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FOREWORD

In 2009, the University of Veterinary Medicine in Košice, the Slovak Republic, celebrated the 60th anniversary of its establishment. This was an excellent occasion to look back and think of past problems and achievements but also try to contemplate future challenges.

Among many various activities of the UVM in Košice an international conference was organized under the heading "School – Science – Practice". The heading itself should by no means be perceived as a succession of steps in one's professional life but rather as interconnected elements inevitable for successful career, professional development, public recognition and, particularly, for personal satisfaction.

Schools of varying level are the institutions that provide basic theoretical knowledge and some practical skills but it is the school of life, the practice, which is the most difficult to pass. In the present rapidly changing situation the challenges are tremendous and only well prepared individuals can stand their ground. For veterinary profession, continuous education is the only way how to keep up with the recent scientific progress, anticipate difficulties and work on solutions.

The role of veterinarians in care of animals, protection of their health and welfare, prevention of zoonotic diseases, monitoring and prevention of residues of xenobiotics in the human food chain and prevention of environmental pollution is essential and publicly recognised. These were also the main topics of the conference which, besides the plenary lectures dealing with general aspects of hygiene and welfare of animal rearing, food safety, animal health control, organisation of state veterinary service and legislative provisions in SR, comprised the following sections:

- *Hygiene and welfare of animal rearing, environmental protection and legislation*
- *Food hygiene*
- *Nutrition, dietetics, feedstuffs, feed-related aspects*
- *Epizootology and laboratory diagnostics.*

The selected papers published in this issue should provide an overview of the topics presented and discussed at the conference "School – Science – Practice I.".

Prof. Ing. Oľga Ondrašovičová, CSc.
Vice-rector of UVM and guarantor of the Conference

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HYGIENE OF ANIMAL REARING AND ITS IMPORTANCE IN PREVENTION OF DISEASES AND SATISFYING WELFARE REQUIREMENTS

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ABSTRACT

Hygiene of animal rearing plays an important role in complying with requirements for welfare and disease prevention. In the sixties of the past century the main stress was put on intensification of rearing and maximum profitability. At the presence, due to increasing public concern about animal and environmental protection, animal hygiene activities focus on optimisation of production processes with the aim to ensure animal health and welfare, keeping in mind effect of animal production on the environment.

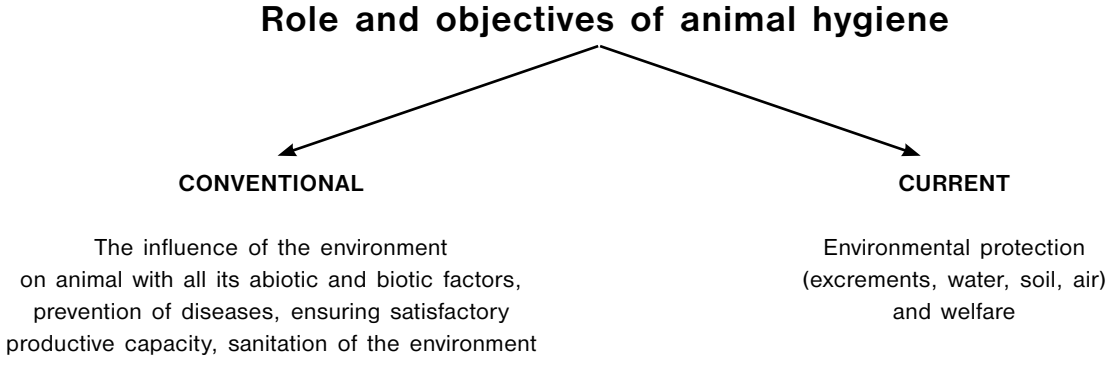
Key words: hygiene of animal rearing; intensive animal production; optimisation of production processes; welfare

IMPORTANCE OF ANIMAL HYGIENE

Hygiene of animal rearing and observation if its rules are the basic factors deciding about preservation of good health of animal herds and also public health through high quality and hygiene level of animal products. It goes without saying that hygiene is the basic precondition of prevention of human infections. However, this rule is not always so self-evident for many animal breeders. In this field we face the tasks that should by addressed to by scientific research and the practical implementation measures related to issues specific for certain periods. Importance of hygiene in animal rearing as a complex of measures was stressed in late sixties and early seventies of the past century in relation to intensification of animal production. Despite politically divided world of that time, on the initiative of leading scientists-teachers in the field of animal hygiene, a statutory meeting of the International Society for Animal Hygiene was held in 1973 in Budapest. One of the founding members of this society was Prof. MVDr. Ján Rosocha, CSc., doyen of animal hygiene in Slovakia, who contributed significantly to development of Society statute and regulation of its activities. The Society has developed progressively, continuing to promote animal health and welfare up to the present time. The principal aims of the Society have included international collaboration of animal hygienists, advertisement of new knowledge related to intensification of animal production in favour of economical production of food but not at the expense of animal health and welfare. Another important public health objective has been the improvement in quality and safety of food of animal origin. Presently the Society focuses also on environmental aspects as the intensive high-productivity systems are attractive for farmers but may have undesirable environmental consequences affecting entire populations. We are by no means contestants of intensification of animal production but, on the other hand, we cannot neglect the risks to animal and human health. Before putting any production unit into practice we must know answers to questions related to environmental protection. This has been the attitude of veterinarians from the very beginning of intensive animal rearing.

In the nineties of the past century, animal hygiene activities focused on the issues related to reconstruction of animal housings and negative effect of wastes from animal production on the environment, resulting from neglect of basic hygiene rules. We have to address these issues even today.

Compared to the original scope of animal hygiene the range of questions that need to be resolved is even wider. The



basic field of observation and evaluation of interactions of the environment with its abiotic and biotic factors and the animal, aimed at prevention of diseases and high productivity, has been extended by welfare issues, i.e. such conditions, technologies and procedures that ensure appropriate animal comfort. Moreover, we must consider also environmental protection and management. The following scheme illustrates the conventional and current understanding and the role of animal hygiene.

Legislative provisions and their importance

In relation to joining the European Union, Slovakia had to ensure approximation of domestic legislation with EU law also in the sector of hygiene of farm animal rearing. The most important legislative provisions regarding requirements on animal rearing include the following: Act No. 245/2003 Coll. on integrated pollution prevention and control, Slovak Republic government orders laying down minimum standards for the protection of calves, layers, pigs and animals kept for farming purposes, protection of animals used for experimental and other scientific purposes, protection of animals in time of slaughter or killing and the Government Order on terms and conditions for the provision of payments for the care of living conditions of animals. Animal welfare issues, embodied in many legislative provisions concerning farm and other animals, are the subject of concern not only of farmers and veterinary administration personnel but also of wide public. Welfare standards for individual species and categories of animals are based on their physiology and ethology and include quality of feeding from the quantitative and qualitative point of view, suitable technology of rearing, level of care, and similar. However, the evaluation of public is sometimes based on a layman's view and suitable conditions are referred to sometimes as cruelty. These issues are addressed to within the pre-graduate and post-gradual studies at the UVM but also within collaboration with professionals abroad through membership in scientific and professional commissions and work on international projects. Experience obtained in this way is very valuable for development of new legislative provisions, pre-graduate and pos-graduate education, obtaining required accreditation in the field of welfare and consultations related to diploma thesis, attestations and PhD. thesis. Observation of welfare aspects is supported considerably by the Slovak Republic Government Order No. 155/2008 Coll. on terms and conditions for the provision of payments for the care of living conditions of animals, based on EU legislation and requiring good agricultural and environmental conditions and good basic management. The mentioned conditions also deal with education of personnel in terms of attendance of accredited courses on living conditions of animals and special care of animals. This Order defines conditions for improvement of life of poultry, cattle, pigs and sheep.

Despite the fact that chemical disinfectants put some load on the environment, they are inevitable preventive and repressive tools in animal production. Minimisation of their influence is possible through their purposeful use. Environmental load is increased by both the use of too low concentrations of disinfectants, which are ineffective against the target micro-organisms, and the use of unnecessarily high concentrations. In addition to appropriate concentration of the active ingredient the volume of the diluted solution applied per unit of surface or volume is a very important factor. Frequently, already when entering the premises to be disinfected, one can tell that the application technique used cannot ensure good performance and effective disinfection. Intensification of animal production and introduction of new biotechnologies is the theme of the present period and failure to observe the rules of correct production practice is reflected in negative influence on the environment and production of safe feed and food which logically raises mistrust of the public. Some infectious diseases of the recent period, such as BSE, avian and novel flu but also FMD and classical swine fever only support this mistrust. Maybe the experience with avian flu in flocks and listeriosis in food production will motivate the farmers and processing facilities to more strict observation of hygiene rules including the black-and-white system and control of everything the animals come into contact with. Our personal experience is that the listeriosis issue resulted in more strict observation of hygienic rules, increased interest in consultations, implementation of hygiene loops, sanitation and current microbiological regimens.

Economic trends in animal production force the producers to become more effective. Productivity of farm animals is increased through breeding but full utilisation of the genetic potential also increases demands on hygiene level of animal rearing involving houses themselves, tending to animals, technologies of feeding, ventilation, removal of manure but also veterinary prevention of metabolic and infectious diseases. Suc-

cessfulness of this process requires application of new scientific knowledge in the field of nutrition, technologies, animal hygiene and welfare and general and special preventive measures. One of the conventional general preventive measures is protection of herd as a production unit against introduction of infectious diseases. This involves observation of "veterinary protection zones" already in the stage of site selection and designing of farms. Our current legislation fails to provide concrete rules and definitions in this area. Requirements well known in the previous period, concerning distances from potential sources of infection or sources of chemical load from animal houses or grazing areas and similar, belong among basic general preventive measures.

Prevention and control of pollution

An important issue of correct agricultural practice is manipulation with excrements which are included among wastes from animal production but, depending on manipulation with these materials, may become valuable organic manure. Their effective handling, storage and processing is important not only from economical but particularly from ecological point of view as they may pollute all components of the environment, i. e. water, soil and air.

Act No. 245/2003 Coll. on integrated pollution prevention and control as amended by Act No. 532/2005 Coll. is an important tool focusing on integrated approach to prevention of emissions into air, water and soil, reduction of production of waste, its reuse and safe disposal with the aim to achieve high protection of the environment. The objective of this Act is to establish a system of granting integrated permits to all intensive operations in order to exclude or limit environmental pollution, i.e. emissions to air, soil and water and pollution arising from wastes, resulting from such operations. From the veterinary and animal production point of view, the Act applies to the following activities: abattoirs with slaughter capacity bigger than 50 t per day, operations processing animal products (with the exception of milk) to produce food or feed with capacity bigger than 75 t of finished products per day, operations processing plant products with production capacity bigger than 300 t of finished products per day, milk processing operations processing more than 200 t milk per day, operations for safe disposal or reuse of animal carcasses and animal wastes with processing capacity exceeding 10 t per day, intensive poultry farms keeping more than 40 000 poultry, pig farms with capacity bigger than 2 000 pigs weighing more than 30 kg or 750 sows. Implementation of this act requires preparation of professionals from among veterinarians able to deal with these issues and authorised to provide professional consultations. This may be an important contribution to coping with the topical problems of environmental protection.

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REQUIREMENTS ON VETERINARY EDUCATION RELATED TO INTEGRATION OF ANIMAL AND PUBLIC HEALTH

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ABSTRACT

Relationship between health problems of animals and humans has been recognised since early human history. Veterinarians protect the public against animal-related hazards and watch over food safety. To maintain good health of world population of animals, including wild-living ones, and to prevent transmission of zoonoses from animals to humans has been the key responsibility of veterinarians. Veterinary education provides students the necessary knowledge to deal with these issues and, at the same time, to investigate and penetrate additional areas to enhance their contribution to the society. The mandate given to veterinary education by the society extends beyond its own priorities but also generates new ones. Veterinary profession must be informed of new requirements and the educational institutions must be able to perceive them and respond to them rapidly. Existing surveys indicate that more than six years are necessary to change a curriculum, which is too long time horizon in the rapidly changing world.

Key words: animal health; public health; veterinary education

INTRODUCTION

Integrated veterinary medicine has been a proven tool for the control of animal and human health via the food chain. From this point of view an integrated, scientifically justified, and practically proved system for ensuring safety of the food chain "from farm to table" is available (2). It is based on extensive and thorough medical knowledge in the veterinary field and application of principles of inter-disciplinary and multi-disciplinary thinking involving all links of the food chain and assurance on the issues of animal health, animal welfare, environmental protection and public veterinary and human health (1). Based on the facts stated above, veterinary profession on international scale has been responsible for food safety and at the same time for penetration to additional areas that help veterinarians to enhance their contribution to professional management of the society. Besides conforming with the basic curriculum the veterinary educational institutions must be ready to implement new professional activities and integrate the latest scientific knowledge into educational schedules.

MATERIALS AND METHODS

The paper deals with the recent requirements on implementation of the principle of integrated veterinary medicine "from farm to table" from the aspect of animal nutrition, welfare, animal health and public health and safety. The role of veterinary medicine and veterinary education has been analysed by virtue of the latest scientific and practical knowledge and experience in this field in Slovakia and EU member countries.

RESULTS AND DISCUSSION

Professional and social requirements on veterinary profession

In the past 20–30 years the role of veterinary surgeon has shifted from a daily therapeutic provider into the

area of consulting and advisory activities. Maintenance of good health of world fauna, including domestic and wild-living animals, and prevention of transmission of zoonoses from animals to humans has been the key professional duty of veterinarians. According to the recent knowledge more than 60 % of human diseases originate in animal population which further stresses the importance of veterinary profession for public health. Historically significant evolutionary changes in profiling the veterinary medicine were recorded, for example, in food animals.

At the turn of the 19th century the main role of veterinary profession was to develop diagnostic procedures for infection diseases of zoonotic nature (tuberculosis, brucellosis, etc.) and to eradicate them gradually. Infectious diseases of such nature had a negative effect on the economy of livestock and on human health.

Later, around 1940, with decreased use of horses as a mean of transport, the veterinary profession focused on food animals. This period, characterised by increased biological and economical value of farm animals, is known for enormous improvement in the principles of veterinary care centred on production of safe food. In those days, owing to better availability of antibiotics, some techniques were developed enabling successful, simple and more demanding operational procedures in all animal species. Prevention based on vaccination schemes gradually became the part of animal health programmes.

In the sixties, more pro-active principles of veterinary medicine were applied at the expense of the reactive ones. In this context, new diagnostic methods for the detection of subclinical forms of diseases, as the cause of production diseases, were developed. Mastitis and infertility prevention programmes were implemented more extensively.

In the eighties, the importance of farm health programmes was increasingly stressed as a tool for ensuring animal health. It was clear that veterinarians should be able to analyse animal health state – from individuals up to the herd diagnostics (evaluation of production and reproduction parameters, morbidity, mortality, quality of animal proteins, etc.) – to identify critical points of health and production and to develop prevention and treatment programmes. Application of integrated veterinary medicine principle "from farm to table" gradually found its position in the control of food safety and public veterinary and human health.

At the beginning of the 21st century, the social responsibility of veterinary profession for food chain safety, especially with regard to zoonoses, was confirmed. Novel methods at the level of cell and cell structure (genetic engineering, nanotechnologies) were introduced in veterinary medicine for diagnostics of serious and economically important diseases and emerging diseases. The use of information and communication technologies within the rapid alert system, intended to warn against dangerous diseases including zoonoses and food hazards, has made the veterinary profession responsible for these issues on a worldwide scale.

Requirements on veterinary education

Philosophy of veterinary education, to be up to society standards, is a complex issue as it covers many areas anticipating high proficiency. By OIE codex definition, veterinary profession is an art dealing with all issues of animals and their relationship with the environment while prioritising certain animal groups on account of demand, such as companion animals or animals kept for production purposes, either for work or for food (e.g. horses, cattle, zebus, fish, etc.) (3). The term "animal-related issues" covers not only areas related to diseases or cruelty but also food safety, public veterinary health, welfare, environment, and others. With respect to this, in international context, veterinary medicine is a branch of science training students to call attention to the above "problems" and, at the same time, to explore and penetrate the areas important for the entire society (national food safety, world trade with animals and food, etc.).

Even though it is very difficult to name nowadays all the areas that veterinary profession may play role in, they must at least include activities requiring veterinary professionals, including those dealing directly with animals and ensuring their health and welfare as the primary objective. However, one should not forget professional activities of veterinarians related to other links of the food chain. Within the scope of specialized technologies one should mention feed and food processing technologies, involving animal health in the stable and animal products from the point of view of food safety, care of animals and inspection before slaughter. Veterinarians practising in the field of specialized technologies are responsible for ensuring good health of animals before slaughter and for inspection of breeding facilities the animals originate from. From this point of view the responsibility of veterinary profession goes far beyond the direct contact with animals. Definition of professional responsibility and related activities of veterinarians clearly emphasizes their important and decisive role in prevention of zoonoses as the majority of new emerging human diseases originate from animals.

Biological inspection of nutritional standards has been an inseparable part of animal health analysis. In many cases animal and plant feed is a source of contamination, introduction of infectious agents or another noxious influences affecting animals and consequently also humans (e.g. BSE, botulotoxins, mycotoxins, intoxications, allergies, etc.). In light of integrated veterinary medicine, the health safety and nutritional value of feed has been a key to animal health, environment and safety of food of animal origin. Feeding meat and bone meal from ruminants, spreading of BSE among cattle and consumption of meat from such animals associated with the incidence of the new variant of Creutzfeldt-Jacob's disease in humans is a typical example. Not less serious public health problem is an uncontrolled use of antimi-

crobials in animals and plants or during food processing which contributes to the increase in antibiotic resistance through diversification of resistant or resistant-genes-producing bacteria entering the human organism with food. Knowledge of genetic engineering predetermines veterinary profession to monitoring genetically modified organisms in animal feed and secondarily at the level of animals and human food. Veterinary training in the field of animal nutrition and feeding, related to animal health, allows veterinary professionals to analyse and control the relevant risk and inform the public.

As the present veterinary activities involve many different areas, it is up to the professional educational institutions to take the responsibility for education of new professionals, state and private specialists at national and international level in the context of new vision of veterinary profession. New trends have not been chosen only by veterinary professionals, but also by consumers, beginning with high buying power of markets and continually spreading worldwide. It is obvious that the consumer is no more satisfied with information limited to safety of the purchased product. Conscious consumer wants to know the history of an animal product from animal's birth through the feed provided to the animal, care of the animal, potential treatments (therapy, medications administered) and its welfare up to the slaughter, i.e. a series of details that were considered unimportant before. It is the result of consumer's high buying power and, consequently, the wish to pay only for the products that are safe to consume. One should consider also the fact that the universal progress resulted in higher life expectancy and thus also in higher awareness of diseases. This is supported by the efforts of producers to put on the market products acceptable to consumers to ensure high economic returns. These relevant investments are often associated with companies that also produce food animals (joint ventures of primary producers and food processing companies).

When satisfying all-society demands, the veterinary education and the role of veterinary professionals has been often underestimated and misinterpreted. On a worldwide scale, the mandate given to veterinary training by the society reaches beyond its own priorities but also generates them. It has to meet social demands and act according to existing needs exceeding its professional framework generally accepted by the final consumer who represents people as a part of society in the globalised world. On the one hand, these demands reflect people's ideas (this would be easy) but, on the other hand, they also protect them in the world with rapidly changing lifestyle which, inevitably, brings even higher guarantee. In response to such situation the veterinary profession must be informed and the educational institutions must be able to react and respond quickly to the changes and new emerging professional demands.

Past experience showed that well determined conceptions were changed by the constantly evolving world (e.g. wild animals, such as birds and their migration played an important role in zoonoses). Therefore individual states must coordinate the training provided by veterinary educational institutions with public health centres and groups professionally dealing with wild animals and their migration as humans have been a target of zoonotic diseases.

Due to ongoing economical, social and bioclimatic changes the market with animals and animal products has been increasingly controlled by the above mentioned conceptions. This phenomenon contributed, on the one hand, to increased long distance trading in animals and food among countries or continents but, on the other hand, increased risk of disease transmission unknown in some regions and having high health and economic consequences or zoonotic nature. To be able to disclose the respective risks and guarantee animal health the veterinary diagnostic activities must be based on technologies operating at molecular or subcellular levels (e.g. nanotechnologies, with matter or processes control involving atoms or molecules under 100 nanometres).

The up-to-date experience points to the need for improvement. This is a challenge for institutions providing postgraduate training courses related to public demands. Educational institutions must accept the fact that education of veterinary professionals is essential for increasing life expectancy. Another reason is the ever increasing occurrence of pathogens related to a great extent to selection pressure of drugs. Another present reality is the need for wildlife control, human behaviour and tendency of humans to disturb the environment. There had been cases when veterinary educational institutions realised that their curricula fell beyond social expectations and thus failed to integrate important aspects into veterinary practice and research. Unfortunately, cooperation with other professionally important branches has been frequently insufficient, even if it would be beneficial to education of new professionals in veterinary medicine and related fields. Development of knowledge in veterinary medicine and related branches indicates that more than six years will be needed to change the curricula which is much too long time in the rapidly changing world.

CONCLUSION

Veterinary educational institutions must be prepared to incorporate rapidly new professional activities and the latest scientific knowledge into their curricula. Veterinary profession reached the stage of full recognition of its professional and social importance on national and international level. According to expectations, resulting from the survey carried out in OIE member countries including the Slovak Republic, this important role of veterinary professionals in the field of food safety and other health issues will become strengthened even more. This will further intensify the responsibility of individual participants, including state and private veterinarians and veterinary educational institutions, that are in the foreground of implementation of new tasks.

ACKNOWLEDGEMENT

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REFERENCES

1. Bíreš, J., 2008: Integrated veterinary medicine from the point of view of good animal nutrition and public health (In Slovak). In *Proc. International conference on the occassion of the 80th birthday anniversary of deceased Prof., DVM Ján Lazar,* UVM, Košice, 36–41.

2. Buncic, S., 2006: *Integrated Food Safety and Veterinary Public Health*. Cromwell Press, Trowbridge, UK, 386 pp.

3. OIE – World organisation for animal health – 74th General Session, Paris, 2006: *Future approaches needed to ensure that veterinary education meets societal demands*. www.oie.int.

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CURRENT TRENDS IN ANALYTICAL METHODS IN DAIRY COWS NUTRITION AND THEIR APPLICATION RELATED TO PRODUCTION HEALTH

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ABSTRACT

The effect of quantity of nutrients on rumen fermentation and the level of metabolic markers in blood serum were analysed simultaneously in dairy cows 21 days before and 21 days after parturition with the aim to diagnose disorders in milk production in the transition period. Results of analysis of health disorders confirmed that low energy concentration in the diet insufficiently saturated with fibrous carbohydrates, followed with rapid change to concentrate type of diet after delivery, resulted in insufficient adaptation of the rumen metabolism before and after rapid transition to production rations after calving. The levels detected indicated acidification of the rumen environment. Investigation of intermediary metabolism confirmed pre- and post-partum lipomobilisation syndrome with increased values of non-esterified fatty acids (NEFA) in 68 % and 54 % of animals, respectively, and increased load on liver in 37 % and 69 % of animals, respectively.

Key words: dairy cow; feeding and nutrition; metabolism; rumen fermentation

INTRODUCTION

The transition period of the cow is generally defined as three weeks before calving to three weeks after calving. During this period the cows undergo extensive metabolic adaptation in glucose, fatty acids, and mineral metabolism to support lactation and avoid metabolic dysfunction (5). Dairy cattle usually experience negative energy balance before calving. This reflects the reduction in DM intake (30 %) and increased energy requirements (23 %) for maintenance and pregnancy during the last month of pregnancy (3). Mammary requirements for glucose, amino acids and fatty acids for a milk yield of 30 $kg.d^{-1}$ at 4 days postpartum are approximately 2.7, 2.0 and 4.5 times those of the gravid uterus during late pregnancy (1). Metabolic disorders and health problems are common during this time and can easily erase the entire profit potential for dairy cow farms (2). The practical goal of nutritional management within this timeframe is to support the respective metabolic adaptations.

The aim of our study was to evaluate the influence of nutrition on production and health of dairy cows in their pre-partum phase (21 days before and 21 days after delivery) in production herds and parallel determination of intake of nutrients from total mixed ration (TMR) in relation to evaluation of rumen fermentation level and metabolites in blood serum of dairy cows and to propose procedure for biological control of nutrition level, adaptation of rumen and intermediary metabolism in the transition period of highly-productive dairy cows.

MATERIALS AND METHODS

Under production conditions, we carried out 3-year investigations in 10 herds of dairy cows with milk yield of 6–8 thousand litres per year for the level of nutrition, degree of rumen adaptation and intermediary metabolism 21-days before and 21 days after parturition. In all investigated herds we analysed the following: nutrient composition of feed and TMR, rumen fermentation level and markers of protein intermediary

metabolism and liver function. In both pre- and post-partum phases we evaluated the nutrition level and fermentation as reflected in nutritionally influenced adaptation of cows during the transition phase.

RESULTS AND DISCUSSION

The 3-year investigation of a large number of samples of TMR, conducted in dairy cows in the pre-partum phase and at the peak of lactation, focused on nutritional parameters including the content, digestibility and energy content of TMR and its influence on rumen fermentation and energy metabolism in dairy cows provided results summarised in Tables 1–3.

Our analysis showed that the level of nutrients in TMR (Tab. 1) reflected decrease in energy concentration in comparison with the recommended values and increased levels of neutral-detergent fibres (NDF) in dairy cow rations prior and after the parturition and at the peak of lactation with mean levels of NDF reaching 411.3 ± 63.5, 361.1 ± 36.7 and 335.6 ± 50.8 g.kg^{-1} dry matter (DM).

Table 1. Carbohydrate composition of dairy cows rations

Parameters	NDF Means ± SD	NFC Means ± SD	NEL Means ± SD	ADF Means ± SD
21 days before parturition	32.0–38.0 % 41.1 ± 6.8 60 % ↑	30.0–35.0 % 35.5 ± 4.2 45 % ↑	6.2–6.5 6.23 ± 0.3 45 % ↓	23.0–25.0 % 25.8 ± 4.5 50 % ↑
21 days after calving	30.0–35.0 % 36.1 ± 4.1 60 % ↑	32.0–38.0 % 38.1 ± 3.9 50 % ↑	6.6–6.8 6.72 ± 0.1 50 % ↓	21.0–23.0 % 23.0 ± 3.8 35 % ↑
Peak lactation	28.0–33.0 % 34.0 ± 4.7 40 % ↑	36.0–42.0 % 39.8 ± 4.0 30 % ↑	6.9–7.1 6.76 ± 0.3 45 % ↓	20.0–22.0 % 20.4 ± 3.7 20 % ↑

NEL – net energy of lactation; ADF – acid detergent fibre

The content of non-fibrous carbohydrates (NFC) in TMR DM reached on average 355.1 ± 41.5 g.kg^{-1} before parturition, 381.8 ± 37.0 g.kg^{-1} after parturition and 397.9 ± 35.5 g.kg^{-1} DM at the peak of lactation. This confirmed that an increased proportion of concentrate feed was supplied to the cows to compensate for the low quality of bulk feed.

State of rumen fermentation directly reflects the composition of feed rations with prevalence of structural carbohydrates and limited portion of non-fibrous carbohydrates before parturition.

In dairy cows, in their preparation for delivery, with considerable proportion of clinically healthy animals, the average values of the analyzed parameters (Table 2) varied around the reference values.

Table 2. Analysed values of rumen fermentation in dairy cows

Index	21 days before parturition			21 days after calving		
	Average n = 60	Individually %		Average n = 60	Individually %	
		Increased	Reduced		Increased	Reduced
pH	6.72 ± 0.3	33	-	6.18 ± 0.4	6	50
NH_3 mg.100 ml^{-1}	17.8 ± 5.9	9	35	19.8 ± 7.3	24	32
VFA mmol.l^{-1}	89.5 ± 21.9	0	33	100 ± 21.9	6	15
Acetic acid %	67.4 ± 4.4	69	0	61.0 ± 16.1	17	41
Propionic acid %	19.6 ± 4.6	13	63	24.6 ± 4.5	44	9
Ratio C_2:C_3	1:3.6	76	11	1:2.6	26	46

The mean level of pH was below the reference value as were the individual results in half of the examined dairy cows after calving. The variability of individual VFA values, increase in propionic acid level in 44 % of animals, decrease in acetic acid in 41 % of cows and a tendency of C_3 : C_2 decline to the lower limit confirmed the trend toward rumen acidification as a consequence of low level of adaptation and rapid transition to the productive type of feeding rations. In the examined animals, propionic acid level before delivery reached on average 17.9 ± 7.4 mmol.l^{-1} and individually, in 65 % of dairy cows, it reached levels < 20 mmol.l^{-1}. Such state of rumen fermentation fails to ensure sufficient production of precursors for dairy cows glucogenesis before delivery. Starch is converted in the rumen to propionic acid and supports development of rumen papillae and proliferation of amylolytic microflora (6, 8). This way supported production of propionic acid, a precursor of glucose synthesis, stabilizes the energetic balance and limits lipomobilisation (7).

The levels of blood serum indices of intermediary metabolism of proteins, carbohydrates and fats, as well as metabolic load on the liver of dairy cows in their pre-partum phase are summarized in Table 3. At serum levels above 0.35 mmol.l^{-1} the liver does not have sufficient capacity to utilize completely non-esterified fatty acids (NEFA) to cover energy requirements or ensure transport of triglycerides to the blood which is a predisposing factor for accumulation of triglycerides in the liver.

The observed pre-partum level in dairy cows restricts the supply of glucogenc sources for saturation of foetus needs and evokes the state of lipomobilization in pre-partum metabolism of dairy cows. When dividing animals

according to the level of propionic acid (Fig. 1) in the rumen content, the degree of lipomobilisation with mean value of NEFA equal to 0.38 ± 0.16 corresponded to the group with level below 20 mmol.l^{-1} while at the reference values of propionic acid above 20 mmol.l^{-1} the values of NEFA reached the level of 0.31 ± 0.1 mmol.l^{-1}.

Table 3. Analysed serum values of nutrient metabolism in dairy cows

Index	21 days before parturition			21 days after calving		
	Average N = 60	Individually %		Average N = 60	Individually %	
		Increased	Reduced		Increased	Reduced
Total proteins g.l^{-1}	69.2 ± 6.9	2	43	73.9 ± 7.8	2	22
Albumin g.l^{-1}	33.5 ± 2.6	0	6	29.4 ± 3.6	0	30
NEFA mmol.l^{-1}	0.49 ± 0.2	68	0	0.56 ± 0.4	54	0
Glucose mmol.l^{-1}	3.56 ± 0.8	17	4	3.02 ± 0.8	6	19
Triglycerides mmol.l^{-1}	0.49 ± 0.3	17	8	0.36 ± 0.2	0	38
Cholesterol mmol.l^{-1}	2.68 ± 1.4	2	0	2.7 ± 0.7	0	0
Bilirubin µmol.l^{-1}	4.69 ± 2.3	37	0	5.43 ± 2.3	46	0
AST µkat.l^{-1}	0.41 ± 0.1	7	0	0.50 ± 0.1	69	0

In the animals with decreased level of propionic acid in the rumen, manifestation of lipomobilization with values of NEFA > 0.35 mmol.l^{-1} was detected in 42 % of dairy cows while in animals with propionic acid exceeding 20 mmol.l^{-1} manifestation of lipomobilisation was observed in 18.3 % of dairy cows pre-partum. The influence on lipomobilisation of propionic acid as the most important precursor of glucogenesis in relation to serum level of NEFA (Fig. 2) was also confirmed by a direct regression relationship (r = 0.447).

Increase in dietary NFC may lead to greater propionate concentration in the rumen, which promotes insulin secretion (4). Because insulin is antilipolytic, an increase in dietary NFC might decrease plasma NEFA and reduce metabolic load of liver. Such state of rumen fermentation does not secure sufficient production of precursors for dairy cows glucogenesis before delivery. The major substrates for hepatic gluconeogenesis in ruminants are propionate from ruminal fermentation, lactate and amino acids from protein catabolism.

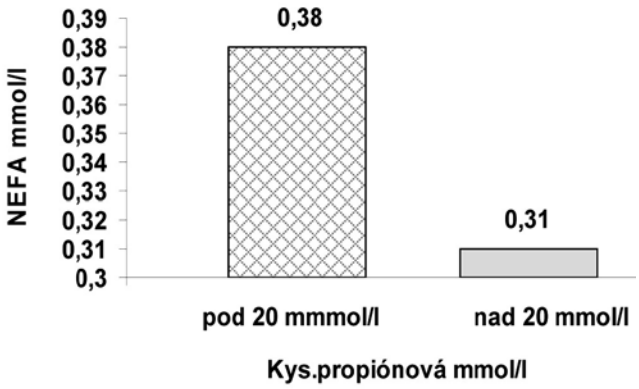

Fig. 1. Values of NEFA in relation to propionic acid level (below 20 mmol.l^{-1} and above 20 mmol.l^{-1})

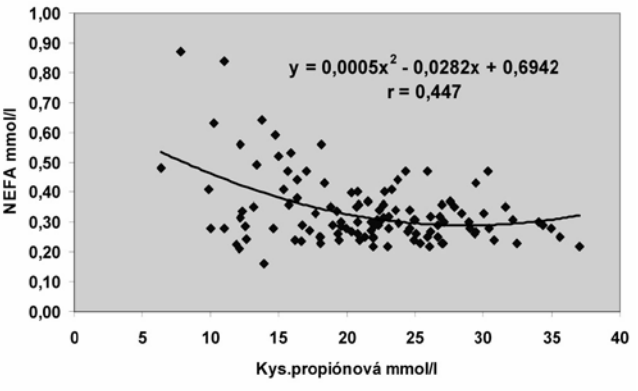

Fig. 2. Regression relationship between rumen propionic acid level and serum NEFA

CONCLUSION

Nutrition and management during the pre-partum phase are essential in determining the profitability of the cow for the rest of its lactation. Proper formulation of rations regarding proteins, energy density, NDF and NFC will help to increase dry matter intake around calving along with management of body condition while excellent quality, nutritional value and digestibility of forages will assure an excellent programme for the high producing dairy cows. Further increase in milk yield and desirable milk components requires nutritional prevention and good management of productive health of herds. This should be implemented through increased intake of forage dry matter and stabilization of rumen and intermediary metabolism of high-productive dairy cows in respective phases of their nutrition.

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REFERENCES

1. Bell, A. V., 1995: Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *J. Anim. Sci.,* 73, 2804–2819.

2. Drackley, J. K., 1999: Biology of dairy cow during the transition period: the final frontier? *J. Dairy Sci.,* 93, 2259–2273.

3. Grummer, R. R., 1995: Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *J. Anim. Sci.,* 73, 2820–2833.

4. Harmon, D. L., 1992: Dietary influences on carbohydrates and small intestinal starch hydrolysis capacity in ruminants. *J. Nutr.,* 122, 203–210.

5. Overton, T. R., Waldron, M. R., 2004: Nutritional management of transition dairy cows: Strategies to optimize metabolic health. *J. Dairy Sci.,* 87, E105–119.

6. Owens, F. N., Secrist, D. C., Hill, W. J. and Gill, D. R., 1998: Acidosis in cattle: A review. *J. Anim Sci.,* 76, 275–286.

7. van Knegsel, A. T. M., van den Brand, H., Graat, E. A. M., Dijkstra, J., Jorritsma, R., Decuypere, E. *et al.*, 2007: Dietary energy source in dairy cows in early lactation: Metabolites and metabolic hormones. *J. Dairy Sci.,* 90, 1477–1485.

8. Yokoyama, M. T., Johnson, K. A., 1988: Microbiology of the rumen and intestine. In *The Ruminant Animal: Digestive Physiology and Nutrition.* **Church, D. C.,** ed., Waveland Press, Inc., Prospect Heights, IL, USA, 125 pp.

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EVALUATION OF SELECTED WELFARE PARAMETERS OF SOWS AND PIGLETS IN FARROWING SYSTEMS

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ABSTRACT

The study evaluated selected welfare parameters of sows and piglets in 2 strawless farrowing systems with fixation crates. The floor of farrowing pens in the first system (PS-1) was divided to a solid and slotted part and in the second system (PS-2) it was slotted except for the part under the sow which was solid with holes for drainage. The sows laid longer on their belly than on their side in both systems (PS-1 and PS-2, $P < 0.05$ and $P < 0.001$, resp.). Piglets in both systems spent more time in the locally heated (52.2% and 63.3%) and much less time at the sow udder ($P < 0.001$). Piglets in the PS-1 system devoted to suckling and massage 2.6% and in the PS-2 system 7.8% of time which corresponded to the time during which the sows laid on their side. Our evaluation showed that behaviour of sows in both farrowing systems differed from the habitual standard, which was reflected partly also in utilization of pen area by piglets.

Key words: farrowing pen; piglet; sow; welfare parameters

INTRODUCTION

The housing system for sows based on individual farrowing pens with varying level of welfare predominates today in Slovakia. Housing of lactating sows in farrowing systems with fixation crates limits free movement, natural behaviour and thereby also welfare of these animals (5). Health problems and difficulties in lying down are relevant indicators of poor welfare in sows confined in farrowing crates (2). Slipping and interruptions while lying down were observed in our study ($P < 0.05$). Slipping was associated with hind feet lesions and overgrown hooves and abnormal lying-down behaviour. Elaborate welfare assessment systems and welfare indicators, as part of ethical assessment of animal husbandry, help farmers to make decisions and provide information about potential problems (6). Indicators of optimal rearing environment (welfare) have to include description of rearing systems, evaluation of behaviour, health of animals and management (1). Different methods for evaluation of pig farm welfare were tested and the results obtained were evaluated (4). Pig welfare parameters include resting (lying) time, animal activity and utilization of pen areas. Generally, in free farrowing pens the sows laid longer on their side than on their belly. This is favourable not only in terms of resting but access of piglets to sow teats (3).

The aim of the paper was to evaluate selected welfare parameters of sows and piglets in two farrowing systems with different housing environment.

MATERIAL AND METHODS

Sows in both farrowing systems were housed in individual strawless pens with fixation crate. The farrowing pens in the first system PS-1 (2.4 × 1.6 m) had floor divided to a solid and slotted part. The area for piglets was heated by an electric panel (0.4 × 1.0 m) and infra-lamp loaded into split cover. Pens in the second system PS-2 (2.4 × 1.8 m) had slatted floor except for the part under the sow which was solid with holes for drainage. The area for piglets was equipped with plastic warm-water panels (0.8 × 0.8 m) built-in in floor. Sows fed from feeders with mechanical discharge. Excrements collected under the slotted

floor were removed by a plug system. Controlled negative pressure ventilation was installed in the farrowing houses.

Condition and behaviour of sows and piglets, condition of dew claws in sows, milk production and live weight of piglets were evaluated. Direct ethological observations of sows and piglets were carried out. Sows were observed for lying on their side (right and left), on their belly, standing and sitting. The way of lying down was subjected to special observation. In piglets we recorded the time spent in locally heated area and other areas of the pen, time at the udder and, especially, their interest in sucking and access to sow teats. In the PS-1 and PS-2 systems we evaluated 14 and 17 sows with their litters, respectively. Analysis of Variance was used for statistical evaluation of the data.

RESULTS AND DISCUSSION

Sows spent lying about 1.8-times more time in the PS-2 than in the PS-1 system (for 86.7 % vs. 48.2 % of time, $P < 0.001$). The sows in both farrowing systems lay longer on their belly than on their side. This was less favourable for nursing because piglets had more limited access to the udder than in the case of longer lying of sows on their side (3). The slotted floor in pens in both farrowing systems was slippery, mostly wet with urine, and because of decreased stability the sows stood up only if it was really necessary. The sows in the PS-2 system lay on their side for 27.1 % and on their belly for 59.6 % of time, in PS-1 pens they laid less (10.5 % and 37.7 % of time). The sows in the PS-2 pens stood (10.3 % vs. 27.2 %, $P < 0.05$) and sat (3.0 % vs. 24.6 %, $P < 0.001$) for shorter time than in the PS-1 pens. In the PS-2 system the sows stood only at feeding, drinking and defecating. In the PS-1 system nursing in standing position was also noticed which increased time of standing in these pens. Longer sitting in PS-1 pens can indicate compromised welfare (1). Sows sat when changing the lying position and consequently laid down on their belly. The proportion of sows with normal condition was 50 % in the PS-1 system and 39.13 % in the PS-2 system. Normal condition of dew claws in PS-1 and PS-2 system was recorded in 81.25 % and 68 % sows, respectively. Limited access of piglets to the udder was registered at older age and especially to sows with big body frame. Better and balanced ability of sows to produce milk was registered in the PS-1 system compared to the PS-2 system (61.730 ± 10.718 kg vs. 43.510 ± 11.438 kg).

Piglets in both systems occupied the locally heated area for the longest time (52.2 % vs. 63.3 % of time). They used the remaining area of the pen for longer time in PS-1 pens than in the PS-2 pens (35.1 % vs. 20.7 %, $P < 0.05$). Occupying these areas included all the activities and resting except staying at the udder. Piglets spent the shortest time at the sow's udder because the sow laid longer on its belly. They suckled (including massage) longer in the PS-2 system then in the PS-1 system (7.8 % vs. 2.6 %) which corresponded to duration of sows lying on their belly. Piglets in the observed litters were in normal condition. During nursing we occasionally noticed that piglets did not find way to sow teat, especially the weaker and less developed animals. We registered also fights of 2 or 3 piglets for access to teats. The mean body weight of piglets and litter uniformity at the age of 21 days was higher in the PS-1 system than in the PS-2 system (6.024 ± 0.719 kg vs. 5.225 ± 1.173 kg).

CONCLUSION

Our observations of welfare parameters indicated that welfare conditions of sows were less favourable in the farrowing pens with slatted floor (PS-2 system) than in the pens with differentiated floor (PS-1 system). It was due to excessive slipperiness of floor and worse stability of sows at standing up, standing and lying down. Although the PS-2 piglets spent more time at the udder of sows and were more interested in suckling, higher body weight was reached by the PS-1 piglets due to better milk production of sows in this system.

REFERENCES

1. Bonde, M., 2003: Welfare assessment in a commercial sow herd: Development, evaluation and report of the method. In *DIAS Report Animal Husbandry,* No. 46, Danish Institute of Agricultural Science, 98 pp.

Bonde, M., Rousing, T., Badsberg, J. H., Sørensen, J. T., 2004: Associations between lying-down behaviour problems and body condition, limb disorders and skin lesions of lactating sows housed in farrowing crates in commercial sow herds. *Livest. Prod. Sci.,* 87, 179–187.

2. Botto, Ľ., Kišac, P., Knížatová, M., Mačuhová, L., Brestenský, V., Hanus, A., Mihina, Š., 2006: Effect of housing environment on sow behaviour in a farrowing house (In Slovak). In *Biotechnology 2006.* The Czech Republic, Scientific Pedagogical Publishing, České Budějovice, CD-ROM, 116–118.

3. Courboulay, V., Foubert, C., 2007: Testing different methods to evaluate pig welfare on farm. *Animal Welfare,* 16, 103–106.

Chaloupková, H., Illmannová, G., 2000: Effect of housing of lactating sows on agonistic behaviuor of piglets (In Czech). In *Proceedings 27th Ethological Conference of the Czech and Slovak Ethological Society,* The Czech Republic, CSEtS, 21.

Sørensen, J. T., Sandøe, P., Halberg, N., 2001: Animal welfare as one among several values to be considered at farm level: The idea of an ethical account for livestock farming. *Acta Agric. Scand.,* Sect. A, Anim. Sci. Suppl., 30, 11–16.

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DUST AS AN ETIOLOGICAL FACTOR OF RESPIRATORY DISEASES IN HORSES, ITS MONITORING AND MANAGEMENT

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ABSTRACT

Organic particulates, especially spores of fungi, forage mites and airborne microbes are important promoting factors of hypersensitivity response in horses suffering from recurrent airway obstruction. Techniques commonly used for monitoring of aerial microbial contamination and dust focus on monitoring of air in animal housings in general, which may not necessarily correlate with microbial contamination and dust in the inhaled air. The presented technique aims to mimic the environment of inhaled air by considering airflow during inhalation and taking samples directly over potential contaminants (hay and straw) taking into account relative position of horse nostrils. Our results suggest importance of proper hay treatment (soaking as compared to quick sprinkling only) on decrease in quantity of airborne dust and the related microbial contamination of inhaled air.

Key words: dust; environmental monitoring; horse; recurrent airway obstruction management

INTRODUCTION

In addition to orthopaedic diseases, recurrent airway obstruction (RAO) is the most common cause of decreased performance of horses. RAO is naturally occurring, dust-induced disease presented as bronchiolitis with mixed-pattern genetic predisposition (5). Exposure of the RAO horses in clinical remission to organic particles (particularly fungi spores) of hay exacerbates clinical symptoms associated with airway inflammation, increased respiratory resistance and lung dysfunction (6). *In vitro* study of bronchial epithelial cells from RAO positive horses proved that pathological response was elicited by hay dust (1). Importance of providing low dust environment and low environmental challenge regarding airborne particles to RAO positive horses is well recognized (1, 6). Preventive measures, including disinfection, are important for protection of animal and human health (7). Other techniques, described elsewhere (1), focus mainly on measurement of dust in stables. Our aim was to evaluate the technique for measurement of dust and microbial composition of aerosol inhaled by a horse during critical time of feeding. We also investigated effectiveness of techniques commonly used to reduce the quantity of dust released from hay (soaking of hay versus sprinkling with water).

MATERIAL AND METHODS

Air samples (aerosol) were collected in close proximity of the hay shaken in a manner similar to that of horse pulling the hay from a feeding net. On the same day, employing the same technique, we sampled hay and straw (just to be used for bedding) and on the following day we sampled the same batch of hay soaked for 12 hours in a barrel with water (3) or sprinkled with a garden hose (the way the hay is treated on local farms as a preventative measure). Microbiological contamination of the air in 1 litre volume was determined with Aeroscope MAS 100 ECO, using Petri dishes with Endo agar, Sabouraud agar and meat-peptone agar. The total quantity of dust particles in the air was determined by membrane filtration (100 l of air, 0.45 μm pore size) using SARTORIUS MD 8. The filters were weighed employing analytic grade balance (METLER AE 160). The airflow produced by Aeroscope and Sartorius equipment was similar to that in the upper respiratory tract of the resting

Table1. Plate counts of microorganisms (CFU.m^{-3}) in an aerosol created by shaking hay and straw

Material	Total CFU × 10^5			Coliforms CFU × 10^4			Fungi CFU × 10^4			Yeasts CFU × 10^4		
	Spr	Sum	Aut	Spr	Sum	Aut	Spr	Sum	Aut	Spr	Sum	Aut
Hay – dry	8.6	7.1	5.5	1.6	1.1	4.8	4.5	2.4	5.6	0.8	1.0	1.2
Hay soaked	1.3	1.4	0.8	1.0	0.2	0.4	1.8	1.2	0.6	0.5	0.6	3.2
Straw – dry	1.1	2.3	0.80	0.4	0.9	0.9	4.4	6.8	8.5	0.7	6.3	0

Spr – spring: T = 10 °C, relative humidity (RH) = 60 %; Sum – summer: T = 17.4 °C, RH = 52 %
Aut – autumn: T = 11.5 °C, RH = 60.5 %

horse, e.g. 100 l.min^{-1}. Air humidity was checked at sampling by hygroscope Testo 625. Samples were taken in spring, summer and autumn. Student *t*-test was used for statistical analysis.

RESULTS AND DISCUSSION

The results obtained (Tab. 1) correlated with other reports (2, 4) implying that straw and dry hay are important sources of dust and fungi in horse stables and their content is influenced by quality of straw and hay rather than by season, temperature or humidity. Significant differences (Tab.2) were observed between dust content of dry and soaked hay ($P < 0.001$), as reported by other authors (3), and also between dust content of soaked and moistened/sprinkled hay ($P < 0.001$). Comparison of dust quantity (Tab. 2) and plate counts of micro-organisms (Tab. 1) indicated correlation between decreased dust in soaked hay and total CFU, coliform bacteria and fungi as compared to dry hay. This decrease was more likely related to decreased production of aerosol by soaked hay than to decreased total count of micro-organisms in the hay which may depend on regular disinfection of the environment. Despite relative simplicity of the technique used compared to that described elsewhere (2), it reflected sufficiently the quality of inhaled air and could become a useful tool in critical points of management of RAO positive horses. While the respirable portion of dust particles could be reduced by moistening of hay, significantly more effective reduction was achieved by hay soaking. The dust content seems to be a more relevant indicator when evaluating dry hay and straw quality as compared to vegetable fungi count.

Table 2. Quantity of dust (μg.m^{-3}) in an aerosol created by soaking the bedding and hay

Season	Straw	Dry hay	Sprinkled hay	Soaked hay
Spring	98.0 ± 29.22	135.75 ± 43.44	47.50 ± 19.87	6.25 ± 2.99
Summer	59.0 ± 29.64	163.25 ± 65.49	43.0 ± 15.51	7.25 ± 3.40
Autumn	100.50 ± 27.67	168.25 ± 40.82	47.50 ± 10.15	5.00 ± 2.16
Average/ all seasons	85.83 ± 32.79	155.75 ± 48.59	46.0 ± 14.37	6.17 ± 2.79

REFERENCES

1. Ainsworth, D. M., Matychak, M., Reyner, C. L., Erb, H. N., Young, J. C., 2009: Effects of in vitro exposure to hay dust on the gene expression of chemokines and cell-surface receptors in primary bronchial epithelial cell cultures established from horses with chronic recurrent airway obstruction. *Am. J. Vet. Res.,* 70, 365–372.

2. Clemens, J. M., Pirie, R. S., 2007: Respirable dust concentrations in equine stables. Part 1: Validation of equipment of various management systems. *Res. Vet. Sci*., 83, 256–262.

3. Clemens, J. M., Pirie, R. S., 2007: Respirable dust concentrations in equine stables. Part 2: The benefits of soaking hay and optimizing the environment in neighbouring stable. *Res. Vet. Sci.,* 83, 263–268.

4. Fleming, K., Hessel, E. F., van den Weghe, H. F. A., 2008: Generation of airborne particles from different bedding materials for horse keeping. *J. Eq. Vet. Sci.,* 28, 408–418.

5. Gerber, V., Baleri, D., Klukowska-Rotzler, J., Swinburne, J. E., Dolf, G., 2009: Mixed inheritance of equine airway obstruction. *J. Vet. Int. Med.,* 23, 627–630.

6. Miskovic, M., Couetil, L. L., Thompson, C. A., 2007: Lung function and airway cytological profile in horses with recurrent airway obstruction maintained in low dust environments. *J. Vet. Int. Med*., 21, 1060–1066.

7. Ondrašovič, M., Hromada, R., Ondrašovičová, O., Halán, M., Sasáková, N., Kudríková, D., Laktičová, K., 2008: Importance of disinfection, insect control and rat control in prevention of zoonoses (In Slovak). In *Proceedings Zoonoses – protection of human and animal health,* Sept. 11–12, Bratislava, 72–77.

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EVALUATION OF SOURCES OF DRINKING WATER IN ENVIRONMENTAL POLLUTED REGION FROM CHEMICAL AND MICROBIOLOGICAL POINT OF VIEW

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ABSTRACT

The aim of the present study was to evaluate individual sources of drinking water in a village located in environmentally polluted Central Spiš region which has been affected negatively by mining activities and subsequent processing of complex Fe and Cu ores. Altogether 20 wells were examined chemically (pH, NH_3, NO_2, NO_3, Cl^-, Cl_2, COD_{Mn}, and 71 elements including heavy metals) and microbiologically focusing on selected indicators of contamination (BC_{22}, BC_{36}, TC and *E. coli*). The results obtained were evaluated on the basis of Slovak Republic Government Order No. 354/2006 Coll. setting requirements on water intended for human consumption.

Key words: chemical parameters; drinking water; heavy metals; hygienic safety

INTRODUCTION

Decrease in water quality on our Earth is a consequence of anthropogenic pressure on the environment and because of that it poses an international problem. People realise that the maximum pollution threshold cannot be exceeded without disturbing the global-ecological balance with all related catastrophic consequences (1, 4).

Quality of water is defined as a set of representative data characterising its physical, chemical and biological properties related to its potential use for various purposes. Since 2006 the Slovak Republic Government Order No. 354/2006 Coll. (2) has been in force defining requirements for water intended for human consumption and quality control of water intended for human consumption. Water complying with this Order will not cause health problems even after long-term consumption. The number of pollutants that may penetrate into ground water and thus also into human food chain increases. Because of increasing demands on drinking water supplies and present deficiency of sources in some locations it is inevitable to increase protection of existing ground and surface sources of drinking water (3, 5) and improve monitoring of their quality

The aim of our study was to examine water from individual sources in endangered Central Spiš region in relation to potential sources of their pollution.

MATERIAL AND METHODS

In the period from October 2005 to December 2006, water samples from 20 wells of various depth (4–14 m) from a village in Central Spiš region not connected to public water supply were collected and examined chemically and bacteriologically. The main sources of pollution in the area were industrial

locations Rudňany, Krompachy and Spišská Nová Ves with mining activities and processing of complex iron and copper ores. Although air pollution decreased in the area in the recent period, contamination of soil with metals, particularly with Hg, Cu, Pb, Cd and Zn, may still affect the quality of surface and ground water.

Chemical examination included determination of pH, NH_3, NO_2, NO_3, Cl^-, Cl_2 and COD_{Mn} (Order No. 354/2006) and 71 elements including heavy metals (AAS method).

Bacteriological examination focused on determination of plate counts of bacteria cultivated at 22 and 36 °C (BC_{22}, BC_{36}), TC and *E.coli* according to respective STN ISO and STN EN ISO.

RESULTS AND DISCUSSION

Our examination showed that only in 4 of 20 examined wells none of the chemical parameters was exceeded at any sampling. The limit for ammonium ions (0.5 mg.l^{-1}) was exceeded in one well (1.44 mg.l^{-1}), for nitrites (0.1 mg.l^{-1}) in 3 wells (W3 – 0.12, W6 – 0.168 and W7 – 0.68 mg.l^{-1}), for nitrates (50 mg.l^{-1}) in 3 wells (W6, W8 and W9, range 55–100 mg.l^{-1}) and chlorides (100 mg.l^{-1}) in 10 wells (W1, 3, 5, 6, 8, 9, 12, 13, 14, 17, range 108–399 mg.l^{-1}). In W7 we detected increased level of active chlorine (0.8 mg.l^{-1} *vs.* max. 3 mg.l^{-1}). Increased level of COD_{Mn} (max. 3 mg.l^{-1}) was detected in 5 wells (W3, 4, 6, 17, 18, range 3.7–8.25 mg.l^{-1}). Increased COD poses a risk to human health if the water is disinfected with chlorine due to production of trihalomethanes with carcinogenic effect. This is particularly important as residual chlorine was detected in 8 examined wells. Evidently, some people disinfected their wells. The water in 4 of 8 wells containing residual chlorine showed increased level of COD_{Mn}. Long-term consumption of such water increases risk of development of cancer. The highest level of COD_{Mn} (8.25 mg.l^{-1}) was determined in W6.

Results of AAS examination (71 elements) showed that only 3 elements, for which the Order No. 354/2006 gives the maximum allowed level (MAL), were exceeded, namely nickel in W6 3 reached 0.027 mg.l^{-1} (MAL = 0.02 mg.l^{-1}), antimony in W16 was detected at the level of 0.012 mg.l^{-1} (MAL = 0.005 mg.l^{-1}) and water in W18 exceeded the levels for both antimony (0.0086 mg.l^{-1} *vs.* 0.005 mg.l^{-1}) and arsenic (0.019 mg.l^{-1} *vs.* MAL = 001 mg.l^{-1}). The maximum allowable levels of Cr and Cd were not exceeded (4). Although adverse health consequences of Ni have been described particularly in relation to contaminated air, long-term exposure to Ni in water can also induce health problems. Chronic toxicity of As is manifested by changes in skin and mucosa, neurologic and haematological changes and mutagenic and carcinogenic effects. Antimony in water is monitored on the basis of WHO recommendations due to its carcinogenic effects.

Very serious were the results of bacteriological examination. Total coliform (TC) bacteria, which serve as an indicator of potential faecal contamination, were recovered from 10 ml of water from all wells over the examination period, occasionally in very high numbers (> 300 CFU). Moreover, *E. coli*, indicating presence of faecal contamination, were also detected in all wells at least at one sampling. From the bacteriological point of view the worst was W6 in which we detected also increased levels of 5 chemical indicators of contamination and increased content of nickel. There was a septic tank not far from this well.

Our results showed that none of the wells could be evaluated as safe from the bacteriological point of view. Although we obtained good results for some wells at one sampling, at other samplings they contained coliform bacteria in 10 ml volumes (none may be present according to Order No. 354/2006). This may be related to the fact that some families in the village kept farm animals and applied their excrements to soil, although the number of animals has been decreasing. Another reason of poor quality of water in investigated wells may be the location of wells and absence of the required adjustment and protection of their immediate surroundings (6).

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REFERENCES

1. Demeterová, B., 2002: *Management of Water Sources* (In Slovak). Košice, SHMÚ.

2. Order No. 354/2006 Coll., 2006: Slovak Republic government order defining requirements for water intended for human consumption and quality control of water intended for human consumption.

3. Podhrázská, J., Toman, F., 2000: Importance of differentiated protection of catchment area of water reservoirs in landscape planning (In Slovak). In *Proceedings Bioclimatology and the Environment*, UVM, Košice.

4. Skalická, M., Naď, P., Demeterová, M., Koréneková, B., 2007: Influence of Cr and Cd on quality of Japanese quails eggs. In *Proceedings 7th International Scientific Conference Risk Factors in Food Chain*, Nitra, The Slovak Republic, 197–199.

5. Directive 91/676/EC, 1991: Directive 91/676/EC on the protection of waters against pollution caused by nitrates from agricultural sources.

6. STN 75 5115, 1993: Water management. Wells for individual water supply (In Slovak).

*

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THE IMPORTANCE OF DISINFECTION AND VALIDATION OF ITS EFFECTIVENESS IN FOOD INDUSTRY

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ABSTRACT

The study focused on hygiene and sanitation of premises for processing of fish. The premises were disinfected with the preparation Topax 66, which was tested under laboratory conditions and directly in operation. Laboratory testing showed that Topax 66 devitalised the test bacterial strains *E. coli* and *S. aureus* already at 0.1% concentration and 5 min exposure time.

Good disinfectant effects of preparation Topax 66 was observed also under practical conditions. Higher plate counts of *E. coli* were detected in swabs taken from surfaces of auxiliary equipment in the section of fish salads before the operation.

Key words: disinfection; food industry; sanitation; Topax 66

INTRODUCTION

Environmental hygiene in food industry is closely related to sanitation and decontamination intended for devitalisation of disease-carrying and hygienically and technologically undesirable micro-organisms and removal of contaminants and harmful substances. Sanitation is a complex of measures which ensures good hygiene, harmlessness and high quality and safety of products and, at the same time, lengthens their shelf life and decreases economic losses related to processing and transport. Objective evaluation of the level of hygiene and sanitation in food processing premises should be the final step of production procedures, taking into consideration the respective specific conditions.

The aim of the study was to evaluate the effectiveness of disinfectant Topax 66 in the laboratory and fish processing premises using *E. coli* and *S. aureus* as test bacterial strains.

MATERIAL AND METHODS

We tested disinfectant Topax 66, based on active chlorine, intended for foam cleaning in food industry at 2 % concentration, using Standard Collection Strains *E. coli* (CCH 5172), *S. aureus* (CCM 2012) and *B. cereus* (CCM 1999).

Suspension test was used under laboratory conditions (1) and bacterial swabs, taken from 10 cm^2 surface in fish processing section, were examined. Samples of air were collected by sedimentation method using exposure time 3 min. After incubation we recalculated the number of colonies to determine CFU per 1 m^3 according to Para *et al.* (4).

RESULTS AND DISCUSSION

The overall contamination of food processing premises involves to a considerable degree residues of processed, hygienically harmless raw materials which form a layer

Table 1. Bactericidal effectiveness of Topax 66 tested by the suspension test

Strain	Exposure (min)	Concentration (%)				
		0.01	0.1	0.5	1.0	2.0
E. coli	5	+	-	-	-	-
	20	+	-	-	-	-
	60	+	-	-	-	-
S. aureus	5	+	-	-	-	-
	20	+	-	-	-	-
	60	+	-	-	-	-
B. cereus	5	+	+	-	-	-
	20	+	-	-	-	-
	60	+	-	-	-	-

Table 2. Mean CFU.10 cm^{-2} detected on surfaces in the frozen fish section

Swabbed surface	Before disinfection			After disinfection		
	Total bacterial count	E. coli	Moulds	Total bacterial count	E. coli	Moulds
Wall	120	110	60	0	0	0
Ceiling	10	0	5	0	0	0
Floor	290	180	100	6	0	1
Table	3	6	170	13	0	0
Equipment	38	13	81	14	0	0

referred to as biofilm. Biofilm is a nutritional layer, allowing the micro-organisms to multiply, but also a protective layer shielding them against disinfectants (2, 3).

Sanitation in fish processing was carried out with Topax 66 heated to 40 °C. The laboratory testing showed that 0.01 % concentration of Topax 66 had no bactericidal effect on the test bacteria. However, 0.1 % concentration was effective against *B. cereus* at 20 min exposure and against *E. coli* and *S. aureus* at 5 min exposure (Tab. 1).

Table 2 shows the results of examination of bacterial swabs taken from surfaces of technical equipment in the frozen fish section.

Bacteriological control of effectiveness of sanitation is required before the operation and after disinfection. The importance of this control is documented in Table 3 which shows the decrease in CFU on surfaces before operation and after cleaning and disinfection.

Results of air contamination in the section of acidic production, production of fish salads and brining section are presented in Table 4 and indicate considerable effect of the disinfectant particularly on *E. coli*.

The role of sanitation in fulfilling the social tasks set in the field of protection of health of human population

Table 3. Mean CFU.10 cm^{-2} recovered from swabs from the surface of auxiliary equipment in the section of production of fish salads (NC – non-countable)

Swabbed surface	Before disinfection			After disinfection		
	Total bact. count	*E. coli*	Moulds	Total bact. count	*E. coli*	Moulds
Carriage 1	NC	10	12	50	0	1
Carriage 2	NC	5	5	0	10	0
Carriage 3	NC	0	22	70	0	10

Table 4. Mean air contamination (CFU.m^{-3}) in fish processing premises

Place of sampling	Number of samplings	Before disinfection			After disinfection		
		Total bact. count	*E. coli*	Moulds	Total bact. count	*E. coli*	Moulds
Acidic products	1	800	10	440	12	0	32
Salad section	1	40	5	320	20	0	11
Brining	1	20	10	10	10	0	8

as well as extent and complexity of all sanitation measures emphasize the need for its comprehensive assurance. It is inevitable to use effective and at the same time relatively non-expensive disinfectants capable of devitalisation of target micro-organisms at low concentrations without leaving residues and putting unnecessary load on the environment.

REFERENCES

1. AOAC, 1984: *Official Methods of the AOAC*, 14th edn., Virginia.

2. Gracey, J. F., Collins, D. S., 1992: *Meat Hygiene*, 9th ed., Bailiere Tindal, London, 129–130.

3. Hoffmann, I., 2000: Sanitation measures and proper sanitation activities are particularly important in meat processing facilities (In Czech). *Maso*,1, 19–22.

4. Para, Ľ., Ondrašovič, M., Ondrašovičová, O., Kottferová, J., 2000: *Practical Lessons in Animal Hygiene*, Vienale, Košice, 63 pp.

*

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THE POSSIBILITIES OF EFFECTIVE ASSURANCE OF THERMAL COMFORT IN POULTRY HOUSINGS

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ABSTRACT

The paper focuses on potential decrease in heat loss based on pre-warming of air used for ventilation in winter and transitional period in poultry housing. Exploitation of solar energy to heat air in the garret space underneath non-insulated tin roof may, with precise regulation, decrease heat loss by ventilation when using this warmed-up air for ventilation during cold, sunny weather. The model calculations show how to decrease heat loss through pre-warming the ventilation air and decreasing air exchange.

Key words: air heating; efficiency; poultry breeding; thermal comfort

INTRODUCTION

Current economic problems put pressure on breeders to re-evaluate energy efficiency of production. In the interest of environment protection, stress is put on more rapid spreading of energetically effective technologies in individual production areas (1). On large-capacity farms with intensive breeding the air conditioning costs make up 50 % of energy costs (4). Thermal comfort in poultry houses differs for different categories and affects profitability of rearing. Investments into thermal comfort of animals improve health situation in rearing, contribute to higher weight gains and thus guarantee more rapid return on investments (3).

The paper deals with potential heat loss decrease in a poultry house based on use of pre-warmed air (solar radiation) for ventilation in winter and transitional periods.

MATERIAL AND METHODS

The aim of the study was to determine the heat loss by ventilation in dependence on air temperature and intensity of air exchange in a model poultry house.

Requirements on heating were established by STN 73 0540-4, based on thermo-technical properties of structures in a model hall (width 20 m, length 100 m, height 3.5 m). Thickness of external wall was 350 mm and thickness of internal polyurethane panel 40 mm. Roof insulation consisted of was constructed from 200 mm layer of mineral wool with heat conduction coefficient $\lambda = 0.04\ W.m^{-1}K^{-1}$. The roof ridge was 1 300 mm above the ceiling. There house was designed for 20 000 broilers and short-chopped straw was used as bedding ($\lambda = 0.16\ W.m^{-1}K^{-1}$).

The heat input needed for heating the ventilated air Q_V may be determined as follows:

$$Q_v = \rho_L.V_e.c_L.(\theta_i - \theta_e^*),\ (kW)$$

where ρ_L is air density ($1{,}2\ kg.m^{-3}$), V_e is the required flow of ventilating air ($m^3.s^{-1}$), c_L is the specific heat capacity of air ($1.01\ kJ.kg^{-1}.K^{-1}$), θ_i is internal calculation temperature of air in °C, θ_e^* is arbitrary real outdoor temperature of air in °C.

RESULTS AND DISCUSSION

When designing the heating system, it is very important to minimize heat losses, particularly heat losses through structures and ventilation air by means of high quality, heat interchangeable structure. In animal houses it is

necessary to ensure suitable microclimate to provide good quality air and avoid to heat stress.

Heat loss was calculated for the model hall in winter and in transition periods ventilating the house with external air with temperatures from –10 to 15 °C, and pre-warmed air (temperature increased by 5–10 °C) exhausted from the garret by ceiling recirculation flap to the breeding space. The starting internal temperature was the highest recommended temperature for poultry chicks (34 °C in the first week) which then decreased gradually to 18–20 °C by days 35–38 (the end of turn fattening). Total heat loss by ventilation with external air and pre-warmed air at the required air exchange (1–5 times per hour), calculated according to Petráš *et al.* (5), is shown in Fig. 1.

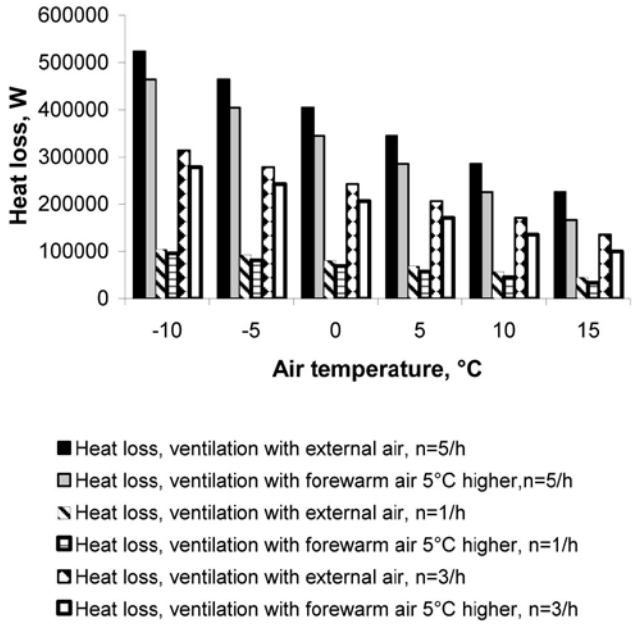

Fig. 1. Heat loss by ventilation with external and pre-warmed air

Microclimate parameters, such as temperature, relative humidity and air flow, as well as number of animals per breeding area, quality of straw bedding, technology system, management of thermal extremes and others, affect significantly the quality of rearing environment and emissions (2). Utilization of solar energy and secondary heating of garret space reduce heat losses of animal houses due to ventilation which must be ensured to guarantee desired air quality also in winter. The calculations showed that the heat loss decreases with increasing temperature of air and decreasing intensity of air exchange (Fig. 1). Although higher air exchange is associated with higher heat losses because of ventilation, the losses are compensated by increasing temperature difference in the garret space. The presented method enables to calculate heat losses also for other objects by means of simple mathematical adaptation.

Results from the present model indicate that effective regulation of the systems of heating and ventilation is very important for optimisation of the ventilation rate and energy consumption. This means that that we proceed with temporary suppression of air exchange intensity only when the instantaneous conditions of solar radiation are unable to preheat sufficiently the ventilation air. Thus it is possible, through high-efficiency heating, cooling and ventilating systems, not only to regulate internal air temperature and modify it according to animal requirements but also to control the total revenues from animal production.

CONCLUSION

Air temperature is an important factor in animal comfort and its stimulation by heating and ventilation systems affects economic results of rearing. Economic management of total input energy requires effective utilization of renewable energy resources which implies also favourable ecological and environmental consequences..

REFERENCES

1. Dalshveen, T., Petráš, D., Hirš, J., 2003: *Energy Audit of Buildings* (In Slovak). Bratislava, Jaga Group, 295 pp.

2. Knížatová, M., Mihina, Š., Karandušovská, I., Orság, J., Šottník, J., 2007: The effect of litter condition on harmful gases production in broiler chicken breeding (In Slovak). In *Environment 2007,* Brno, Institute of Botany of the ASCR and TOCOEN, 40–44.

3. Novák, P., Novák, L., Šlégerová, S., Vokřálová, J., 2003: Thermal insulation properties of poultry breeding houses – health, welfare and economic presuming of breeding (In Czech). In *3rd Conference of Czech Branch of WPSA – 30th Czech Poultry Conference „Poultry 2003*". VETEDUCA Brno, 32–35.

4. Para, Ľ., Ondrašovič, M., Ondrašovičová, O., Vargová, M., Kottferová, J., 2001: Applicability of calculated values in the management of bioclimate in housing of broilers (In Slovak). In *Proceedings Bioclimatological Workshop 2001 – Weather Extremes as a Limiting Factor of Biometeorological Processes.* Račková dolina, Sept. 2001, SAU Nitra, CD, 5 pp.

5. Petráš, D., Kalús, D., Koudelková, D., 2006: *Heating Systems* (In Slovak). Bratislava, SUT, 298 pp.

*

Selected papers from the International Conference "School – science – practice I." held at the University of Veterinary Medicine in Košice on September 24, 2009, on the occasion of the 60th anniversary of its establishment.

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LAMENESS AND MASTITIS AS MAJOR WELFARE PROBLEMS ON SLOVAK DAIRY FARMS

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ABSTRACT

We monitored 18 dairy farms in Slovakia for prevalence of lameness and mastitis which is widely regarded as a major welfare problem. On each farm we chose 50 cows at random and monitored them for mastitis and lameness using SCC as a mastitis indicator. On the same day we took samples for SCC analysis and carried out assessment of lameness. The cows with SCC higher than 200 000 were assumed to have mastitis. The prevalence of lameness was 12–87 % with the mean of 27 ± 15 %. The prevalence of mastitis was 27–58 % the mean level reaching 30 ± 11 %.

Key words: lameness; mastitis; monitoring of farms

INTRODUCTION

Two main production diseases of dairy cows can be observed directly on farms. They are mastitis and laminitis. These two diseases affect animal welfare in general and reduce cow comfort and performance.

Lameness is a disease often observed in loose-housed dairy cattle herds. It can be assessed on the basis of locomotion scoring. Lameness causes big economical losses in dairying (3, 15). It is widely regarded the major welfare problem of dairy cows (7) and its incidence has been included in a number of on-farm animal welfare assessment schemes.

Locomotion scoring is a relatively quick and simple qualitative assessment of the ability of cows to walk normally. Sprecher *et al.* (11) has developed a 5-point lameness scoring system that assesses gait and places a novel emphasis on back posture. If locomotion score is determined regularly (e. g. monthly), it can identify specific cows at risk of becoming clinically lame, which should help to determine the cause of their lameness.

Mastitis or inflammation of the mammary gland is the most common and most expensive disease of dairy cows throughout the world (10). Clinical mastitis is characterized by observable inflammatory changes in the mammary gland, such as heat or swelling, and alterations in the appearance of milk. It means that mastitis is a welfare problem, too. Studies have demonstrated that even mild cases of mastitis resulted in alteration of pain processing. It is therefore important that cases of mastitis are identified and treated rapidly. Mastitis can be judged on the basis of SCC in milk. This parameter is used for milk quality control.

MATERIAL AND METHODS

Our investigations were carried out on 18 dairy farms in Slovakia. On each farm we selected 50 cows at random and assessed them for the following:

- SCC – Somatic cell count in 1 ml of milk
- Lameness

SCC was used as mastitis indicator. The cows with SCC higher than 200 000 were assumed to have mastitis.

In parallel with taking samples for SCC analysis we assessed the cows for lameness using a 5-point system developed by O'Callaghan *et al.* (8) and modified by Thomsen *et al.* **(13).** According to this ordinal lameness scoring system, each

cow was assigned a respective lameness score (13): 1 – Normal; 2 – Uneven gait; 3 – Mild lameness; 4 – Lameness; 5 – Severe lameness.

The locomotion scorings were done before milking in the straight 10–15 meters long and 1.2–1.5 m wide lanes which lead to the milking parlors.

All data were assessed and analyzed using statistical software Statistics v. 8.

RESULTS AND DISCUSSION

Lameness prevalence was 12–87 % with the mean value of 27 ± 17 %. Esslemont and Kossaibati (4) reported 24 % lameness in a DAISY survey of 90 herds in 1992–1993, while in another survey (6), performed on 50 farms during 1995–1996, lameness reached 38 %. The Farm Animal Welfare Council (7) reported that current levels of herd lameness in the UK were unacceptably high. Herd lameness has been estimated at 22 % by recent studies undertaken in the UK (14) and Wisconsin, USA (2). Clarkson (1) performed a survey of 37 dairy farms in Wales to assess the prevalence of lameness in the cows between May 1989 and September 1991. The prevalence of lameness was determined by regular visits at which locomotion was scored on a scale of 1 to 5 and the prevalence of lameness was calculated for each visit as the proportion of cows with scores of 3 or more. The mean annual prevalence over the whole period was 20.6 % and ranged from 2.0 to 53.9 %. The mean prevalence during summer and winter reached 18.6 and 25.0 %, respectively. Our findings of lameness prevalence (12–87 %, with mean value of 27 ± 15 %) are in accordance with these authors.

Prevalence of mastitis in our study was 27–58 % with the mean of 30 ± 11 %. This is in accordance with other authors. According to Ferguson *et al.* (5) mastitis prevalence in Sicily reached 35.4 %, Tenhagen *et al.* (12) reported 26.4 % prevalence in Germany and Pitkälä *et al.* (9) found 30.6 % prevalence in Finland.

REFERENCES

1. Clarkson, M. J., Downham, D. Y., Faull, W. B., Hughes, J. W., Mason, F. J., Merritt, J. B. *et al.*, 1996: Incidence and prevalence of lameness in dairy cattle. *Vet. Rec.*, 138, 563–567.

2. Cook, N. B., 2003: Prevalence of lameness among dairy cattle in Wisconsin as a function of housing type and stall surface. *J. Am. Vet. Med. Assoc.*, 223, 1324–1328.

3. Enting, H., Kooij, D., Dijkhuizen, A. A., Huirne, R. B. M., Noordhuizen-Stassen, E. N., 1997: Economic losses due to clinical lameness in dairy cattle. *Livestock Prod. Sci.*, 49, 259–267.

4. Esslemont, R. J., Kossaibati, M. A., 1996: Incidence of production diseases and other health problems in a group of dairy herds in England. *Vet. Rec.,* 139, 486–490.

5. Ferguson, J. D., Azzaro, G., Gambina, M., Licitra, G., 2007: Prevalence of mastitis pathogens in Ragusa, Sicily, from 2000 to 2006. *J. Dairy Sci.*, 90, 5798–5813.

6. Kossaibati, M. A., Esslemont, R. J., 1999: The incidence of lameness in a group of dairy herds in England. In *Proceedings of British Society for Animal Science*, 221 pp.

7. Farm Animal Welfare Council, 1997: *Report on the Welfare of Dairy cattle.* The Farm Animal Welfare Council, London.

8. O'Callaghan, K. A., Cripps, P. J., Downham, D. Y., Murray, R. D., 2003: Subjective and objective assessment of pain and discomfort due to lameness in dairy cattle. *Animal Welfare*, 12, 605–610.

9. Pitkälä, A., Haveri, M., Pyörälä, S., Myllys, V., Honkanen-Buzalski, T., 2004: Bovine mastitis in Finland 2001. Prevalence, distribution of bacteria, and antimicrobial resistance. *J. Dairy Sci.*, 87, 2433–2441.

10. Rajala, P. J., Grohn, Y. T., 1998: Disease occurrence and risk factors analysis in Finnish Ayrshire cows. *Acta Vet. Scand.*, 39, 1–13.

11. Sprecher, D. J., Hosteler, D. E., Kaneene, J. B., 1997: A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. *Theriogenology*, 47, 1179–1187.

12. Tenhagen, B. A., Köster, G., Wallmann, J., Heuwieser, W., 2006: Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. *J. Dairy Sci.,* 89, 2542–2551.

13. Thomsen, P. T., Munksgaard, L., Togersen, F. A., 2008: Evaluation of a lameness scoring system for dairy cows. *J. Dairy Sci.*, 91, 119–126.

14. Whay, H., 2002: Locomotion scoring and lameness detection in dairy cattle. *Practice,* 24, 444–449.

15. Whitaker, D. A., Kelly, J. M., Smith, E. J., 1983: Incidence of lameness in dairy cows. *Vet. Rec.,* 113, 60–62.

*

Selected papers from the International Conference "School – science – practice I." held at the University of Veterinary Medicine in Košice on September 24, 2009, on the occasion of the 60th anniversary of its establishment.

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FACTORS AFFECTING SAFETY AND QUALITY OF GAME MEAT FROM THE CONSUMER'S POINT OF VIEW

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ABSTRACT

Various factors (environmental, microbiological, and chemical) affecting the safety and quality of game meat are characterised in this study. The emphasis is given on the chemical elements, mycotoxins, veterinary drug residues and biogenic amines. The post-mortem changes in game meat during handling and storage are evaluated from the consumer's point of view. This review summarises the most important results obtained within the project Safety and quality of wild game meat and farmed game meat.

Key words: consumer; game meat; quality; risk analysis; safety

INRODUCTION

Game meat as a food for humans is produced in an environment with a great variability of external effects (14). Owing to increased level of risk chemical elements, wild game can serve as a bioindicator of environmental pollution (1, 5). Due to control of parasitic diseases, e.g. coccidiosis, meat from farmed game must be monitored for drug residues (4, 6). Farmed game can be contaminated also by mycotoxins through low quality feed (9). Detailed knowledge of meat ripening process is required for safety and quality of game meat production. Lactic acid level reflects quantitative transformation of glycogen and indicates typical or atypical processes of meat ripening. Inadequate handling or storage conditions can influence oxidation of lipids and production of free radicals and result in decreased quality of game meat (10). Microbiological contamination can also affect safety and quality of the product.

MATERIAL AND METHODS

Liver and muscles of wild (hares) and farmed game (rabbits, pheasants) were examined for the level of Cd, Pb, Ni, Cu and Zn by atomic absorption spectrophotometry, Aflatoxin B_1 and Ochratoxin A by thin layer chromatography, coccidiostats by Premi®Test and STAR methods, lactic acid by electrophoretic analyser and oxidative changes in fat by spectrophotometry. Biogenic amines were determined by amino acids analyser and bacteriological examination involved plate counts of family *Enterobacteriacae*, *Pseudomonas* spp. and *Salmonella* spp.

RESULTS AND DISCUSSION

Minimum differences were observed between mean levels of Cd in breast and thigh muscle of shot (7) and killed (6) pheasants. The highest mean levels of Cd were recorded in the liver of shot pheasants (0.04 $mg.kg^{-1}$). Significantly higher (0.85 $mg.kg^{-1}$) levels of Pb ($P \leq 0.05$) were found in breast muscle of shot compared to killed pheasants (0.07 $mg.kg^{-1}$), slightly higher in thigh muscle while liver levels showed minimum differences. Ni level (0.55 $mg.kg^{-1}$) was significantly higher ($P \leq 0.05$) in breast muscle of shot compared to killed pheasants (0.22 $mg.kg^{-1}$). The results indicated that shooting had a great impact on Pb and Ni but Cd accumulation was related to local environmental pollution (7). Increased levels of Zn and Cu were observed in thigh compared to breast muscles of pheasants (15). The highest levels of Zn and Cu were in the liver of pheasants (221.14 and 15.22 $mg.kg^{-1}$, resp.). The

effect of essential elements on bonding of toxic elements to metalothionein is not clearly understood regarding the kinetics. Knowledge of these interactions is useful for evaluation of higher levels of essential elements in regions with industrial pollution. Dynamics of lactic acid during 16 days of storage of hare meat at 4 °C showed a peak on day 3 in back muscle. Significant decrease was observed on day 9 in leg ($P \leq 0.01$), shoulder ($P \leq 0.05$) and back muscles ($P \leq 0.001$) (8). Tissue analysis of coccidiostats on day 0 of the withdrawal period (WP) and day 1 after WP showed significantly ($P \leq 0.001$) higher level in broiler chickens compared to pheasants. This indicates the need for re-evaluation of WP which is the same for both bird species (12). Analysis of 10 rabbits, pheasants and fodder detected ochratoxin (2–50 $\mu g.kg^{-1}$) in fodder and the liver of rabbits and pheasants. Aflatoxins were not detected (2). Higher microbial contamination was found in thigh compared to breast muscles of shot, uneviscerated pheasants (13). *Enterobacteriaceae c*ounts were significantly higher ($P \leq 0.01$). High counts of all bacteria were detected in thigh muscles of frozen pheasants (-18 °C) most likely due to handling before freezing. The highest changes in lipids were observed in hunted uneviscerated pheasants and the lowest in frozen pheasants. The extent of deterioration of game meat lipids can be influenced by handling after hunting and conditions of storage (10). The highest levels of biogenic amines were detected in leg muscle of hunted uneviscerated pheasants and were not affected by the length of storage (3).

In conclusion, the improvement of knowledge of game meat ripening process can help to identify optimum handling and storage conditions, inhibit adverse microbial and chemical changes in meat and contribute to quality and safety of game meat.

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REFERENCES

1. Almášiová, V., Cigánková, V., Holovská, K., 2008: Spermatogenesis of Japanese quails. In *Proceedings Ecology and Veterinary Medicine,* Košice, 96–100.

2. Cicoňová, P., Laciaková, A., Máté, D., Koréneková, B., 2009: Occurrence of aflatoxins and ochratoxins in hares and rabbits. In *Proceedings Hygiena Alimentorum,* The High Tatras, 309–310.

3. Dičáková, Z., Sokol, J. 2008: Analysis of biogenic amines by using of ion chromatography in meat of pheasants. In *Proceedings Analytical Methods and Human Health,* Nový Smokovec, 1–4.

4. Goldová, M., Letková, V., Čurlík, J., Kočišová, A., Soroka, J., Miško, J., 2004: Endoparasites of farm breeding pheasants and partridges. In *Proceedings Wild Fauna,* The High Tatras, 31–33.

5. Kimáková, T., 2000: Mercury content in muscle of various species of animals. *Slov. Vet. J.,* 4, 213–216.

6. Kočišová, A., Lazar, P., Letková, V., Čurlík, J., 2008: Occurrence and importance of ratite game in Slovakia. In *Czech and Slovak Parasitological Days,* Sezimovo Ústí, 42–43.

7. Koréneková, B., Skalická, M., Kožárová, I., Nagy, J., Máté, D., Naď, P., 2008: Comparison of Cd, Pb, Ni accumulation in liver, breast, leg muscles of shot and killed pheasants. *Slov. J. Anim. Sci.*, 41, 184–186.

8. Koréneková, B., Nagy, J., Mačanga, J., Paulsen, P., 2009: Comparison levels of lactic acid during ripening process of hare's meat. In *Proceedings Hygiena Alimentorum,* The High Tatras, 350–351.

9. Laciaková, A., Dičáková, Z., Pipová, M., Laciak, V., Pastorová, B., 2006: Occurrence of toxigenic micromycetes, mycotoxins in food. In. *Proceedings Biotechnology,* České Budějovice, 278–280.

10. Marcinčák, S., Sokol, J., Bystrický, P., Popelka, P., Turek, P., Bhide, M., Máté, D., 2004: Determination of lipid oxidation level in broiler meat by lipid chromatography. *J. AOAC Int.*, 87, 1148–1152.

11. Marcinčák, S., Koréneková, B., Nagy, J., Mačanga, J., 2009: Deterioration changes of lipids during storing of pheasants. In *Proceedings Hygiena Alimentorum*, The High Tatras, 339–334.

12. Mačanga, J., Kožárová, I., Goldová, M., Major, P., Koréneková, B., 2009: Statistical evaluation of occurrence of anticoccidial residues in tissues of chickens and pheasants. In *Proceedings Hygiena Alimentorum,* The High Tatras, 290–292.

13. Pipová, M., Jevinová, P., Soroka, J., Nagy, J., Sütőová, I., 2009: Changes of micro flora in breast and leg muscle of pheasants during chilling storing. In *Proceedings Hygiena Alimentorum,* The High Tatras, 275–277.

14. Sasáková, N., Vargová, M., Ondrašovičová, O., Ondrašovič, M., Kottferová, J., Skalická, M., 2006: Environmental pollution and health risk related to metals in the solid fraction and effluent from waste water treatment. *Bull. Environ. Cont. Toxicol,* 76, 671–676.

15. Skalická, M., Koréneková, B., Kožárová, I., 2009: Observation of Cu and Zn levels in tissues of pheasants. In *Proceedings Hygiena Alimentorum,* The High Tatras, 302–304.

*

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DETECTION OF SULPHAMETHAZINE RESIDUES IN THE MUSCLES AND LIVER OF RABBITS BY HPLC METHOD

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ABSTRACT

Sulphamethazine (SMZ) residues in the muscles and liver of rabbits up to day 15 of the withdrawal period (WP) were identified and quantified by HPLC method. The minimal residual concentration of SMZ in the liver was detected on day 12 of the WP (0.09 mg.kg^{-1}) and in the muscles on day 10 of the WP (0.02 mg.kg^{-1}). These levels were below the established maximum residue limit (MRL) of 0.1 mg.kg^{-1}.

Key words: HPLC analysis; rabbit tissues; residues; sulphonamides

INTRODUCTION

Sulphonamides (SA) are the oldest group of antimicrobial agents and belong among the first synthetic substances used in medicine. They inhibit bacteria, chlamydia, toxoplasma and other protozoan agents, especially coccidian, in poultry, rabbits and other economically important animal species. SAs are effective against many respiratory and gastrointestinal infections, mastitis, septiceamia, peritonitis, etc. (5, 7).

Administration of these substances to food-producing animals raises risk of transfer of their residues to the food chain. Food originating from animals treated with veterinary drugs for therapeutic, prophylactic, or subtherapeutic purposes are not allowed to contain residues that present risk to consumer's health (4, 6). Therefore SAs are included among substances with valid maximum residue limit. Current legislation (2) establishes the MRL for SAs (all compounds of the SA group) in foods of animal origin at the level of 0.1 mg kg^{-1}.

High performance liquid chromatography (HPLC) is generally used in food analysis as a confirmatory method preferred to immunological or microbial inhibition screening tests for the determination of additives, contaminants and natural compounds (1).

MATERIAL AND METHODS

Test material. Sixteen 16-weeks-old meat line HY+ rabbits (farm Hylapa s.r.o., SR), weighing 2.60–2.90 kg were used in the experiment. SMZ (Sulfadimidin PG plv. sol. a. u. v., Pharmagal, SR) was administrated in the drinking water for 3 consecutive days at a dose of 2 g.l^{-1} of water. After withdrawal of the drug, one rabbit per day was slaughtered up to day 15 of the WP. Individual bagged samples of muscle and liver were stored until analysis at –18 °C. Six untreated rabbits served as SMZ-free control (negative control).

HPLC analysis. SMZ residues in the muscle and liver of rabbits were detected in compliance with the method approved for screening of animal products for veterinary drug residues in Slovakia (3). All solvents and reagents were analytical or HPLC grade. Samples were analysed by a TSP liquid chromatograph (Thermo Separation Products, USA) with a variable UV-VIS detector, UV 3000 HR (Hewlett-Packard, USA). The separation was performed on a LiChroCART RP-18e column (125–4 (5 µm)) (Merck, Germany) using acetonitrile/acetate buffer (pH 4.6) (25 : 75, v/v) as a mobile phase at a flow-rate of 0.8 ml.min^{-1} at +40 °C and volume of extract 25 µl. The detection was conducted at 275 nm.

RESULTS AND DISCUSSION

The limit of detection (LOD) of the method for SMZ was 0.004 mg.kg^{-1} with a respective limit of quantification (LOQ) of 0.010 mg.kg^{-1}. Samples from negative control with the addition of SMZ standard at the concentration of 0.3–3 mg.kg^{-1} were used to determine recovery of the method (83–88 %). The SMZ residues were detected with a UV-VIS detector at 275 nm at retention times between 6.958 and 7.731 min.

Table 1 shows the results of residual concentrations of SMZ in the liver and muscles of rabbits during 15 days of WP. The decrease in residual concentrations of SMZ in both tissues was gradual and very slow. On the 1st day of the WP the mean residual level of SMZ was 0.56 mg.kg^{-1} in the liver and 0.42 mg.kg^{-1} in muscles. SMZ residues were detectable up to day 12 of the WP at the concentration of 0.09 mg.kg^{-1} in the liver and up to day 10 of the WP at the concentration of 0.02 mg.kg^{-1} in the muscles. SMZ residues were below the LOD of the method on day 11 of the WP in the muscles and on day 13 of the WP in the liver. No residues of SMZ were found in muscles and liver obtained from the SMZ-free control group.

Table 1. SMZ residual levels (mg.kg^{-1} ± SD) in rabbit muscle and liver after oral administration up to day 15 of the withdrawal period detected by HPLC

Day of WP	Concentration of SMZ ± SD (mg.kg^{-1})	
	Liver	Muscle
1	0.56 ± 0.021	0.42 ± 0.046
2	0.49 ± 0.032	0.38 ± 0.011
3	0.41 ± 0.017	0.33 ± 0.033
4	0.39 ± 0.009	0.33 ± 0.015
5	0.39 ± 0.011	0.27 ± 0.007
6	0.31 ± 0.013	0.25 ± 0.004
7	0.27 ± 0.015	0.21 ± 0.025
8	0.25 ± 0.008	0.18 ± 0.016
9	0.21 ± 0.050	0.15 ± 0.002
10	0.21 ± 0.070	**0.02 ± 0.004**
11	0.19 ± 0.006	ND
12	**0.09 ± 0.003**	ND
13–15	ND	ND

Bold numbers – days of WP when the lowest SMZ residual levels were reached; ND – not detectable

Wang *et al.* (8) reviewed several screening and confirmatory methods for determination of SAs in edible animal products published by different authors. According to this review HPLC has been the technique most frequently used for detection of SA residues in pork, beef, poultry and milk. Authors recommend HPLC for confirmation but not for screening of large numbers of samples because of time consuming and expensive preparation of samples and use of toxic solvents.

Marcinčák *et al.* (6) detected SMZ residues on the first day of the WP at the level of 37.22 ± 3.42 mg.kg^{-1} in muscles and 8.116 ± 0.75 mg.kg^{-1} in the liver of laying hens. On day 6 of the WP the concentration of SMZ residues was 0.043 ± 0.008 mg.kg^{-1} in the muscles and 0.099 ± 0.009 mg.kg^{-1} in the liver.

Our results demonstrate that HPLC methods detected SMZ residues in both examined matrices at levels below the established MRL. Based upon these results HPLC appears to be a suitable confirmatory method for the detection of SMZ residues in animal products.

REFERENCES

1. Bovanová, L., Brandšteterová, E., 2000: Direct analysis of food samples by high-performance liquid chromatography. *J. Chromatogr. A*, 880, 149–168.

2. EC. 1990. Council Regulation (EEC) No. 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. *Off. J. Eur. Commun. L.*, 224, 1–8.

3. CH 6. 2. 2004. Determination of sulphonamides by HPCL method. In List of official methods for laboratory diagnostics of food and feed. *Bulletin of the Ministry of Agriculture of the Slovak Republic*, 36, 97–99.

4. Kožárová, I., Janošová, J., Máté, D., Pipová, M., Jevinová, P., 2005: A utilization of para-aminobenzoic acid (PABA) for the presumptive identification of sulphadimidine at its residue screening by using the microbiological Four-plate test. *Bull. Vet. Inst. Pulawy*, 49, 59–64.

5. Kriška, M., Božeková, L., 2000: Sulphonamides. In *Pharmacology and Clinical pharmacology of Antimicrobial Substances,* **Kriška, M., Božeková, L. *et. al.***, Comenius University in Bratislava. Asklepios, 69–73.

6. Marcinčák, S., Hussein, K., Zdolec, N., Janošová, J., 2006: Premi®Test – fast screening test for detection of sulphadimidine residues in poultry tissues. *Maso*, 5, 276–279.

7. Prescott, J. F., Baggot, J. D., 1993: *Antimicrobial Therapy in Veterinary Medicine*, (2nd ed.), Ames, Iowa State University Press, 564–565.

8. Wang, S., Zhang, L., Wang, L., Duan, Z. J., Kennedy, I., 2006: Analysis of sulphonamides residues in edible animal products: A review. *Food Addit. Contam.*, 23, 362–384.

*

Selected papers from the International Conference "School – science – practice I." held at the University of Veterinary Medicine in Košice on September 24, 2009, on the occasion of the 60th anniversary of its establishment.

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THE RIPENING PROCESS IN HUNTED AND EVISCERATED PHEASANTS (*Phasianus Colchicus*)

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ABSTRACT

The aim of our study was to observe the dynamics of lactic acid and phosphoric acid by capillary electrophoresis and changes in pH in the muscle of pheasants (*Phasianus colchicus*) in the monitored muscles during the ripening process. We detected no marked changes in pH during the 14 days of ripening. Dynamics of both mentioned acids in the monitored muscles was similar. Their concentrations increased till day 7 and subsequently decreased till day 14. The lactic acid level was significantly higher in the breast muscle throughout the ripening process (day 1 – $P \leq 0.05$; day 7 – $P \leq 0.001$; day 14 – $P \leq 0.001$) while the level of phosphoric acid was significantly higher in the breast muscle only on day 7 ($P \leq 0.05$).

Key words: lactic acid; pheasant; phosphoric acid; pH; ripening process

INTRODUCTION

Ripening process is a term used to denote a complex of biochemical changes in almost all parts of meat resulting in characteristic organoleptic features (3). The result of these changes is formation of organic acids which decrease pH (5). In our study we monitored the levels of lactic acid, phosphoric acid and pH during the ripening process which influence the final quality of game meat.

MATERIALS AND METHODS

Hunted and eviscerated pheasants (n = 9) from Rozhanovce were divided to 3 groups, 3 pheasants in each, and stored until analysis in a refrigerator at 4 °C. From each group we sampled breast and thigh muscles (1st group at 24 hours, 2nd on day 7 and 3rd on day 14 after slaughter). Ten grams of muscle were homogenized and the monitored acids were extracted with water. The pH of the extract was measured with a pH meter InoLab WTW 720. The samples were diluted 100-fold and analysed by an Electophoretic analyser EA102 with conducting detector (Villa Labeco, SR). The leading electrolyte consisted of 10 mM HCL, β-alanin and 0.1 % mHEC and the terminating electrolyte of 5 mM caproic acid and 5 mM TRIS. Results were evaluated by software ITPPro 32. All results of acid concentration were expressed in $g.100g^{-1}$ of sample.

RESULTS AND DISCUSSION

Dynamics of lactic acid in pheasant meat during 14-day ripening process is presented in Fig. 1. The level of lactic acid in breast muscle 24 h after slaughter reached 1.181 ± 0.327. Within 7 days of ripening it increased significantly ($P \leq 0.001$; 1.930 ± 0.280) and, subsequently, showed a significant decrease by day 14 ($P \leq 0.001$; 0.842 ± 0.116).

Dynamic of lactic acid in thigh muscle was the same as in breast muscle. Its level at 24-h after slaughter reached 0.817 ± 0.178, on day 7 was increased significantly ($P \leq 0.001$; 1.248 ± 0.154) and by day 14 decreased to

Fig. 1. Dynamic of lactic acid during the ripening process

Fig. 2. Dynamic of phosphoric acid during the ripening process

0.428 ± 0.113 ($P \leq 0.001$). Dynamics of lactic acid level during ripening reflects a quantitative conversion of glycogen to lactic acid (2).

Throughout the ripening, the level of lactic acid in breast was significantly higher than in the thigh muscle (day 1 – $P \leq 0.05$; day 7 – $P \leq 0.001$; day 14 – $P \leq 0.001$). The differences can be explained by the fact that breast muscle contains larger quantity of glycogen (1).

The concentration of phosphoric acid in breast (0.670 ± 0.098) and thigh muscles (0.647 ± 0.095) was almost the same (Fig. 2.). By day 7 we observed an increase in phosphoric acid (breast 1.038 ± 0.103, $P \leq 0.001$; thigh 0.897 ± 0.122, $P \leq 0.01$) and by day 14 a significant reduction in both breast and thigh muscles ($P \leq 0.001$) (breast 0.686 ± 0.079; thigh 0.596 ± 0.100). Phosphoric acid in muscles arises from energy-rich phosphates in the process of degradation of glycogen to lactic acid (6). It is possible that its content depends on the amount of glycogen before slaughter.

No significant changes in pH were observed during the ripening (Table 1). On the other hand, differences between the muscles were significant ($P \leq 0.001$) and pH in thigh muscles was higher than in breast muscles throughout the monitored period. Our results are comparable with the results of Paulsen *et al.* (4).

Table 1. Level of pH in breast and thigh muscles during the ripening

Muscle	Day 1	Day 7	Day 14
Breast	5.70 ± 0.12	5.75 ± 0.02	5.74 ± 0.01
Thigh	6.43 ± 0.06	6.42 ± 0.16	6.37 ± 0.05

During the ripening process the muscle is subjected to biochemical changes and related changes in meat. There is a degradation of glycogen and energy-rich phosphates. This results in production of lactic acid and phosphoric acid which is related to the change in pH, improvement in organoleptic and technological properties of meat and some protection against micro-organisms.

Our results allowed us to conclude that the dynamics of both acids during the ripening process had a typical course. The pH was not changed significantly. This may be caused by the fact that muscle of pheasants contains relatively low level of glycogen. The quantity of lactic acid, which affects the pH to the greatest extent, was probably unable to produce significant pH changes.

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REFERENCES

1. Balsyte, G., Turek, P., Nagy, J., Cabadaj, R., 1998: Effect of temperature on Dynamics of lactic acid in poultry meat (In Slovak). In *Proceedings Hygiena alimentorum XIX*., Košice, Oct. 1998, 25–26.

2. Kopřiva, V., Steinhauser, L., Matyáš, Z., 1992: Evaluation of course of ripening process of meat (In Czech). *Veterinářství*, 9, 348–349.

3. Kopřiva, V., Steinhauser, L., 1992: Development of ripening process of vacuum packed meat (In Czech). *Průmysl potravin*, 1, 13–14.

4. Paulsen, P., Nagy, J., Popelka, P., Ledecký, V., Marcinčák, S., Pipová, M. *et al.,* 2008: Influence of storage conditions and shotshell wounding on the hygienic condition of hunted, uneviscerated pheasant (*Phasianus colchicus*). *Poult. Sci.*, 87, 191–195.

5. Šimek, J., Vorlová, L., Steinhauser, L., 2002: Variations of meat quality and their identification (In Czech). *Maso*, 4, 24–27.

6. Winkelmayer, R., Lebersorger, P., Zedka, H. F., Forejtek, P., Vodňanský, M., Večerek, V. *et al.*, 2005: *Hygiene of Venison* (In Czech). Central European Institute of Animal Ecology Wien-Brno-Nitra, 168 pp.

*

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DETECTION OF SUSCEPTIBILITY TO ANTIBIOTICS IN ISOLATES OF STAPHYLOCOCCI BY AGAR DILUTION METHOD

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ABSTRACT

Sixty-six strains of staphylococci were isolated in accordance with the requirements of STN EN ISO 6888-1 from the muscles of 9 rabbits (*Oryctolagus cuniculus)* and 4 herrings (*Clupea harengus)*. Among them, 66 isolates were susceptible to ampicilin. On the other hand, majority of isolates showed resistance to vancomycin (66 strains) and tetracycline (61 strains). Moreover, simultaneous resistance to two antibiotics was detected in 23 isolates of staphylococci.

Key words: agar dilution method; antibiotics; antimicrobial resistance; fish; rabbits; staphylococci

INTRODUCTION

A regular increase in antibiotic resistance is recorded every year in many bacterial species, including representatives of the genus *Staphylococcus.* One of the major routes of transmission of bacterial antibiotic resistance among human and animal population is the food chain. Bacteria resistant to certain antibiotics enter with food the digestive tract of the consumer, where this property can be transferred to other, previously susceptible bacteria.

Therefore, the aim of this study was to determine the susceptibility of coagulase-negative isolates of staphylococci to five antibiotics (erythromycin, tetracycline, ampicillin, gentamicin and vancomycin) using agar dilution method.

MATERIAL AND METHODS

Samples for microbiological examination were taken from muscles of 9 rabbits (*Oryctolagus cuniculus*) from the farm in Rozhanovce and from 4 herring fillets (*Clupea harengus*) originating from Norway. Sampling complied with the requirements of ISO 3100-2 (4), preparation of the basic suspension and further decimal dilutions followed the standard procedure set by ISO 6887-1 (5). Staphylococci were isolated from the samples according to ISO 6888-1 guidelines (6), the isolates were further tested for their ability to produce plasmocoagulase (STAFYLO PK, IMUNA, Šarišské Michaľany). The susceptibility of different isolates of staphylococci to selected antibiotics was determined by agar dilution method using the test plates with the following final concentration of antibiotics: erythromycin 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 $mg.l^{-1}$; tetracycline 2.0, 4.0, 8.0, 16.0 $mg.l^{-1}$; ampicillin 0.25, 0, 5, 1.0 $mg.l^{-1}$; gentamicin 2.0, 4.0, 8.0, 16.0 $mg.l^{-1}$ and vancomycin 2.0, 4.0, 8.0, 16.0, 32.0 $mg.l^{-1}$. Susceptibility, moderate susceptibility or resistance of individual isolates of staphylococci were evaluated according to the criteria set by CLSI (2005) (1).

RESULTS AND DISCUSSION

Sixty-six strains of staphylococci were obtained after the cultivation of samples on selective agar media. All staphylococci isolates were coagulase-negative.

Resistance to tetracycline and vancomycin was confirmed by agar dilution method in 40 isolates and resistance to erythromycin in one isolate from rabbits

(Tab. 1). Resistance to gentamicin and ampicillin was not determined in any isolate tested. Moderate susceptibility to erythromycin was confirmed in 39 strains and to gentamicin in 25 strains of staphylococci isolated from rabbit meat.

Among 26 isolates of staphylococci from herring fillets, the resistance to vancomycin was confirmed in 26 strains, to tetracycline in 21 strains and to gentamicin in 2 strains. Resistance to erythromycin and ampicillin was not detected in any isolate of staphylococci.

Moderate susceptibility to erythromycin was confirmed in 26 strains, to gentamicin in 16 strains and to tetracycline in 5 strains isolated from fillets of herring (Tab. 1).

Table 1. Susceptibility (S), moderate susceptibility (I) and resistance (R) of staphylococci isolates from rabbits and herring fillets to selected antibiotics

Antibiotics	*Oryctolagus cuniculus*			*Clupea harengus*		
	R	I	S	R	I	S
Tetracycline	40	0	0	21	5	0
Vancomycin	40	0	0	26	0	0
Erythromycin	1	39	0	0	26	0
Ampicillin	0	0	40	0	0	26
Gentamicin	0	25	15	2	16	8

The final evaluation of 66 staphylococcal isolates from rabbits and fillets of herring confirmed resistance to vancomycin (100 %) and to tetracycline (92 %). On the other hand, the highest susceptibility among staphylococcal isolates was observed to tetracycline (100 %). Furthermore, moderate susceptibility to gentamicin, erythromycin and tetracycline was also confirmed among isolates of staphylococci. Simultaneous resistance to two antibiotics was observed in 23 isolates of the staphylococci tested.

Similar results have already been published by several authors. Based on the high level of antimicrobial resistance of staphylococci, isolated from rabbits and fillets of herring in this study, it is recommended to test other staphylococcal isolates from different kinds of food in order to protect the consumer's health.

Shitandi and Mwangi (3) tested 216 isolates of *Staphylococcus aureus* from milk samples collected from different areas in Kenya. The study confirmed resistance to penicillin (72.2 %), sulphametazin + trimethoprim (59.2 %), tetracycline (57.9 %), erythromycin (21.3 %), chloramphenicol (46.8 %) and methicillin (7.8 %).

Another study published by Kaszanytzky *et al.* (2) was performed in 2001 in Hungary on 806 staphylococcal isolates from humans, food and different animal species. Authors reported that of 428 staphylococcal isolates from foods of animal origin 7 % showed resistance to erythromycin and 13 % to tetracycline.

CONCLUSION

The results of this study confirmed that the resistance of staphylococci to antibiotics is still a current problem. To prevent its further spread it is necessary to ensure rational use of antibiotics as well as permanent and detailed monitoring of antibiotic resistance.

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REFERENCES

1. CLSI (Clinical and Laboratory Standards Institute), 2006: *Performance Standards for Antimicrobial Susceptibility Testing.* 16th Informational Supplement. CLSI document M100-S16. Wayne, PA, USA, 92 pp.

2. Kaszanyitzky, E. J., Jánosi, S. Z., Egyed, Z. S., Ágost, G., Semjén, G., 2001: Antibiotic resistance of staphylococci from humans, food and different animal species according to data of the hungarian resistance monitoring system in 2001. *Acta Vet. Hung.*, 51, 451–464.

3. Shitandi, A., Mwangi, M., 2004: Occurrence of multiple antimicrobial resistance among *Staphylococcus aureus* isolates from Kenyan milk. *Journal of Food Technology in Africa*, 9, 23–25.

4. STN ISO 3100-2, 1999: Meat and meat products. Sampling and preparation of test samples. Part 2: Preparation of test samples for microbiological examination (In Slovak). SÚTN.

5. STN EN ISO 6887-1, 2001: Microbiology of food and animal feeding stuffs. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 1: General rules for the preparation of the initial suspension and decimal dilutions (In Slovak). SÚTN.

6. STN EN ISO 6888-1, 2001: Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 1: Technique using Baird-Parker agar medium (In Slovak). SÚTN.

*

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CONTROL OF NUTRITION AND ENERGY METABOLISM IN COWS IN THE TRANSITION PHASE OF NUTRITION

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ABSTRACT

The results obtained indicated decreased quality and nutritional value of bulk feed harvested in late vegetation phase. Increased proportion of concentrate in feed rations was manifested by acidification of the rumen and tendency to decreased fat content in milk. Nutritional prevention in herds may be achieved through increase in dry matte intake by bulk feed that should stabilize the rumen and intermediary metabolism of dairy cows.

Key words: forage quality; NDF; NFC; rumen fermentation

INTRODUCTION

Profile of rumen fermentation of carbohydrates is important for diagnostics of rumen function in relation to nutritional level of dairy cows. The quantity and ratio of metabolites of rumen fermentation of carbohydrates reflects the response of rumen microflora to the diet regarding the quantity and ratio of fibrous and non-fibrous carbohydrates in TMR in the pre-partum phase of dairy cow nutrition (3). Evaluation of relationships and interactions of diet nutrients, level of rumen fermentation and influence on markers of internal energy metabolism allows one to evaluate the intake and control of metabolism of nutrients in the phase of negative energy balance and to determine nutrition strategy focused on prevention of metabolic load and on proper management of productive health of high-yield animals.

MATERIAL AND METHODS

Twenty herds of dairy cows with mean milk yield of 7–9 thousand litres were investigated under breeding conditions to validate the analytical and software capacity of optimization of carbohydrate composition of rations in relation to biological evaluation of animal response focusing on the following relationships: nutrition → rumen fermentation → blood metabolism → production and health and evaluation of the respective feedback.

RESULTS

Evaluation of energy metabolism in investigated herds using markers of rations, rumen fermentation and blood metabolites provided characteristic metabolic relations for individual transient phases at the current system of nutrition and rearing of dairy cows:

The phase of preparation for parturition – the relationships obtained are summarised in Table 1. Analysis of feed rations showed a bulk character of TMR with increased values of neutral-detergent fibres (NDF) in 60 % of the herd indicating that both the mean 41.1 ± 6.3 % and marginal values of non-fibrous carbohydrates (NFC) 35.5 ± 4.1 % could not ensure sufficient supply of energy in the pre-partum phase.

Table 1. Level of energy metabolism in dairy cows 21 days before parturition

Parameter			Reference values	Mean ± SD	Individual %	
					Increased	Decreased
Number of herds				20		
TMR	NDF	%	32.0–38.0	41.1 ± 6.3	60	30
	NFC	%	30.0–35.0	35.5 ± 4.1	45	15
Number of animals				121		
Rumen	pH		6.4–6.6	6.58 ± 0.33	41	22
	VFA	mmol.l^{-1}	80–130	102.8 ± 22.4	15	10
	Acetic acid	%	60.0–65.0	66.7 ± 3.6	57	2
	Propionic acid	%	20.0–25.0	20.8 ± 3.0	2	38
	$C_2 : C_3$		2.5–3.0 : 1	3.3 ± 0.7	53	4
Blood	Glucose	mmol.l^{-1}	2.0–4.1	3.24 ± 0.6	7	3
Serum	NEFA	mmol.l^{-1}	< 0.35	0.34 ± 0.1	30	-
	AST	µkat.l^{-1}	0.22–0.5	0.47 ± 0.2	40	-
	Bilirubin	µmol.l^{-1}	0.17–5.13	3.49 ± 2.0	13	-

Rumen fermentation with the mean values of pH 6.58 ± 0.33 was at the marginal level. The sum of volatile fatty acids (VFA) reached on average the medium level of 102.8 ± 22.4 mmol.l^{-1}. Increased relative proportion of acetic acid (C_2) with mean value of 66.7 ± 3.6 % and mean level of propionic acid (C_3) close to the lower limit (20.8 ± 3.0 %) confirmed that the character of fermentation corresponded to the bulk-type rations with low degree of adaptation of rumen fermentation in the phase of preparation for parturition.

Energy metabolism – internal environment with mean values of blood glucose was 3.24 ± 0.6 mmol.l^{-1}. The mean level of non-esterified fatty acids (NEFA) was 0.34 ± 0.1 mmol.l^{-1} as a manifestation of lipomobilisation. The mean value of AST was close to the upper limit and reached 0.47 ± 0.2 µkat.l^{-1} and the mean level of bilirubin was 3.49 ± 2.0 µmol.l^{-1}.

The postpartum phase – the relationships are summarised in Table 2. The sudden change to the concentrate type of TMR with increased mean level of NFC reaching 38.2 ± 3.7 % and low level of adaptation of rumen fermentation before calving resulted in a decrease in mean rumen pH to 6.22 ± 0.41. The system of feeding after calving was accompanied with increased mean level of VFA equal to 117.6 ± 18.8 mmol.l^{-1} and individually increased levels due to reduced absorption of VFA. The relative proportion of acetic acid (C_2) reached mean value of 63.0 ± 4.5 %. The mean proportion of propionic acid (C_3), close to the upper limit (24.4 ± 3.9 %), indicated different adaptation of rumen fermentation in dairy cows to the concentrate-type of diet after calving.

Table 2. Level of energy metabolism in dairy cows up to 21 days after calving

Parameter			Reference values	Mean ± SD	Individually %	
					Increased	Decreased
Number of herds				20		
TMR	NDF	%	30.0–35.0	36.1 ± 3.6	60	5
	NFC	%	32.0–38.0	38.2 ± 3.7	50	25
Number of animals				121		
Rumen	pH		6.3–6.5	6.22 ± 0.41	41	50
	VFA	mmol.l^{-1}	80–130	117.6 ± 18.8	23	10
	Acetic acid	%	60.0–65.0	63.0 ± 4.5	30	20
	Propionic acid	%	20.0–25.0	24.4 ± 3.9	31	12
	$C_2 : C_3$		2.5–3.0 : 1	2.68 ± 0.6	37	22
Blood	Glucose	mmol.l^{-1}	2.0–4.1	2.81 ± 0.6	-	10
Serum	NEFA	mmol.l^{-1}	< 0.8	0.42 ± 0.2	3	-
	AST	µkat.l^{-1}	0.22–0.5	0.59 ± 0.2	64	-
	Bilirubin	umol.l^{-1}	0.17–5.13	4.55 ± 1.8	33	-

DISCUSSION

The concentration and physical structure of NDF is related to the activity of rumination and maintenance of rumen pH (1). The limiting factor for intake of feed and energy is the quantity and mutual ratio of diet carbohydrates and frequency in which the character of NDF in rations. The extent and rate of rumen degradation of carbohydrates depends on composition of feed and nutrients in TMR and directly affects the quantity and ratio of VFA in the rumen fluid (6). Energy intake with regard to the quantity and ratio of carbohydrates in TMR is particularly important in the transient phase of dairy cow nutrition with impact on rumen metabolism, milk yield and animal health (5). Both source of energy for dairy cows in the phase of negative energy balance at deficient supply of glucogenic nutrients stimulate lipomobilisation (7) which is confirmed diagnostically

by increase in values of NEFA and their incomplete oxidation in liver and lipid infiltration of liver (4).

CONCLUSION

Evaluation of energy metabolism by analysis of selected markers and looking for mutual relationships in the transient phases proved the following:

• **In the period of preparation for parturition –** bulk character of rations with increased values of NDF was observed in 60 % of herds. The increased level of acetic acid (in 57 % of animals) and decreased level of propionic acid (in 38 % of animals) with characteristic fermentation of the bulk-type of TMR, limited markedly the adaptation of rumen metabolism to the concentrate-type of rations after parturition. Such a state of fermentation results in negative energy balance with tendency to lipomobilisation (30 % of animals) and manifestation of load on the liver (40 % of animals).

• **In the postpartum phase –** transition to the concentrate type of TMR with increased levels of NFC in 50 % of herds and low level of adaptation of rumen microflora resulted in rumen acidification (50 % of animals) and load on the liver (64 % of dairy cows) corresponding to the functional load on the liver related to negative energy balance, lipomobilisation, and liver lipid infiltration in the pre-partum phase.

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REFERENCES

1. Allen, M. S., 1997: Relationship between fermentation acid production in the rumen and requirement for physically effective fibre. *J. Dairy Sci.,* 80, 1447–1462.

2. Allen, M., 2001: Formulating lactating cow diets for carbohydrates. In *Proceedings 5th Western Dairy Management Conference*, April 2001, Las Vegas, Nevada.

3. Crooker, B. A., Carriquiry, M., Weber, W. J., 2007: Energy balance of the periparturient dairy cows. In *Four State Dairy Nutrition and Management Conference*, June 2007, Dubuuque, Iowa, 1–6.

4. Duffield, T., 2000: Subclinical ketosis in lactating dairy cattle. *Vet. Clin. North Am. Food Anim. Pract.,* 16, 231–253.

5. Rabelo, E. R. L., Rezende, S., Bertics, J., Grummer, R. R., 2003: Effects of transition diets varying in dietary energy density on lactation performance and ruminal parameters of dairy cows. *J. Dairy Sci*., 86, 916–925.

6. Sutton, J. D., Dhanoa, M. S., Morant, S. V., France, J., Napper, D. J., Schuller, F., 2003: Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. *J. Dairy Sci*., 86, 3620–3633.

7. Van Knegsel, A. T. M., van den Brand, H., Graat, E. A. M., Dijkstra, J., Jorritsma, R., Decuypere, E., Tamminga, S., Kemp, B., 2007: Dietary energy source in dairy cows in early lactation: Metabolites and metabolic hormones. *J. Dairy Sci,*. 90, 1477–1485.

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PERFORMANCE OF CHICKENS FED DIETS CONTAINING FULL-FAT SOYBEAN AND NATURAL HUMIC COMPOUNDS

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ABSTRACT

The effect of toasted full-fat soybean (FFSB) (22% in weeks 1–5 and 27.5% in week 6, in proportion to substitute soybean meal and oil) in diets supplemented with natural humic substances (7 g per kg – group FFSB + H_1 throughout whole period and group FFSB + H_2 in weeks 3–6) on performance, nutrient utilization and carcass yield were studied in broiler chickens under experimental conditions. Lower body weight, weight gain, European Efficiency Index ($P < 0.001$), carcass yield ($P < 0.05$) and higher feed conversion rate ($P < 0.05$, $P < 0.01$) were observed in the FFSB group in comparison to the control. Supplementation of FFSB diets with natural humic compounds tended to improve body weight, weight gain, carcass yield and European Efficiency Index in comparison to the FFSB group.

Key words: broiler; carcass; full-fat soybean; humic compounds; performance

INTRODUCTION

The use of high quality feed ingredients to balance nutrient content in diets is the principal assumption of meeting nutrient requirements of poultry. Nutrient utilization depends to a great extent on the control of available feed ingredients in relation to the miscellaneous sources and technological processes used in feed processing. Soybean meal is currently a principal plant protein source in poultry diets. The possibility of using toasted full-fat soybean, a combination of high quality protein source and energy in the form of oil, has been investigated recently. Its processing aimed at inactivation of anti-nutritional factors and better utilization of energy and proteins is essential for its dietary use. Humic substances are natural organic compounds formed by decomposition of plant material and by microbial activity. The positive effect of humates on performance of poultry after their application in diets or in drinking water was described by a number of authors (4, 7, 9).

The objective of the present study was to investigate the effect of supplementation of natural humic compounds on performance, nutrient utilization and slaughter characteristics in broiler chickens fed diets containing toasted full-fat soybean.

MATERIAL AND METHODS

Two hundred one-day old broiler chicks (Ross 308 hybrid) were divided to 4 groups (50 chicks per pen) and fed *ad libitum* the diets based on corn and soybean meal according to the growth phases (control group). Diets in three experimental groups were supplemented with full-fat soybean (FFSB) (22% in starter and grower, and 27.5% in finisher) in quantities meeting the fat level reached in control diet by using oil. Two experimental groups were given natural humic substances (total humic acids 60%, free humic acids 48%, calcium formate) in the concentration of 0.7%, in group FFSB + H_1 throughout the whole experiment (42 days) and in group FFSB + H_2 in the 2nd and 3rd phase of the experiment (the same mixture as in FFSB group was used in the 1st phase). Neither antibiotic growth promoters nor anticoccidials were added to the diets. All diets were balanced to the nitrogen and energy content. All birds were weighed and the feed consumed was recorded

weekly. Feed conversion (FCR) was determined as the ratio between the feed intake and weight gain at each phase of the trial. Health status of the chickens was evaluated by examining clinical parameters, such as vitality, feed intake, quality of droppings and mortality rate. The following equation was used for the evaluation of European Efficiency Index (EEI):

EEI = (livability × live weight in kg/length of fattening period in days × FCR) × 100

At the end of the trial, the birds were left for 10–12 h without feed, weighed and slaughtered. Diet composition was analyzed according to AOAC (1). One-way ANOVA and Tukey-Kramer multiple comparison tests were used for statistical evaluation.

RESULTS AND DISCUSSION

The results of performance variables in chickens under different treatments in respective phases of the trial are summarised in Table 1.

No significant differences in body weight were observed between the treatments in the respective phases of fattening. The addition of toasted FFSB to the diets led to lower body weight and weight gain in all observed phases. Supplementation of FFSB diets with humic substances (FFSB + H_1 and FFSB + H_2) positively influenced the body weight and weight gain after 35 and 42 days of the trial but the levels recorded in the control group were not reached. The FCR was higher in all experimental groups in comparison to control. The differences were significant in the period of 0–14 days in all experimental groups ($P < 0.001$), in the period of 0–35 days in group FFSB + H_1 ($P < 0.05$) and in the period of 0–42 days in groups FFSB and FFSB + H_1 ($P < 0.05$). The highest FCR was observed in the FFSB + H_1 group. This confirmed that humic substances used throughout the trial affected positively the body weight and weight gain through higher intake of feed. The values of EEI in experimental groups were lower compared to the control group after 35 as well as 42 day periods (significant differences after 42 days, $P < 0.001$). The use of toasted full-fat soybean caused a significant decrease in hot carcass yield in the FFSB group after 42 days of the trial in comparison to control birds. Supplementation of diets with humic substances showed tendency toward improvement of the above mentioned variables without significant differences in comparison to control group. No significant differences were observed in hot carcass yield and abdominal fat pad weight between male and female birds from different treatments. One of the reasons for lower growth intensity and higher FCR in experimental groups can be the higher urease activity in FFSB (0.48). Lower than 0.1 urease activity of processed soybean is considered an optimum for maximum production (2, 6). The recommended level of FFSB used in poultry diet is up to 20 % for grower and up to 15 % for starter (5). Yeo and Kim (8) explained the FCR improvement after the use of humic substances by stimulative

Table 1. Performance variables in broiler chickens fed diets supplemented with full-fat soybean and humic substances

Variables	Days	Control	FFSB	FFSB+H_1	FFSB+H_2
Body weight (g)	14	359.6 ± 6.06	349.4 ± 7.45	335.4 ± 7.40	356.7 ± 7.49
	35	1912.1 ± 28.5	1884.7 ± 31.5	1914.8 ± 38.3	1901.3 ± 35.9
	42	2519.2 ± 43.8	2377.3 ± 46.2	2484.9 ± 52.7	2456.2 ± 45.2
Weight gain (g)	0–14	316.5	305.3	293.0	312.5
	0–35	1869.1	1840.7	1872.4	1857.1
	0–42	2476.2	2333.2	2442.5	2412.1
Feed conversion (kg.kg^{-1})	0–14	1.32	1.39**	1.51***	1.42***
	0–35	1.66	1.77	1.83*	1.79
	0–42	1.85	1.99*	2.01*	1.95
EEI	0–35	329.1	303.7	293.1	286.2*
	0–42	324.9	284.4***	288.5***	282.1***
Hot carcass yield (%)	42	75.81 ± 0.33	74.04 ± 0.56*	75.15 ± 0.35	74.23 ± 0.41
male		75.49 ± 0.42	74.24 ± 0.43	75.21 ± 0.52	73.94 ± 0.52
female		76.11 ± 0.45	74.14 ± 1.02	72.78 ± 2.36	74.53 ± 0.66
Abdominal fat (% hot carcass)	42	1.58 ± 0.11	1.47 ± 0.11	1.29 ± 0.12	1.44 ± 0.08

*– $P < 0.05$, **– $P < 0.01$, ***– $P < 0.001$

effect on metabolic processes of digestion and nutrient utilization. The diversity of humic substances preparations were considered the reason for different results obtained during the study of their effects on animal performance (4).

Lower body weight, weight gain, EEI and hot carcass yield and higher feed conversion were observed in broilers fed toasted full-fat soybean (FFSB) in comparison to the control group. Supplementation of FFSB-containing diets with humic substances had a positive effect on body weight, weight gain, hot carcass yield and EEI in birds and this effect was more pronounced in the group continually fed the humic substances.

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REFERENCES

1. AOAC, 2001: *Official Methods of Analysis.* 17th ed., **Horwitz, W.** (ed.)**:** AOAC Inc., Arlington, USA, 2200 pp.

2. Batal, A. B., Douglas, M. W., Engram, E. A., Parsons, C. M., 2000: Protein dispersibility index as an indicator of adequately processed soybean meal. *Poult. Sci.,* 79, 1592–1596.

3. Bates, L. S., 1991: Poultry and full-fat soybean meal. *Roasting Technology*, 1, 1–3.

4. Kocabagli, N., Alp, M., Acar, N., Kahraman, R., 2002: The effects of dietary humate supplementation on broiler growth and carcass yield. *Poult. Sci*., 81, 227–230.

5. Lázaro, R., Mateos, G. G., Latorre, M. A., Piquer, J., 2002: *Whole Soybeans in Diets for Poultry*, American Soybean Association, 1–43.

6. Ruiz, N., de Belalcázar, F., Díaz, G. J., 2004: Quality control parameters for commercial full-fat soybeans processed by two different methods and fed to broilers. *J. Appl. Poult. Res.,* 13, 443–450.

7. Vaško, L., Szanyi, G., Jeníková, J., 2007: The influence of feeding humic acids on production and economic parameters of farm animals (In Slovak). *Slovenský veterinársky časopis*, 2, 106–108.

8. Yeo, J., Kim, K., 1997: Effect of feeding diets containing an antibiotic, a probiotic, or jucca extract on growth and intestinal urease activity in broiler chicks. *Poult. Sci.,* 76, 381–385.

9. Yörük, M. A., Gül, M., Hayrli, A., Macit, M., 2004: The effects of supplementation of humate and probiotic on egg production and quality parameters during the late laying period in hens. *Poult. Sci.*, 83, 84–88.

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BIOCHEMICAL RESPONSES AND PERFORMANCE OF EARLY-WEANED PIGLETS FED DIFFERENT PROTEIN LEVEL DIETS

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ABSTRACT

The study was conducted to determine the effects of low protein diets supplemented with crystalline amino acids (AA) on biochemical parameters and performance of 10 crossbred piglets weaned at 28 days of age (2 groups of 5 animals, 8.8 ± 0.6 kg and 8.6 ± 0.7 kg body weight). The treatments were a control diet containing 21.1 % crude protein (CP) and low protein diet containing 18.6 % CP, supplemented with crystalline amino acids (AA), lysine, threonine and methionine, to achieve an ideal AA pattern. Blood from all piglets was taken to determine biochemical parameters at 5 weeks after weaning. The decrease in the diet CP was manifested by significant ($P < 0.01$) decrease in blood urea (mean levels 2.61 mmol.l^{-1} and 4.21 mmol.l^{-1}, resp.) which indicated increased biological value of the respective mixed feed.

Key words: amino acids; excretion; nitrogen; nutrition; urea; weaned piglets

INTRODUCTION

Protein source is a very important factor for growing piglets because poor amino acid and protein nutrition affects profoundly their health and development. Diets with high crude protein (CP) content are commonly supplied to early-weaned pigs. This kind of diet can improve growth performance of piglets but is always associated with diarrhoea (5). Proteins are composed of amino acids and it is actually the amino acids that are the essential nutrients. The primary factors affecting bioavailability are the efficiency 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 level 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 of 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 is decreased.

The objective of the present experiments was to determine the effects of reduction of the dietary CP content from 21.1 to 18.6 % on serum biochemical parameters.

MATERIAL AND METHODS

The experiment was conducted on 10 crossbred piglets (Slovakian White x Landrace), with initial mean body weight (BW) of 8.8 ± 0.6 and 8.6 ± 0.7 kg, weaned at 28 days of age. At weaning, the piglets were divided to two groups (5 animals in each) with equal number of females and castrated males. The experimental diets were formulated with 2 levels of CP (21.1–18.6 %). The low CP diet was supplemented with lysine, methionine, threonine. The experiment was carried out in the facilities of the Institute of Animal Nutrition and Dietetics at the University of Veterinary Medicine in Košice in compliance with EU regulations concerning protection of experimental animals. The diets were analyzed for dry mater (DM), crude protein (CP), crude fibre (CF), neutral detergent fibre (NDF),

ether extract (EE) and ash by AOAC methods (1). Blood serum levels of biochemical parameters, total proteins, albumin, urea, glucose, total lipids, cholesterol, aspartate aminotransferase (AST) and alkaline phosphatase (ALP), were determined spectrophotometrically using commercial Bio-La-Tests (Pliva-LaChema Brno Ltd.; The Czech Republic.). Differences between the groups were evaluated by paired t-test.

RESULTS AND DISCUSSION

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

The biochemical parameters in blood serum of weaned piglets varied in relatively wide ranges of physiological values for piglets, presented in literature (4, 6). The mean values of biochemical parameters, such as total proteins, albumin, glucose, total lipids, cholesterol, AST and ALP did not differ significantly within the groups. However, throughout the experiment, serum urea nitrogen was significantly lower ($P < 0.01$) in piglets fed low CP diet supplemented with lysine, methionine, threonine, compared to those which consumed the higher CP diet. Urea excreted in urine is the main nitrogenous end-product of amino acids catabolism in pigs and plasma or serum

Table 1. Chemical composition of experimental diets

Parameters	Control diet		Experimental diet	
Dry mater g.kg^{-1}	903.70	1000	902.70	1000
CP g.kg^{-1}	210.80	233.26	186.40	206.49
NDF-CP g.kg^{-1}	11.07	12.25	10.40	11.52
EE g.kg^{-1}	13.30	14.72	12.90	14.29
CF g.kg^{-1}	38.60	42.71	39.50	43.76
NDF g.kg^{-1}	161.00	178.16	169.9	188.21
ADF g.kg^{-1}	49.80	55.11	47.50	52.62
Ash g.kg^{-1}	67.80	75.02	65.40	72.45
NFE g.kg^{-1}	573.20	634.29	598.50	633.01
Lys g.kg^{-1}	12.60	13.94	13.00	14.40
Tre g.kg^{-1}	7.90	8.73	8.00	8.86
Met.+cys. g.kg^{-1}	6.70	7.41	6.90	7.64

Table 2. Effect of dietary CP on biochemical parameters in blood serum of piglets

Parameters	Control diet (21.1 % CP)					Experimental diet (18.6 % CP)				
Week	1	2	3	4	5	1	2	3	4	5
Total protein g.kg^{-1}	51.92 ± 2.85	52.94 ± 2.96	55.86 ± 3.40	54.70 ± 3.27	50.92 ± 5.23	50.74 ± 1.89	53.74 ± 2.69	53.30 ± 3.82	49.61 ± 1.33	51.20 ± 4.3
Urea mmol.l^{-1}	2.92^{a} ± 0.11	4.52^{a} ± 0.48	4.39^{a} ± 0.32	4.99^{a} ±0.51	6.63^{a} ± 0.58	1.69^{b} ± 0.35	3.06^{b} ± 0.23	2.63^{b} ± 0.28	3.06^{b} ± 0.40	4.67^{b} ± 0.27
Albumin g.l^{-1}	30.28 ± 1.09	33.44 ± 1.79	34.82 ± 1.03	32.69 ± 2.00	32.96 ± 2.29	29.07 ± 2.33	33.15 ± 1.30	32.56 ± 2.41	30.62 ± 2.04	31.62 ± 2.28
Glucose mmol.l^{-1}	5.30 ± 0.51	4.92 ± 0.23	5.99 ± 0.47	3.77 ± 0.79	4.85 ± 0.46	5.22 ± 1.86	5.03 ± 0.67	6.01 ± 2.42	3.81 ± 0.32	5.25 ± 0.72
Total lipids g.l^{-1}	1.68 ± 0.08	1.62 ± 0.49	1.84 ± 0.25	1.83 ± 0.20	2.44 ± 0.30	1.76 ± 0.87	1.59 ± 0.39	1.80 ± 0.49	1.64 ± 0.21	2.20 ± 0.28
Cholesterol mmol.l^{-1}	1.85 ± 0.35	2.28 ± 0.38	1.93 ± 0.22	2.18 ± 0.21	2.20 ± 0.45	1.75 ± 0.24	2.06 ± 0.25	1.97 ± 0.12	1.99 ± 0.04	1.89 ± 0.07
AST ukat.l^{-1}	0.26 ± 0,02	0.29 ± 0.08	0.36 ± 0.04	0.24 ± 0.02	0.22 ± 0.02	0.22 ± 0.05	0.37 ± 0.03	0.40 ± 0.03	0.24 ± 0.01	0.24 ± 0.02
ALP ukat.l^{-1}	5.49 ± 0.65	5.25 ± 0.59	4.66 ± 1.47	5.17 ± 0.61	5.05 ± 0.27	6.06 ± 0.03	5.86 ± 0.28	5.68 ± 0.96	5.70 ± 0.09	6.60 ± 0.48

^{ab} – Significant differences ($P < 0.01$)

urea concentrations may be indicative of N excreted in urine (6). Serum or plasma urea nitrogen can be used to quantify in various animal species N utilization and excretion rates in various animal species. Lower blood urea nitrogen indicated higher availability of dietary nitrogen (3).

In conclusion, our study showed that decreased CP content in the diet of piglets resulted in a significant decrease ($P < 0.01$) in blood urea level indicative of increased biological value of the mixed feed.

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REFERENCES

1. AOAC (Association of Official Analytical Chemists International), 2001: *Official Methods of Analysis,* 17th ed., **Horwitz, W.** (ed.), AOAC Inc., Arlington, USA.

2. Bindas, Ľ., 2008: Phase nutrition as a limiting factor in pig rearing (In Slovak). *Slovenský Veterinársky Časopis,* 5, 311–313.

3. Figueroa, J. L., Lewis, P. S., Miller, R. L., Fishcher, R. S., Diedrichsen, R. M., 2002: Nitrogen metabolism and growth performance of gilts fed standard cor-soybean meal diets or low-crude protein, amino acid supplemented diets. *J. Anim. Sci.,* 80, 2911–2919.

4. Kraft, W., Durr, M. U., 2001: *Clinical laboratory Diagnostics in Veterinary Medicine* (In Slovak/Czech). **Hajko, Hajková** (ed.), Bratislava, 365 pp.

5. Qiao, R. Y., 2003: Low protein diets balanced with amino acids in piglets. *Feed ind.,* 6, 1–5.

6. Vrzgula, L., 1990: *Metabolic Disorders in Farm Animals and their Prevention* (In Slovak), Publ. House Príroda, Bratislava, 494 pp.

7. Yue, L. Y., Qiao, Y. S., 2008: Effects of low-protein diets supplemented with crystalline amino acids on performance and intestinal development in piglets over the first 2 weeks after weaning. *Livest. Sci.,* 115, 144–152.

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EFFECT OF INTERACTION OF ZINC AND CADMIUM ON DISTRIBUTION OF ELEMENTS IN THE TISSUES OF TURKEYS

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ABSTRACT

Experiments were conducted to determine the extent of tissue accumulation of Cd in the turkeys exposed to 2 different doses of Cd. Cadmium was found especially in the livers and kidneys. The level of Cd in turkey kidneys 19 times and in the liver 14 times higher compared to muscles. The differences were related to the time of exposure. Administration of higher doses of Zn together with Cd eliminated accumulation of Cd in the tissues of turkeys.

Key words: cadmium; distribution; interaction; turkey; zinc

INTRODUCTION

Saturation of the animal body with mineral elements is process that should be studied at the level of soil. The content of mineral substances in plants and forage is influenced by various factors. They include, for example, soil acidity, the relationship among elements, excess or deficiency of mineral substances in soil and the type of feed. There are firstly the physiological conditions in the digestive tract, the proportional representation of mineral substances in rations, form of chemical elements bonds, interactions as well as occurrence of risk elements. Cadmium is one of the most toxic elements. It is an important environmental contaminant which presents risk to humans and animals (4, 5, 6). Migration of Cd in the environment and its effect on animals depend on its interaction with biogenic micro- and macro-elements, particularly Ca, Zn, Cu, Fe and Se (9, 1, 3). An important antagonist of Cd is Zn. The distribution of Cd and Zn in tissues and organs of quails and turkeys and the effect of increased doses of Zn and Se were observed in this study.

MATERIAL AND METHODS

Seven weeks old turkeys (n = 70) were divided to 5 groups: control group (C) administered no additional elements, group with addition of 2.0 mg $Cd.day^{-1}$ per bird (Cd1), group with addition of 1.0 mg $Cd.day^{-1}$ per bird (Cd2), group with addition of 72 mg $Zn.day^{-1}$ per bird (Zn) and group exposed to combination of Cd and Zn (CdZn). Cadmium was administered as $CdCl_2$ and zinc as Zn_2SO_4 for 71 days in water solution. At the beginning of the experiment the turkeys were fed mixed feed HYD-15 and for two weeks before finishing the experiment they were fed HYD-16. Tissue samples were taken and examined at the end of the experiment.

The samples of breast muscles, leg muscles, liver and kidneys were digested in a microwave oven Milestone 1200 and analysed for Cd, Zn and Cu by atomic absorption spectrophotometry employing Unicam Solar, Model 939.

The results were analysed statistically Student's *t*-test at significance levels $P < 0.05$, $P < 0.01$ and $P < 0.001$.

RESULTS AND DISCUSSION

Results obtained in the experiment on turkeys are shown in Table 1.

Table 1. Mean content of Cd and Zn in turkey tissues and organs (mg.kg^{-1})

Element	Sample	C	Cd1	Cd2	Zn	CdZn
Cd	Breast muscle	0.026	0.057^{***1}	0.045^{***1**2}	0.024^{***2**3}	0.030^{***2**3}
	Leg muscle	0.014	0.033^{***1}	0.019^{***1,2}	0.016^{***2**3}	0.028^{**1,4*3}
	Liver	0.071	0.781^{***1}	0.492^{***1,2}	0.072^{***2,3}	0.409^{***1,2,4}
	Kidney	0.073	1.091^{***1}	0.717^{***1,2}	0.134^{***1,2,3}	0.627^{***1,2,4*3}
Zn	Breast muscle	20.53	17.79	10.19^{***1,2}	36.26^{***1,2,3}	20.67^{***3,4}
	Leg muscle	19.46	15.93	13.21	31.56^{***1,2,3}	19.04^{**3,4}
	Liver	25.66	20.81	21.30	29.23^{***2,3}	19.93^{***4}
	Kidney	23.02	19.44	20.46	25.09^{**2,3}	20.41^{**4}

*** – P < 0.001, ** – P < 0.01; * – P < 0.05
^{1} – C: Cd1, Cd2, Zn, CdZn; ^{2} – Cd1: Cd2, Zn, CdZn
^{3} – Cd2: Zn, CdZn; ^{4} – Zn: CdZn

Significantly higher mean level of Cd (P < 0.001) was detected in leg muscle in Cd1, Cd2 and CdZn groups (0.033, 0.019 and 0.028 mg.kg^{-1}, resp.) in comparison to the control group C and Zn group (0.014 and 0.016 mg.kg^{-1}, resp.). Similar tendency was observed in kidney and liver. The highest mean level of Cd (1.09 mg/kg) was found in kidneys in the of Cd1 group.

The mean levels of Cd in the liver in Cd1 and Cd2 groups (0.78 and 0.49 mg.kg^{-1}, resp.) were significantly higher (P < 0.001 and P < 0.05, resp.) compared to the CdZn group (0.40 mg. kg^{-1}). A significant increase (P < 0.001) was found also in kidneys. Cadmium level in kidneys in Cd1 and Cd2 groups (1.09 and 0.71 mg.kg^{-1}, resp.) were higher than in the CdZn group (0.62 mg.kg^{-1}). This suggests an important protective effect of Zn against Cd as Cd was decreased by about one half in the liver and kidneys in the CdZn group.

The highest mean level of Zn in breast muscle was recorded in the CdZn group (36.25 mg.kg^{-1}) and the lowest (10.19 mg. kg^{-1}) in the Cd1 group. The highest content of Zn in leg muscle was analysed in the Zn group (31.55 mg.kg^{-1}) and the lowest (13.21 mg.kg^{-1}) in the Cd2 group. The highest level of Zn in the liver (29.22 mg.kg^{-1}) was found in the Zn group and the lowest in Cd2 and CdZn groups (20.81 and 19.93 mg.kg^{-1}, resp.). The tendency observed in the liver resembled that in kidneys, however, at lower level of significance. The highest mean levels of Zn

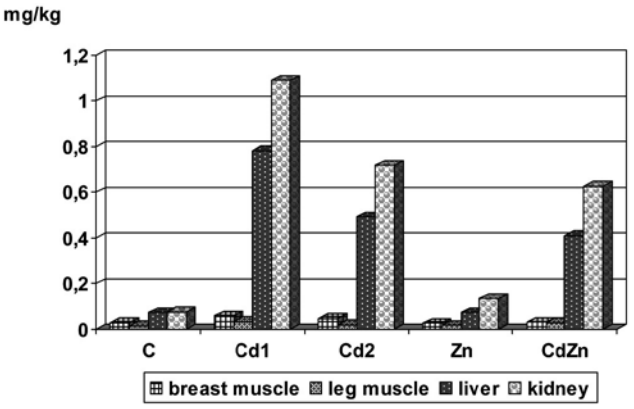

Fig. 1. Mean levels of cadmium in breast muscle, leg muscle, liver and kidneys of turkeys

were recorded in the Zn group (25.08 mg.kg^{-1}) and the lowest in Cd1 and ZnCd groups (19.44 and 20.41 mg.kg^{-1}, resp.).

Low level of Zn is an important factor affecting absorption and retention of cadmium (7). Fox *et al.* (2) reported that low content of Zn in feed (11 mg.kg^{-1}) caused increase in Cd in the liver of quail. Similar findings were present also by Waalkes *et al.* (9). Supply of 200 mg Cd.kg^{-1} in feed at low doses of Zn (7 mg.kg^{-1} feed) caused considerable increase in Cd content in the liver and kidneys of rats. According Swiergosz and Kowalska (8) the content of Cd in the liver of pheasants depended on doses of Cd and the length of exposure.

CONCLUSION

Our study indicates a protective effect of supplementation of Zn to birds or adequate supply of this element in feed of animals with regard to cadmium. It is possible to use this approach for effective elimination of Cd from animal body and should be utilized particularly in areas with serious risk of Cd exposure.

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REFERENCES

1. Beyersmann, D., 1994: Interactions in metal carcinogenicity. *Toxic. Letters*, 72, 333–338.

2. Fox, M. R. S., Tao, S. H., Stone, C. L., Fry, B E., 1984: The effect of Zn, Fe, Cu deficiencies on Cd levels in tissues of Japanese quail. *Env. Health Persp,* 54, 57–65.

3. Grosicki, A., Domańska, K., 1997: Dietary Zn-heavy metal interactions. *Polish J. Env. Stud.*, 6, 5–9.

4. Kolesarova, A., Slamecka, J., Jurcik, R., Tataruch, F., Lukac, N., Kovacik, J., Capcarova, M., Massanyi, P., 2008: Environmental levels of Cd, Pb and Hg in brown hares and their relation to blood metabolic parameters. *J. Env. Sci. Health – Part A Toxic/Haz. Subst. Env. Eng.*, 43, 653–657.

5. Massanyi, P., Weis, J., Lukac, N., Trandžík, J., Bystrická, J., 2008: Cadmium, zinc, copper, sodium and potassium concentrations in rooster and turkey semen and their correlation. *J. Env.Sci. Health – Part A Toxic/Haz. Subst. Envir. Eng.,* 43, 563–565.

6. Massányi, P., Lukáč, N., Uhrín, V., Toman, R., Pivko, J., Rafay, J. *et al*., 2007: Female reproductive toxicology of Cd. *Acta Biol. Hung.* 58, 287–299.

7. Panemangalore, M., 1993: Interaction among Zn, Cu, and Cd in rats: effect of low Zn and Cu diets and oral Cd exposure. *J. Trace Elem. Exper. Med.,* 6, 125–139.

8. Swiergosz, R. and Kowalska, A., 2000: Cadmium accumulation and its effects in growing pheasants *Phasianus colchicus. Env. Toxic. Chem.,* 19, 2742–2750.

9. Waalkes, M. P., Kovatch R., Rehm, S., 1991: Effect of chronic dietary Zn deficiency on Cd toxicity and carcinogenesis in the male Wistar rat. *Toxic. Appl. Pharm.*, 108, 448–456.

*

Selected papers from the International Conference "School – science – practice I." held at the University of Veterinary Medicine in Košice on September 24, 2009, on the occasion of the 60th anniversary of its establishment.

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THE PREVALENCE OF *DIROFILARIA* SPP. IN DOMESTICATED AND WILD CARNIVORES IN SLOVAKIA

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ABSTRACT

Dirofilariosis is a zoonotic parasitic disease which was characteristic for the countries around the Mediterranean Sea only. Recently many factors have contributed to extended geographical range of *Dirofilaria* worms and the risk of infection for both animals and humans. They include climate changes related to global warming, increasing abundance of mosquitoes and increased movement of uncontrolled cats and dogs across Europe between endemic and non-endemic countries. Due to the existing environmental and climatic modifications, this vector-borne disease should be regarded emergent and therefore important. However, although it was widely studied in pets, it was poorly investigated in wild animals including foxes. Wild carnivores are suitable hosts of the filariae in nature and serve as an important source of the parasite for vectors which afterwards could infect dogs or humans. The study performed epidemiological survey of dirofilariosis and parasite prevalence in domesticated and wild carnivores in Slovakia. A total of 300 dogs and 30 foxes from various regions were examined. Dirofilariosis was detected in 29 dogs (9.67%) and 10 foxes (33.33%).

Key words: *Dirofilaria immitis*; *Dirofilaria repens*; dogs; foxes; Slovakia

INTRODUCTION

Dirofilariosis is a disease of dogs, cats and rarely of wild carnivores caused by zoonotic species of the *Dirofilaria* genus. *Dirofilaria immitis* inhabits pulmonary arteries, the right chamber and atrium of the heart and often cause serious cardiovascular disease. Less pathogenic *Dirofilaria repens* parasites in the skin and subcutis. Experimental studies showed that nematodes of the genus *Dirofilaria* are able to develop completely or incompletely in many arthropods, but mosquitoes are the most important vectors for their spread. Representatives of the family *Culicidae* belong to the main group of the vectors of these pathogens. Mosquito calamities but also common populations of mosquitoes are plentiful in almost all areas of the country and therefore can be expected that this infection is more prevalent. The first case of canine dirofilariosis in the Slovak Republic was detected in 2005 (6). In 2007 also the first autochthonous case of human dirofilariosis was confirmed (2).

MATERIAL AND METHODS

Diagnosis of *Dirofilaria* spp. is based mainly on the evidence of circulating microfilariae in the blood. Blood of 300 dogs was examined in cooperation with a number of veterinary practitioners from various Slovak regions. We collected 3 ml of venous blood into tubes with EDTA which enabled subsequent processing of the samples. Microfilariae were detected using the method developed by Knott (3).

Species determination was carried out by histochemical staining based on different somatic distribution of acid phosphatase activity in individual species of *Dirofilaria*. *Dirofilaria immitis* microfilariae show two acid phosphatase activity spots localized around the anal and excretory pores. *Dirofilaria repens*

microfilariae show only one acid phosphatase activity spot localized around the anal pore. For this purpose commercial kit test Lucognost SP® (Merck) is used nowadays (4).

PCR analysis with species-specific primers was used as a very specific method suitable for detection of the pathogen and for confirmation of the species differentiation (6).

At the same time 30 blood samples from red foxes (*Vulpes vulpes*) hunted in the districts of Košice, Vranov nad Topľou and Stropkov were examined. The Knott's test was conducted on blood obtained from the hearts and the presence of adult worms was determined by helminthological autopsy. Commercial kit was used to isolate DNA from spleens for the PCR method.

RESULTS AND DISCUSSION

Dirofilariosis was confirmed in 29 of 300 examined dogs (9.67 %). Monoinfection by *D. repens* was detected in 26 cases (8.67 %) while mixed infection with *D. repens* +*D. immitis* was confirmed only in three cases (1 %).

Diagnosis of dirofilariosis in foxes is relatively complicated. The presence of the adult filariae was not confirmed and examination by Knott's test of blood from the hearts of cadavers showed insufficient because of low interception. This method identified reliably only one positive sample out of 30. PCR is considered the only reliable diagnostic method for diagnosis of dirofilariosis in wild carnivores. Ten out of 30 foxes (33.33 %)were infected with *D. repens*. Mixed infection and monoinfection by *D. immitis* was not detected.

In Slovakia dirofilariosis in dogs has been recorded repeatedly since 2005. The main cause is probably the movement of dogs across countries and their return to Slovakia without effective prevention. But presently the disease is detected in dogs which never travelled, that means they acquired infection in our territory. Our investigations showed that *D. repens* is the dominant species in our territory. Causativel agent of the heartworm disease, *D. immitis,* was diagnosed in only three dogs and it was also mixed infection with *D. repens*. One explanation of the low incidence of *D. immitis* was presented by Genchi *et al*. (1) who dealt with the mutual relations between both *Dirofilaria* genera and pointed out that the spreading of *D. immitis* is repressed in areas with high prevalence of *D. repens* (1).

CONCLUSION

Dirofilaria immitis and *Dirofilaria repens* are among the major causative agents of parasitic disease which spread from subtropical and tropical to temperate zones, thus also to Slovakia. Our survey showed that *Dirofilaria repens* is the dominant filariae species in Slovakia. One should also consider the recently increasing zoonotic importance of these parasites.

Diagnosis of dirofilariosis in foxes is more complicated than in dogs but our results showed that these wild carnivores are a very suitable reservoir for this disease. We should mention the increasing population of foxes and their more frequent movement in the vicinity of villages or cities which increases the risk of transmission of dirofilariosis to domestic animals and humans.

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REFERENCES

1. Genchi, C., Solari Basano, G., Bandi, C., DI Sacco, B., Venco, L., Vezzoni, A., Cancrini, G., 1995: Factors influencing the spread of heartworms in Italy: interaction between *Dirofilaria immitis* and *Dirofilaria repens*. In *Proceedings of Heartworm Symposium '95.* American Heartworm Society, Baravia, IL., 65–71.

2. Jalili, N., Kozbova, D., Novák, I., Dubinský, P., Babál, P., 2007: The first case of human dirofilariosis in Slovakia. *Acta Tropica*, 5, 75–78.

3. Knott, J., 1939: A method for making microfilarial survey on day blood. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 33, 191.

4. Peribañez, M. A., Lucientes, J., Arce, S., Morales, M., Castillo, J. A., Gracia, M. J., 2001: Histochemical differentiation of *Dirofilaria immitis*, *Dirofilaria repens* and *Acanthocheilonema dracunculoides* microfilariae by staining with a commercial kit, Leucognost-SP®. *Vet. Parasitol.*, 102, 3, 173–175.

5. Rishniw, M., Barr, S. C., Simpson, K. W., Frongillo, M. F., Franz, M., Dominguez Alpizar, J. L., 2006: Discrimination between six species of canine microfilariae by a single polymerase chain reaction. *Vet. Parasitol.*, 135, 303–314.

6. Svobodová, V., Svobodová, Z., Beladičová, V., Valentová, D., 2005: First case of canine dirofilariosis in Slovakia: a case report. *Veterinární medicína*, 50, 510–511.

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THE EFFECT OF *S. ENTERITIDIS* PT4 AND *E. Faecium* EF55 ON CHICKEN INTESTINE MUCUS PRODUCTION AND SOME HAEMATOLOGICAL PARAMETERS

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ABSTRACT

The experiment was carried out on 40 chickens of ISA BROWN hybrid, divided to four groups. It lasted 22 days. Intestinal segments were collected on days 8 and 22 and mucus production was determined quantitatively. The number of erythrocytes and haemoglobin concentration were determined in 22-day old chickens. Salmonella infection and probiotic culture affected production of mucus in the intestinal segments of chickens and selected hematologic parameters. The results obtained indicated the potential of *E. faecium* EF55 to optimise chicken intestinal microflora.

Key words: chicken; *Enterococcus faecium;* intestine; mucus; *Salmonella enteritidis*

INTRODUCTION

Salmonella enterica, serotype Enteritidis, causes gastroenteritis in humans and animals worldwide. Especially vulnerable to colonization by pathogens are chickens at an early stage as their intestinal microflora is not stabilized. Probiotics optimize gut microflora in animals and chickens and thus create an effective barrier against pathogens (3). The well-studied probiotic organisms include probiotic, bacteriocin-producing strain *Enterococcus faecium* EF55 (7). Levkut *et al.* (5) monitored the protective effect of EF55 against *Salmonella enterica,* serovar Enteritidis type 4 (SEPT4), in chickens and confirmed its inhibitory effect on Salmonella infection, as reflected by significant reduction in the number of SEPT4 in the liver, spleen, caecum and faeces. The first line of defence against the penetration of microorganisms is the mucus which covers the intestinal mucosa.

The aim of our study was to investigate the effect of probiotic culture *E. faecium* EF55 and Salmonella infection (strain PT4) on mucus production in chicken intestine and potential changes in the number of erythrocytes and haemoglobin concentration.

MATERIAL AND METHODS

The 22-day experiment was carried out on 40 ISA BROWN chickens divided to four groups (C – control, SE – *S. enteritidis*, EF – *E. faecium*, EFSE – *E. faecium* + *S. enteritidis*). Water and feed HYD-04/a was available *ad libitum*. EF55 was applied at a dose of 10^9 CFU.ml^{-1}, 3 g per group, and SE PT4 at a dose of 10^8 CFU per 0.2 ml PBS once, *per os*. Intestinal segments (duodenum – D, jejunum – J, ileum – I, caecum – Cc) were collected (n = 5) on days 8 and 22 and mucus was quantified by

the method of Corne *et al.* (1) modified by Smirnov *et al.* (6) and subsequently by Thompson *et al.* (8). The quantity of Alcian blue (AB) absorbed to mucus was calculated using a standard curve and expressed as μgAB.cm^{-2} intestine. The number of erythrocytes and haemoglobin concentration were determined in 22-day old chickens. Differences between groups were evaluated by one-way ANOVA and Tukey post-test and Dunnett test was used for statistical analysis of bacteria.

RESULTS AND DISCUSSION

Production of mucus in 8-day old chicks (Tab. 1) in the SE group was increased in the duodenum, jejunum and caecum in comparison with the control. Deplancke *et al.* (2) pointed out that pathogenic bacteria can use mucin as a substrate. Therefore salmonella infection could provoke higher production of mucus, particularly in the caecum, the predilection site of salmonella localization. The quantity of mucus was increased in duodenum and jejunum in the EFSE group compared to the control and SE group. On the other hand, it was decreased in caecum compared to the control and the decrease compared to the level in SE was 3-fold. Adherence of probiotic micro-organisms to intestinal mucosa is one of the mechanisms affecting intestinal pathogens (4). Such action of EF55 in the EFSE group could prevent adhesion of salmonellae bacteria and subsequently increase production of mucus in caecum, the principal location of the infection.

The differences in mucus production in 22-day old chickens (Tab. 2) were less pronounced which could be related to the age, as salmonella infections affect most the lower age categories causing high mortality. This may explain insignificant differences between the groups in the caecum. In both age categories the EF group showed tendency of reduced mucus production in all intestinal segments compared to SE and EFSE groups.

Salmonella infection and probiotic culture affected both production of mucus in the intestinal segments of chickens and selected hematological parameters. We believe that the decline in the number of erythrocytes (Tab. 3) at salmonella infection could be caused by haemorrhagic processes which usually accompany the infection.

In conclusion, results obtained in our study indicate the suitability of *E. faecium* EF55 strain for optimization of intestinal microflora in chickens.

Table 1. The effect of SEPT4 and EF55 on mucus production in 8-day old chickens (μgAB.cm^{-2})

Intestinal segment	Control (C)	*S. enteritidis* (SE)	*E. faecium* (EF)	EF55 + SEPT4 (EFSE)
Duodenum	28.68 ± 1.66^{ab}	68.30 ± 4.63^{ace}	33.20 ± 1.86^{cd}	97.00 ± 4.88^{bde}
Jejunum	52.00 ± 3.61^{ab}	71.30 ± 2.73^{ace}	37.40 ± 1.86^{cd}	90.90 ± 3.03^{bde}
Ileum	41.20 ± 3.74	48.80 ± 1.96	43.00 ± 3.02	57.40 ± 3.07
Caecum	81.40 ± 2.53^{abd}	161.50 ± 3.81^{ace}	44.10 ± 1.74^{cd}	51.90 ± 3.9^{be}

Legend (Tables 1–3): Significant differences in the rows are marked with the same letter; $P < 0.05$; mean ± SD; n = 5; AB – Alcian blue

Table 2. The effect of SEPT4 and EF55 on mucus production in 22-day old chickens (μgAB.cm^{-2})

Intestinal segment	Control (C)	*S. enteritidis* (SE)	*E. faecium* (EF)	EF55 + SEPT4 (EFSE)
Duodenum	40.80 ± 3.17^{a}	62.70 ± 4.85^{abc}	23.40 ± 2.63^{b}	41.40 ± 2.70^{c}
Jejunum	28.60 ± 1.60^{ad}	49.10 ± 1.79^{ac}	31.80 ± 1.71^{b}	78.90 ± 3.41^{bcd}
Ileum	33.80 ± 3.14^{a}	70.60 ± 2.68^{abc}	34.70 ± 2.48^{b}	51.10 ± 2.57^{c}
Caecum	51.90 ± 5.54	42.50 ± 1.61	50.00 ± 2.65	57.90 ± 3.63

Table 3. The effect of SEPT4 and EF55 on the number of erythrocytes (Er) and haemoglobin (Hb) concentration in 22-day old chickens

	Control (C)	*S. enteritidis* (SE)	*E. faecium* (EF)	EF55 + SEPT4 (EFSE)
Er (T.l^{-1})	2.13 ± 0.07^{acd}	1.59 ± 0.03^{ab}	1.86 ± 0.03^{bd}	1.67 ± 0.04^{c}
Hb (g.l^{-1})	79.11 ± 2.57^{a}	91.20 ± 1.26^{a}	92.52 ± 0.61^{b}	85.40 ± 2.03^{b}

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REFERENCES

1. Corne, S. J., Morissey, S. M., Woods, R. J., 1974: Proceedings: A method for the quantitative estimation of gastric barrier mucus. *J. Physiol*., 242, 116–117.

2. Deplancke, B., Vidal, O., Ganessunker, D., Donovan, S. M., Mackie, R. I., Gaskins, H. R., 2002: Selective growth of mucolytic bacteria including *Clostridium perfringes* in a neonatal piglet model of total parenteral nutrition. *Am. J. Clin. Nutr*., 76, 1117–1125.

3. Herich, R., Levkut, M., 2002: Lactic acid bacteria, probiotics and immune system. *Vet. Med. Czech,* 47, 169–180.

4. Lauková, A., Stromfová, V., Simonovová, M., Michlovičová, G., Marcin, A., Herich, R. *et al*., 2008: In **Levkut *et al*.**: *Prevention and Control of Salmonellosis*, Editorial Centre UVM Košice, Slovakia, 128–139.

5. Levkut, M., Pistl, J., Lauková, A., Révajová, V., Herich, R., Ševčíková, Z. *et al*., 2009: Antimicrobial activity of *Enterococcus faecium* EF55 against *Salmonella enteritidis* in chicks. *Acta Vet. Hung*., 57, 13–24.

6. Smirnov, A., Sklan, D., Uni, Z., 2004: Mucin dynamics in the chick small intestine alterated by starvation. *J. Nutr.,* 134, 736–742.

7. Stromfová, V., Lauková, A., 2007: *In vitro* study on bacteriocin production of enterococci associated with chickens. *Anaerobe*, 13, 228–237.

8. Thompson, K. L., Applegate, T. J., 2006: Feed withdrawal alters small-intestinal morphology and mucus of broilers. *Poult. Sci.*, 85, 1535–1540.

*

Selected papers from the International Conference "School – science – practice I." held at the University of Veterinary Medicine in Košice on September 24, 2009, on the occasion of the 60th anniversary of its establishment.

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LABORATORY DIAGNOSTICS OF SWINE MYCOPLASMOSIS

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ABSTRACT

***Mycoplasma hyopneumoniae* is a widespread respiratory pathogen in pigs considered a primary agent of enzootic pneumonia, one of the most chronic diseases in pig herds. The PCR test, used to detect the presence of the pathogen in live animals, should be easy to perform, rapid, inexpensive, and should provide data on implementation of control of the occurrence of *M. hyopneumoniae*. Nested PCR appears to be the most reliable method capable of best demonstration of the infectious agent even at low concentration. Ideal detection of *M. hyopneumoniae* extends the serological methods (ELISA) and provides a broader view of the emergence and development of disease.**

Key words: diagnostics; ELISA; enzootic pneumonia of swine; *Mycoplasma hyopneumoniae*; PCR; swine

INTRODUCTION

Good health of pigs is one of key success factors in production of pork. Early detection of diseases throughout the breeding period is crucial for health of the entire herd (11). Enzootic pneumonia of pigs is caused by the bacterium *Mycoplasma hyopneumoniae.* As the majority of mycoplasma diseases of pigs, enzootic pneumonia is of great economic importance, particularly in terms of low weight gain in animals carrying the mild form of disease. The economic losses may increase under poor environmental conditions due to secondary bacterial flora, particularly *Pasteurella multocida* type A (3). Other agents of swine mycoplasmosis are *Mycoplasma hyosynoviae*, populating upper airways and occasionally causing polyarthritis, and *Mycoplasma hyorhinis,* causing polyarthritis and polyserositis. Enzootic pneumonia of pigs occurs in all pig-keeping areas throughout the world. Morbidity is high but mortality is close to zero (6). Methods of mycoplasma diagnosis could be divided to field testing or diagnostics and laboratory diagnostics. Field diagnostics is based on testing on farms and at slaughterhouses. Laboratory diagnostics is crucial for isolation and identification of the agent of swine mycoplasmosis.

MATERIAL AND METHODS

Sampling of biological material – samples suitable for the purpose, in appropriate quality and number – precedes laboratory diagnosis.

Bacteriological cultivation tests use swabs. There is a variety of sampling systems and transport media suitable for difficult-to-cultivate bacteria and collection of tissues from affected organs, particularly the pathologically changed lungs. Antibodies against *M. hyopneumoniae* can be detected in blood serum and plasma collected at least two weeks after infection. Antibodies are usually demonstrable in the early and middle fattening periods using ELISA detection method. PCR diagnostics uses lung tissue, bronchial lavage or swabs of the nasal cavity. Indirect immunofluorescence method and immunohistochemical methods are used to test the affected lung tissue.

RESULTS AND DISCUSSION

The use of liquid or solid growth media for mycoplasma cultivation is considered a *"Gold standard"* for detection of *M. hyopneumoniae.* Isolation of microorganisms is difficult and requires special growth media. Usual source of sterol is blood serum, which is necessary for most species of mycoplasma, where the requirement may be pig serum negative for antibodies to *M. hyopneumoniae*. The source of growth factors for mycoplasmas is yeast extract. For the purpose of inhibiting bacterial contaminants Penicillin, Thallium Aceticum and Amphotericin B must be added. Specific antiserum against commensal mycoplasmas may be added to culture media to facilitate the selective isolation of certain pathogenic mycoplasma species. Investigated samples are optimally inoculated simultaneously into liquid and solid media. Incubation is preferably performed at 37 °C in an atmosphere with 5–10 % CO_2, for at least 3 to 7 days. Thacker (12) reported that cultivation often takes more than 4 to 8 weeks. Soli growth media are assessed under a light microscope. Bacterial colonies can be differentiated using several criteria. They are usually smaller (diameter 50–500 μm), of „fried egg" shape and stained according to Diens (6). Claims for isolation make the method under-utilized for diagnosis, and failure to isolate the agent often does not confirm or deny the presence of the etiological agent in the pig herd (12).

The serological methods used to diagnose mycoplasmas include ELISA and agglutination reaction by indirect agglutination test. A comparison of different serological tests showed that the most appropriate method of proof of specific antibodies is the ELISA. In serological testing, ELISA is preferred to the complement fixation test (KFT) because it is sensitive and does not give false positive reactions as KFT (8). To monitor enzootic pneumonia in pigs herds in which we want to achieve eradication, it is recommended to examine colostral antibodies of sows and piglets instead of blood serum. The most reliable results are obtained with serological testing of pigs 2 to 4 weeks after infection. Antibodies are detected in infected animals 4–5 weeks after infection, with peak titres 8–9 weeks after infection (7, 9).

Another important diagnostic method is the polymerase chain reaction (PCR). On the basis of analysis of DNA sequences of *M. hyopneumoniae* and cloning, Artiushin *et al.* (2) prepared the first primers already in 1993. It was demonstrated that the test is species-specific, without cross-reactions with other mycoplasma species. PCR was used to detect *M. hyopneumoniae* in the respiratory tract and lungs from lung swabs. The PCR reaction allows to prove the presence of *M. hyopneumoniae* in the sample of air from contaminated stables (10). While conventional PCR detected positivity in less than 4 % of infected pigs, with the nested PCR the number of positive results increased to 55 %. Investigation of bronchial swabs by nested PCR can demonstrate positive results in up to 50 % of the pigs showing indistinctive pathohistological findings (5). Owing to the high sensitivity of this diagnostic method it is the most frequently used laboratory method. Until recently, routine examination of the lung tissue for *M. hyopneumoniae* was carried out by direct and indirect fluorescence tests using polyclonal antibodies. In the direct test, the proof of antigen in the frozen section is obtained by means of conjugate, which is the antibody-linked fluorescein isothiocyanate. *M. hyopneumoniae* is detected on the surface of epithelial cells, bronchi and bronchioli. The test is not very sensitive. Positive reaction can be demonstrated only for extensive pneumonias. Immunofluorescent tests are not specific. False positive results also demonstrate the infection with *M. flocculare* and *M. hyorhinis* (1, 4).

CONCLUSION

Technical implementation and time complexity of conventional cultivation methods focused our attention on the quality and rapidity of PCR methods. Nested PCR seems to be the most reliable method due to its higher sensitivity even at low concentrations of infectious agents. Ideal approach to detection of *M. hyopneumoniae* is extension of the serological methods (ELISA, which gives us a broader view of the emergence and development of disease.

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REFERENCES

1. Armstrong, C. H., Freeman, M. J., Sands-Freeman, L., 1987: Cross-reactions between *Mycoplasma hyopneumoniae* and *Mycoplasma flocculare*: practical imlications for the serodiagnosis of mycoplasmal pneumonia. *Mol. Cell Probes*, 371–376.

2. Artiushin, S., Stipkovits, L., Minion, F. C., 1993: Development of polymerase chain reactions primers to detect *Mycoplasma hyopneumoniae*. *Mol Cell Probes,* 7: 381–385.

3. Beníšek, Z., 2006: Swine Mycoplasmoses (In Slovak). In **Švrček, Š., Bajová, V., Beníšek, Z., Čížek, M., Kapitančik, B., Levkutova, M., et. al.**: *Infectious Diseases in Animals Part 1, Bacterial and Mycotic Diseases* (In Slovak), UVL Košice, 487 pp.

4. Bolske, G., Strandberg, M. L., Bergstrom, K., Johansson, K. E., 1987: Species-specific antigens of *Mycoplasma hyopneumoniae* and the cross reactions with other porcine Mycoplasmas. *Curr. Microbiol*., 15: 233–239.

5. Calsamiglia, M., Collins, J. E., Pijoan, C., 2000: Correlation between the presence of enzootic pneumonia lesions and detections of *Mycoplasma hyopneumoniae* in bronchial swabs by PCR. *Vet. Microbiol*., 76, 299–303.

Goll, Jr., F., 1994: Identification of mycoplasmas isolated from domestic animals. In **Whitford, H. W., Rosenbusch, R. F.,**

Lauerman, L. H.: *Mycoplasmosis in Animals: Laboratory Diagnosis*, Iowa State University Press, Ames, Iowa, 15–30.

6. Kobisch, M., Blanchard, B., Le Potier, M. F., 1993: Mycoplasma hyopneumoniae infection in pigs: duration of the disease and resistance to reinfection., *Vet. Rec*., 24, 67–77.

7. Pilipčinec, E., Švický, E., Mikula, I., Šnirc, J., 1998: Porcine respiratory disease complex and the interest of bacterial pathogens in its development (In Slovak). *Slovenský Veterinársky Časopis*, 23, 192–199.

8. Sörensen, V., Barfold, K., Feenstra, A. A., Feld, N. C., 1994: The humoral immune response to experimental *Mycoplasma hyopneumoniae* infection in pigs in relation to clinical signs and patological lesions. In *13th IPVS Congress Bangkok*, 190 pp.

9. Stark, K. D. C., Nicolet, J., Frey, J., 1998: Detection of *Mycoplasma hyopneumoniae* by air sampling with a nested PCR assay. *Appl. Env. Microbiol*., 64, 543–548.

10. Svoboda, M., Drábek, J., 2005: *Veterinary Care of Pig Herds* (In Czech), VFU Brno, CR, 197 pp.

11. Thacker, E. L., 2004: Diagnosis of *Mycoplasma hyopneumoniae*, *J. Swine Health Prod.*, 12, 252–254.

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EMISSIONS OF GREENHOUSE GASES AND AMMONIA FROM INTENSIVE PIG BREEDING

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ABSTRACT

Concentrations of ammonia (NH_3) and greenhouse gases (CH_4, N_2O, CO_2, H_2O) were monitored in intensive pig breeding during two fattening cycles (summer, winter). Total emissions of NH_3, CO_2 and N_2O reached the highest values in winter and were influenced especially by high concentrations of gases in the housing area. Total emissions of CH_4 and H_2O showed approximately stable trends in both fattening periods. Emission factors for given breeds and categories of animals were determined from total emissions of monitored gases.

Key words: ammonia; emissions; greenhouse gases; pigs

INTRODUCTION

Agricultural production bears a large degree of responsibility for pollution of the environment, namely by production of toxic ammonia (NH_3) and greenhouse gases (CH_4, N_2O, CO_2, H_2O) (1). The process of releasing ammonia into the atmosphere depends on methods of livestock husbandry, nutrition conditions, manipulation with slurry and manure and its storage and land application. The main source of ammonia is the urine of mammals and urea occurred therein. In poultry it is uric acid eliminated by faeces (4). In addition to ammonia emissions, agriculture contributes significantly to methane emissions. Therefore it is necessary to take measures to reduce methane and other greenhouse gases.

MATERIAL AND METHODS

The experiment was carried out in intensive pig breeding facilities. Pigs were housed on slatted floor during two fattening cycles (summer – 348 animals, initial weight 25 kg, finishing weight about 110 kg, 105 days of fattening; winter – 352 animals, starting and finishing weight as in summer, 121 days of fattening). Ventilation was provided by three exhaust ceiling fans and inlet flaps located along the sidewalls of the building. Ventilation was controlled automatically and run after exceeding the pre-set temperature in the housing area. Slurry was stored in space under slats and removed at the end of the fattening cycle or during its course.

To calculate the emissions of NH_3, CH_4, N_2O, CO_2 and H_2O it was necessary to determine their concentrations ($mg.m^{-3}$) in the housing space and to identify the volume of emitted air (m^3). The concentrations of gases was measured with 1312 Photoacoustic Multi-Gas Monitor with multi-channel sampling and dosing gas analyzer Multipoint Sampler 1309 (Innova Air Tech Instruments, Denmark) (2.). The volume of emitted air was monitored by a measuring fans and air temperature was recorded by thermocouples. Sampling tubes installed at measuring points (3 ceiling fans, animal zone and external zone) transported air from the measuring points to the analyzer. Thermocouple probes, scanning air temperature, were also installed at these points and measuring fans were installed at three ceiling fans. The measurement apparatus was placed outside the housing area. Suction of air was ensured by compressors.

The data measured were stored in a database at 12 min intervals (gases) or three times per hour (temperature, air volume) during both fattening cycles.

RESULTS AND DISCUSSION

The summer cycle

Concentrations and emissions of all monitored gases showed an upward trend and the highest values were recorded in the IIIrd fattening phase (FP) (Tab. 1 and 2). The increase in emissions in the IInd and particularly in the IIIrd FP was associated with an increase in concentration of gases in the housing space in these phases (filling up the slurry space, surfaces for evaporation). It was also influenced by ventilation capacity, however, its reduction was not reflected in their decrease during the IIIrd FP. The main source of emissions was slurry degradation but the emissions of CO_2 and H_2O were affected also by oxygen demands of animals related to their growth. (3.).

The winter cycle

Emissions of CO_2, N_2O a CH_4 showed the highest values in the Ist FP. This was associated with their high concentrations during this FP (full slurry space at the onset of monitoring, the highest temperature) and also, in the case of CO_2, with heating of the house. Emissions of NH_3 were the highest in the IIIrd and Ist FPs due to high concentrations of this gas in the housing space (full slurry space at the onset of monitoring, higher temperatures). Emissions of H_2O were the highest in the IInd and Ist FPs in relation to the highest concentration in the housing space in the Ist FP and also due to inadequate drying of the housing after disinfection prior to the fattening cycle in the winter months (Tab. 1 and 2).

Internal temperature during the summer cycle showed an uniform course in all FPs and thus had no significant effect on concentrations or emissions of monitored gases. During the winter cycle the temperatures differed during individual FPs which could affect enzymatic processes in slurry and result in decreased or increased concentrations and emissions of monitored gases.

During the summer cycle, the demands on ventilation were the highest in the first two FPs while in the IIIrd FP they were reduced due to decreased outside temperature and necessity to maintain desired temperature in the housing space. During the winter cycle the demands on ventilation were the highest in the IIIrd FP as the animals were bigger and had lower temperature demands. The fans were operated at the lowest capacity in the Ist FP

Table 1. Concentration ($mg.m^{-3}$) and emissions (kg) of NH_3, CO_2 and N_2O during three phases of two fattening cycles

Fattening phases	NH_3				CO_2				N_2O			
	concentration		emission		concentration		emission		concentration		emission	
	s	w	s	w	s	w	s	w	s	w	s	w
Phase I	2.1	9.6	31.7	83.4	983	4196	12756	37354	0.2	1.2	2.6	10.7
Phase II	4.1	8.9	53.6	82.2	1576	3381	20887	31239	0.3	1.0	4.6	8.9
Phase III	5.9	11.4	73.4	129.7	2139	2292	26525	26369	0.5	0.6	6.2	6.6

s – summer, w – winter

Table 2. Concentration ($mg.m^{-3}$) and emissions (kg) of CH_4 a H_2O during three phases of two fattening cycles

Fattening phases	CH_4				H_2O			
	concentration		emission		concentration		emission	
	s	w	s	w	s	w	s	w
Phase I	23	55	299	474	2012	4807	26079	40175
Phase II	32	52	422	480	2959	5240	39185	48404
Phase III	36	29	442	340	3623	3401	44860	39090

s – summer, w – winter

due to lower body weight of animals and higher demands on air temperature in the of housing space.

Emissions of all monitored gases (except CH_4) were higher during the winter cycle compared to the summer one (Tab. 3). The highest differences were recorded for emissions of N_2O (increase by 69.4 %), NH_3 (increase by 61 %) and CO_2 (increase by 37 %) and the lowest for emissions of H_2O (increase by 0.6 %). Only CH_4 emissions decreased slightly (by 3.4 %) during the winter cycle. The increase during the winter cycle was related to higher concentrations of the respective gases in the housing space. As the output of fans was reduced also, this reduction was not reflected in decreased emissions. The increase in CO_2 concentrations and emissions could be related to heating of the housing space during Ist FP in order to ensure the desired air temperature. The slight increase in concentration and emissions of H_2O during the winter cycle could be related to disinfection before the fattening cycle and insufficient drying up for 3 days at lower winter temperatures. Higher concentration of N_2O and NH_3 during the winter cycle could contribute to higher emission rates of these gases. The principal source of N_2O and NH_3 was the slurry stored under slatted floor. In this space, due to technical difficulties, the temperature, humidity and air flow were not monitored.

Table 3. Total emissions and emission factors

Gas	Total emission (kg)			Emission factor (kg.head^{-1}.year^{-1})	
	summer (105 days)	winter (121 days)	winter (105 days)	summer	winter
NH_3	159	295	256	1.6	2.5
CO_2	60168	94962	82405	601	81.4
N_2O	13.4	26.2	22.7	0.1	0.2
CH_4	1162	1294	1122	11.6	11.1
H_2O	110124	127669	110787	1100	0.2

CONCLUSION

Total emissions of NH_3, CO_2 and N_2O were higher during the winter cycle. Total emissions of CH_4 and H_2O were approximately stable during both fattening cycles (recalculation per 105 fattening days). Decrease in intensity of ventilation capacity during the winter cycle had no negative effect on total emissions of monitored gases. Probably high concentration of these gases (filled up slurry space, temperature, humidity, air flow) affected significantly the total emission rates.

Our calculations of emission factors allowed us to conclude that one fattening cycle is insufficient for exact determination of emission factors per year (especially for NH_3 and CO_2) because emission factors determined for winter and summer cycle differed significantly. One-year measurement period is necessary for objective determination.

REFERENCES

1. Hartung, J., Phillips, V. R., 1994: Control of gaseous emissions from livestock buildings and manure stores. *J. Agric. Eng. Res.*, 57, 173–189.

2. Ni, Ji-Qin, Heber, A. J., 2001: *Sampling and Measurement of Ammonia Concentration at Animal Facilities*. ASAE Meeting Presentation, Sacramento, CA, USA.

3. Osada, T. *et al.*, 1998: Continuous measurement of nitrous oxide and methane emission in pig units by infrared photoacoustic detection. *American Society of Agricultural Engineers*, 41, 1109–1114.

4. Rotz, C. A., 2004: Management to reduce nitrogen losses in animal production. *J. Anim. Sci.*, 82, E 119–137.

*

Selected papers from the International Conference "School – science – practice I." held at the University of Veterinary Medicine in Košice on September 24, 2009, on the occasion of the 60th anniversary of its establishment.