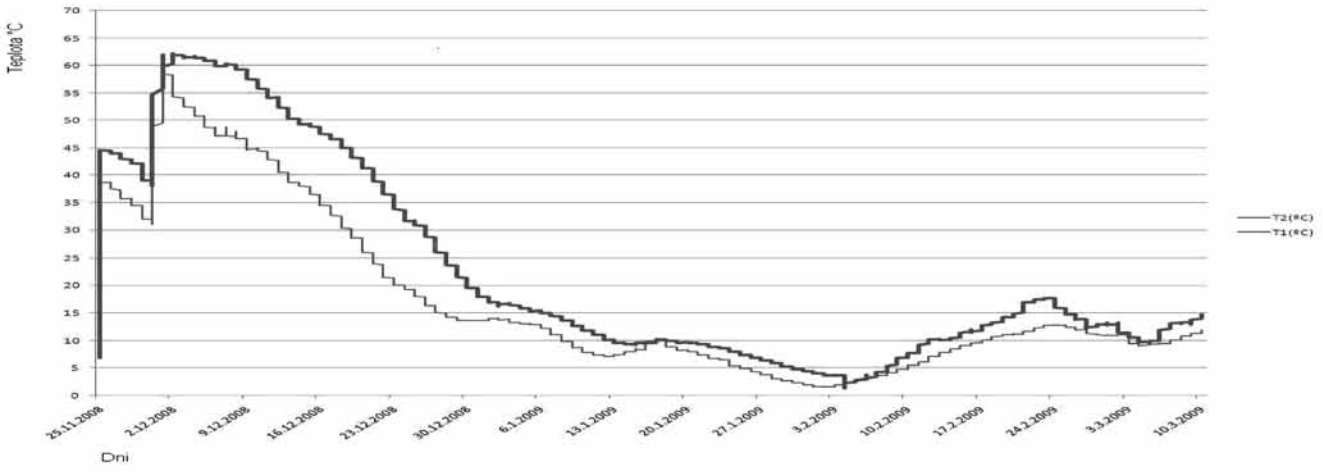


**Fig. 1. The course of core temperature (T1) and surface layer temperature (T2) during 190-day composting ($P < 0.0001$)**

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# ASSESSMENT OF DOG-HUMAN ATTACHMENT USING AINSWORTH'S STRANGE SITUATION TEST

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## ABSTRACT

**A human psychological test of Ainsworth's Strange Situation (ASS) was used to investigate the state of emotional attachment in dog-human relationship. Ten adult dog – owner pairs were observed in an unfamiliar test room, introduced to a human stranger and subjected to 8 short episodes. The dogs exhibited a range of attachment behaviours, i.e. search and proximity seeking of their owner, following, vocalizing. Finally, the dogs' behaviour in ASS has shown a wide range of types and degree of attachment and was very similar to that reported in chimpanzees and human infants. This fact confirmed a strong behavioural correlation between the canine group tested by our team and the children's group tested by Ainsworth *et al.***

**Key words: Ainsworth's strange situation; attachment; behaviour; dog**

## INTRODUCTION

The bond between dog and man is one of the primary reasons why people keep dogs as companion animals (4). Up to now, from the beginning of historical time, dog with man walked side by side. Their interspecies emotional bond has risen to a higher level of social attachment which is as strong as we can observe in the adult-child bond (2, 4). The infant-caretaker attachment relationship is evolutionary-important: with behaviours like proximity seeking, vocalization and following the infant ensures the presence of the caretaker, nursing mother or another individual (4). This relationship is also a necessary component for living in a social group and helping to keep group members together (7). A wide spectrum of social and socio-cultural environment in early ontogenesis opens a wider space for flexible choosing from this spectrum (6). Attachment is claimed to be a basic organizational factor for any species' social structure leading to group formation (2). If we can say in general that dependency of the attached individual manifests itself in behavioural preferences indicated by special behaviour patterns in a choice situation (2), than we can use it as an indicator which possibly shows us that the pattern for formation of the mechanism of the emotional bond is the same for all social individuals, and possibly others, as well. The influence of healthy social and natural environment with access to a higher information source which doses information hierarchically according to their understanding, needing and wanting by any individual from lower species, can catalyse the evolution of any species. The irrational aspect of psychoanalyticaI conception can be best explained with the presence of experimental evidence.

## MATERIALS AND METHODS

Ten dogs (1 to 10 years old, 5 non castrated and 2 castrated males, 3 sterilised females) – owner (7 men and 3 women, 21 to 37 years old) pairs were observed in an unfamiliar test room, introduced to a human stranger and subjected to 8 short 3 minutes periods. All the dogs were kept for companionship only. None of them had ever shown aggression towards a human and their vaccinations were valid. Owners of dogs were recruited from the clients of the University clinic. The dogs were tested in a standardised „strange" experimental room (Fig. 1; 4.30 m × 5.30 m) with a camera system connected to a monitor in an adjacent room so that each session could be independently observed. For recording we used an Unimo UDR 4004 system with 4 camera plugs. The video was analysed using Observer XT software (7th version).

Pre-experimental phase: The owner and the dog were escorted to a waiting room where the procedure was described but in order not to influence the owner's behaviour, they were told that the investigation focus on exploratory behaviour instead of the attachment.

Experimental phase: The episodes 1–7 were according to ASSP (1). We included an additional eighth episode to verify the assumption of stronger orientation on articles of clothing left by the owner on a chair comparing to the ones left by the stranger. The episodes: 1) Owner and Dog (3 min); 2) Owner, Dog and Stranger (3 min); 3) Stranger and Dog (3 min, 1st separation); 4) Owner and Dog (3 min, 1st reunion); 5) Dog alone (3 min, 2nd separation); 6) Stranger and Dog (3 min, 3rd separation). Toward the end of the episode, the stranger removed shoes and placed them near a chair and also left an article of clothing; 7) Owner and Dog (3 min, 2nd reunion); 8) Dog alone (3min, 4th separation and 3rd reunion).

After the observation in the ASS, the behavioural markers for attachment analysis were chosen. Decision was according the results from Ainsworth's test with 57 one-year old human infants (1). Each behaviour was marked by frequency on a 7-point scale. For reaching an understandable interpretation of the results, the points were recalculated on a percentage scale and are presented in Table 1. The column with children represents the average result of all infants from the study group.

## RESULTS AND DISCUSSION

The aim of our study was to compare the inter- and intra-species behaviour inside the emotional bond with focus on behaviour related to attachment. The external behavioural expressions were specifically individual and emotionally very significant.

In the observation room during the separation period only one dog have shown interest in owner's shoe. Attached individuals tend to maintain proximity and contact and become distressed when involuntary separation occurs (3). The results of dogs' ethogram during the ASS together with the comparison of children's behavioural reactions


## ACKNOWLEDGEMENTS

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**Table 1. Comparison of behavioural reactions in children and dogs during the ASSP**

| Dog number:/ Dog's behaviour | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Dog $\bar{x}$ | Child $\bar{x}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Exploration time** | ++ | +++ | +++ | ++ | +++ | + | +++ | ++ | ++ | +++ | ++ | +++ |
| **Proximity** | +++ | ++ | +++ | +++ | +++ | ++ | ++ | ++ | +++ | +++ | **+++** | **+++** |
| **Initiation of contact** | ++ | ++ | +++ | +++ | +++ | ++ | ++ | +++ | ++ | +++ | **+++** | **+++** |
| **Play** | ++ | ++ | + | ++ | +++ | ++ | ++ | ++ | ++ | +++ | **++** | **++** |
| **Vocalization in presence of owner** | + | + | + | + | ++ | + | + | ++ | + | ++ | **+** | **+** |
| **Vocalization in presence of stranger** | ++ | ++ | + | ++ | +++ | ++ | ++ | + | ++ | + | **++** | **++** |
| **Vocalization while is alone** | ++ | ++ | ++ | +++ | +++ | ++ | ++ | ++ | + | + | ++ | +++ |

The percentage evaluation is marked by a cross with value of one third part from 100 %

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# HUMIC ACIDS IN REDOX REGULATION

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## ABSTRACT

**Natural high-molecular substances, humic acids (HAs), have been used as plant growth stimulators, active substances in prophylaxis and as therapeutical drugs in veterinary practice in Europe. Isoflavones are structuraly related to widespread occurring plant flavonoids. Mostly they are responsible for estrogenic activity or even act as strong pro-oxidants. We investigated composite action of both substances on mitochondria activities in relation to energy and reactive oxygen species (ROS) production. We observed humic acid repealing effect on activities evoked by selected isoflavones.**

**Key words: antioxidants; humic acids; isoflavones; mitochondria**

## INTRODUCTION

Overall positive effects of humates on health and even productivity of animals are known from animal feeding trials (6). The mechanism of humic acids (HAs) action either in vivo or in vitro is not clear. Via oxidative generation and reduction in the activities of antioxidant enzymes in human erythrocytes, HAs induced echinocyte transformation and oxidative DNA damage in human peripheral blood lymphocytes (3). They were observed to stimulate rat liver mitochondria respiration (7). Higher plant polyphenolic secondary metabolites, such as flavonoids, were effective in the defence against free radicals in organism (4). Isoflavones, mostly known for their estrogenic activity, are structurally related to flavonoids and are produced from a branch of the general phenylpropanoid pathway.

We assessed the effect of 2'-, 3'- and 4'-methoxy substituted isoflavones on mitochondria respiration and antioxidant defenders (glutathione peroxidase and superoxide dismutase). Parallel measurements by addition of humic acids to the mixture were done.

## MATERIALS AND METHODS

2'-, 3'- and 4'-methoxy substituted 3-(X-benzylidene)-4-chromanones as A, B, C were synthesized and purified as described before (4). Male Wistar rats (Velaz, Prague, CR) weighing 200–250 g were used. Adhering to procedures approved by the University of Košice Animal Care and Use Committee, the animals were sacrificed by cervical displacement and decapitation. Liver mitochondria were isolated using the method by Johnson and Lardy (5). Mitochondrial protein was determined as described by Bradford (2). Polarographic measurement of oxygen consumption of isolated mitochondria was carried out with a Clark electrode (WTWoxi 325, Germany) at experimental temperature of 25 °C. Measurements were carried out in a respiratory medium supplemented with 70 nmol chalcone per mg mitochondrial protein, 50 mM sodium succinate, 0.5 mM ADP and 20 mg.ml$^{-1}$ mitochondrial protein. The test compounds were added in dimethyl sulfoxide (DMSO), a dose that did not affect control rates of respiration. The activities of glutathione peroxidase (GPx, E.C. 1.11.1.9) and superoxide dismutase (SOD, E.C. 1.15.1.1) were measured by means of GPx-Assay-Kit (Sigma-Aldrich, Germany) and SOD-Assay Kit-WST (Fluka, Japan) according to kit user manual. Statistical significance was determined by Student t-test with $P < 0.05$ level considered as significant.

## RESULTS AND DISCUSSION

Mitochondria, as the central site of oxygen consumption in the cell during the process of oxidative metabolism, are also a main source of reactive oxygen species (ROS) production. The dual nature of oxygen as a vital electron acceptor in oxidative phosphorylation and as a dangerously reactive molecule has created pressure for the cell to evolve powerful antioxidant defences that convert ROS into harmless products to maintain a predominantly reduced redox environment (1). All three compounds lowered coupling, however 2'-, 4'-methoxy isoflavones displayed clear respiration inhibition (Fig 1). Different degree of mitochondria respiration defects resulted in significantly increased superoxide dismutase activities (Fig. 2). Under 2'-, 4'-methoxy analogues treatment reduced glutathione depletion jointly a glutathione reductase significant increase (data not published) corresponding to mitochondria destruction were observed. Still, with nearly unchanged glutathione peroxidase activities (Fig. 2). Quantity of experimental work deals with the possibility of direct thiol oxidation by superoxide radicals giving the sulfinyl radicals. Come to a conclusion, two of selected compound with stated prooxidant effect evoked glutathione peroxidase inhibition in excess. Collated 3'-methoxy analogue seemed to have a beneficial effect.

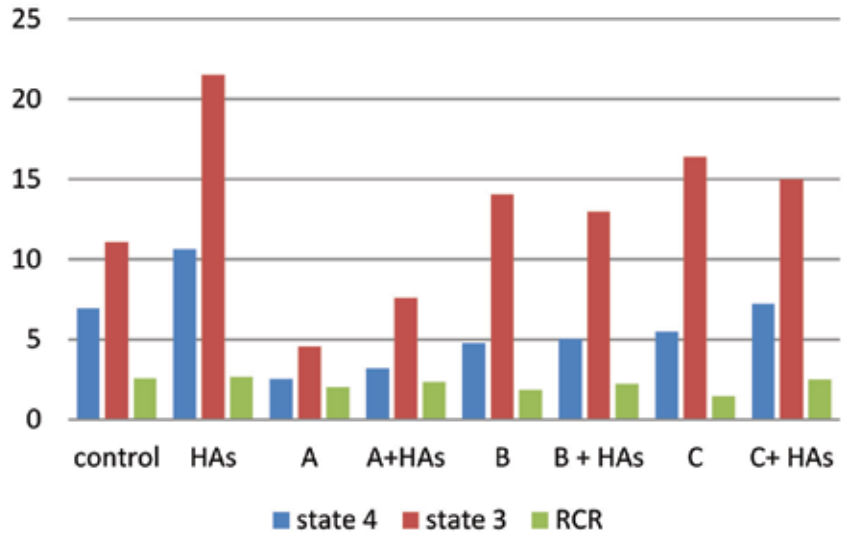


**Fig. 1. Mitochondria respiratory competence after treatment with HAs ($O_2$ consumption in nmol $O_2$ $min^{-1}$. $mg^{-1}_{prot}$)**

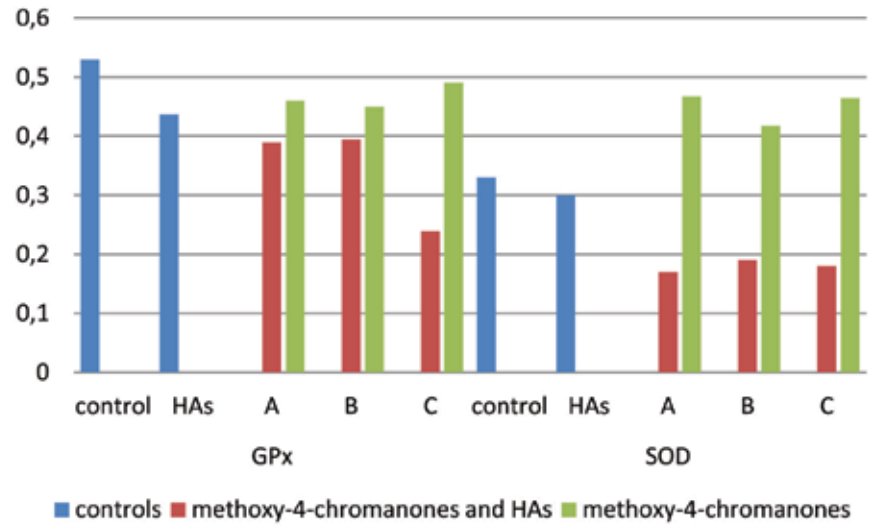


**Fig. 2. Glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities in mitochondria after treatment with HAs (U.$mg^{-1}$ prot)**

## CONCLUSIONS

Measurements of ADP-limited respiration showed tendency to elevate state 4, when humic acids are present. Estimation the state 3, reflecting the maximal activity of respiratory chain showed wholly identical tendency to increase. The observed increase in state 4 respiration may indicate loose coupling by influence the dissipation of the proton gradient through uncoupling proteins. This partial uncoupling effect alone could prevent increased phosphorylation that would inhibit respiration so that effected preventively to mitochondrial electron transport chain inhibition. Antioxidant enzymes activities were estimated to embody lowered level of oxidative stress conditions when HAs present. Despite relatively low solubility in aqueous solutions, humic acids participated in redox regulation.

## ACKNOWLEDGEMENT

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# THE IMPORTANCE OF SANITATION IN MEAT PROCESSING INDUSTRY

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## ABSTRACT

**Surface cleaning and disinfection of equipment in meat processing is essential for minimisation of microbial contamination of products and their high quality and safety. The study investigated hygiene and sanitation in meat processing industry focusing on disinfectant Topax 66. The disinfectant was tested under laboratory and practical conditions. Under laboratory conditions, devitalisation of all bacterial strains tested, exposed to Topax 66, occurred within 5 min. when using 0.5 % solution. Under practical conditions, *E. coli* was detected sporadically on the surface of weighing scales and cleavers used in the cutting section before further processing.**

**Key words: cutting section; disinfection; fish processing; hygiene; Topax 66**

## INTRODUCTION

According to the HACCP system, hygiene and thorough cleaning of premises and equipment is the principal precondition of high quality of final products (6). Cleaning procedures should remove effectively remains of food and other residues that may contain microorganisms or support microbial growth. Elimination of the majority of microbes is inevitable for good effectiveness of disinfection. Well selected disinfectant used at appropriate concentration and acting for sufficient time will support high quality of products and ensure their safety for consumers (2).

The aim of the study was to test the bactericidal action of Topax 66, intended for food industry, under laboratory conditions and in practice.

## MATERIAL AND METHODS

Topax 66 is a disinfectant based on active chlorine, intended for food industry in 2 % concentration and exposure time of 30 min. Laboratory testing was carried out with Standard Collection strains *Escherichia coli* (CCH 5172), *Staphylococcus aureus* (CCM 2012) and *Bacillus cereus cereus* (CCM 1999) using a suspension test (1). Under practical conditions, we tested microbiological swabs taken before and after disinfection from 10 $cm^2$ surface from the cutting section of fish processing for total bacterial counts (TC), *E. coli* and moulds. We determined TC, *E. coli* and moulds in the air in the cutting section using.

## RESULTS AND DISCUSSION

Sanitation in the fish processing facility was carried out with Topax 66 heated to 40 °C and allowed to act for 30 min. Testing under laboratory conditions using 0.5 % concentration of Topax 66 and exposure time 5 min showed good effectiveness of this disinfectant against all tested strains (Table 1).

Table 2 shows mean plate counts of bacteria and moulds on individual surfaces in the cutting section before and after disinfection. Before disinfection we obtained non-countable (NC) results and higher counts of *E. coli* on floor and table but after disinfection the results were negative or acceptable.

Table 3 shows unacceptable recovery of *E. coli* from scales and cleaver even after disinfection, which indicates that the disinfection was inadequate. This stresses the importance of the control of disinfection effectiveness which can

**Table 1. Batericidal effectiveness of Topax 66 against the tested bacterial strains (suspension test)**

| Concentration (%) | Exposure (min) | Tested strains | | |
|---|---|---|---|---|
| | | *E. coli* | *S. aureus* | *B. cereus* |
| **0.01** | 5 | + | + | + |
| | 20 | + | + | + |
| | 60 | + | + | + |
| **0.1** | 5 | + | + | + |
| | 20 | + | + | + |
| | 60 | + | + | + |
| **0.5** | 5 | – | – | |
| | 20 | – | – | – |
| | 60 | – | – | – |
| **1.0** | 5 | – | – | – |
| | 20 | – | – | – |
| | 60 | – | – | – |
| **2.0** | 5 | – | – | – |
| | 20 | – | – | – |
| | 60 | – | – | – |

reveal inadequacies in sanitation and enables their retroactive elimination (4).

Results of determination of airborne micro-organisms in the cutting section are presented in Table 4. Disinfection reduced plate counts of all observed groups.

Testing of disinfectants under laboratory and practical conditions is justified as confirmed by the results of suspension test. Topax 66 was effective in 0.5 % concentration at 5 min exposure on all tested bacterial strains (*E. coli*, *S. aureus* and *B. cereus*). This is important because the use of insufficient concentrations or too short exposure time may not result in desired devitalisation of pathogens or other harmful microbes.

Disinfection of premises and equipment in the cutting section of fish processing with Topax 66 was effective with the exception of weighing scales and the cleaver from the swabs of which we recovered *E. coli* (5 CFU.10 $cm^{-2}$). Other surfaces provided negative results.

The aim of disinfection is to decrease surface populations of viable cells after cleaning and to prevent growth of micro-organisms on surfaces before subsequent operation (5). In any operation it is advantageous to ensure permanent monitoring of hygiene level and obtaining results of laboratory examination so quickly that some corrective measures can be taken, if necessary, and potential contamination of produc-

**Table 2. Colony forming units (CFU) of bacteria and moulds on surfaces in the cutting section before disinfection and after application of Topax 66 (CFU.10 $cm^{-2}$)**

| Swabbed location | Number of swabs | Before disinfection | | | After disinfection | | |
|---|---|---|---|---|---|---|---|
| | | TC Ø | *E. coli* Ø | Moulds Ø | TC Ø | *E. coli* Ø | Moulds Ø |
| **Floor** | 10 | NC | 205 | 4 | 11 | 0 | 2 |
| **Wall** | 10 | 12 | 0 | 1 | 0 | 0 | 0 |
| **Table** | 10 | NC | 30 | 0 | 18 | 0 | 0 |
| **Shelf** | 10 | 22 | 120 | 3 | 0 | 0 | 0 |
| **Wash basin** | 10 | 32 | 10 | 5 | 1 | 0 | 0 |

NC – not countable

**Table 3. Plate counts (CFU) of bacteria and moulds on surfaces in the cutting section before disinfection and after application of Topax 66 (CFU.10 $cm^{-2}$)**

| Technological line | Number of swabs | Before disinfection | | | After disinfection | | |
|---|---|---|---|---|---|---|---|
| | | TC Ø | *E. coli* Ø | Moulds Ø | TC Ø | *E. coli* Ø | Moulds Ø |
| **Saw** | 10 | 0 | 2 | 0 | 0 | 0 | 0 |
| **Scales** | 10 | NC | 212 | 4 | 12 | 5 | 0 |
| **Cleaver** | 10 | 35 | 22 | 0 | 0 | 5 | 0 |
| **Steriliser** | 10 | 5 | NC | 15 | 1 | 0 | 0 |

NC – not countable

**Table 4. Mean plate counts of airborne microorganisms (CFU. m$^{-3}$) before disinfection and after application of Topax 66**

| Place of sampling (cutting section) | Number of samples | Before disinfection | | | After disinfection | | |
|---|---|---|---|---|---|---|---|
| | | TC Ø | *E. coli* Ø | Moulds Ø | TC Ø | *E. coli* Ø | Moulds Ø |
| **Entry** | 10 | 155 | 10 | 11 | 8 | 0 | 2 |
| **Central part** | 10 | 56 | 1 | 0 | 0 | 0 | 0 |
| **Rear part** | 10 | NC | 0 | 22 | 0 | 0 | 4 |

NC – not countable

tive premises and damage to the products are prevented. This prevents both more extensive economic losses and risk to consumer health or development of alimentary diseases (3).

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