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ANIMAL BIOTECHNOLOGY 2016

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RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Institute of Genetics and Reproduction of Farm Animals
NPPC - Research Institute for Animal Production Nitra
Slovak Republic

Faculty of Biotechnology and Food Science
Slovak University of Agriculture in Nitra
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Faculty of Agrobiotechnology and Food Resources
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Main topic of the conference: **Laboratory and small farm animals**

which will involve the following animal species: ***rabbit, poultry, bee, mouse, rat***

December 8th, 2016

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Dear Participants and Dear Colleagues,

We are very pleased to welcome you at the 4th International Scientific Conference **"Animal Biotechnology 2016"**. The conference is organized by the Research Institute for Animal Production Nitra, National Agricultural and Food Centre in cooperation with the Faculty of Biotechnology and Food Science and Faculty of Agrobiotechnology and Food Resources, Slovak University of Agriculture Nitra.

The aim of the conference is the presentation of actual research from the field of animal biotechnology, with a special focus on the laboratory and small farm animals. The topic areas of the conference will involve the use of animal species as rabbit, poultry, bee, mouse and rat in genetics, reproduction, nutrition and health research. Moreover, the conference will provide an opportunity to gather researchers engaged in this and adjacent fields of research in order to exchange their skills and experience as well as to establish potential collaboration in a given task. We would appreciate the attendance and participation at this conference of colleagues from various research institutions and universities.

We wish you cordial and warm atmosphere at our conference for presentation, creative and fruitful discussion and inspiring ideas for future research.

Nitra, December 8th, 2016

Peter Chrenek

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

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THE RABBIT AS A MODEL AND FARM ANIMAL AT THE RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA: A REVIEW

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ABSTRACT

Use of the rabbit as a model and farm animals builds on the rich farming tradition in Slovakia. It is associated with the name of Jaroslav Zelnik, who put down the foundations for zootechnical and biological research of the rabbit at the early seventies of the 20th century. He built and managed the Department of Experimental Biology and Genetics, which had its own experimental farm for rabbit breeding. In the beginning of the existence of this department, the research program was focused mostly on the use of the rabbit as productive animals. The performance parameters of individual breeds were evaluated, the effects of inbreeding and hybridization on production abilities of rabbits were investigated and first rabbit broiler populations were created. Later, research activities were expanded to include physiological, immunological and behavioral experiments. At the turn of the eighties and nineties, genomic approaches and methods of biotechnology, especially in the area of reproduction, viability and genetic manipulations began to be used. In recent time, farming rooms have been reconstructed to meet the actual requirements of animal welfare. Concomitantly, the department has got a new laboratory equipment allowing experiments performed in a larger range of biological goals.

Key words: rabbit; breeding; model organism; biotechnological methods

INTRODUCTION

The institualization of research in rabbit breeding follows the years-long breeding tradition in Slovakia. The founder of this field of zootechnical and biological research was Jaroslav Zelník, who towards the end of the 70s established a specialized department for experimental rabbit husbandry within the Research Institute for Animal Production (RIAP) Nitra. At the beginning, this department focused on the zootechnical aspects of broiler rabbit production; later the experiments in the spheres of genetic, physiological, behavioral, immunological, morphological, and genomic biological research were conducted. The use of biotechnological and genomic techniques allowed the development of new methods, which expanded the existing knowledge on gene structure, function and expression. Nowadays this knowledge is extensively implemented in the development of rabbit husbandry.

Chronological development of rabbit research at the RIAP

Rabbit, a zoological species (*Oryctolagus cuniculus*), is an unexampled animal in consequence of several applications and uses similar importance and various economic values that depend on countries or economic interests. From this point of view there are five main directions of rabbit exploitation: 1) rabbit as livestock species kept mainly for meat, fur, skin and wool production; 2) rabbit as a hobby animal with broad possibilities for breeding process and competition in rabbit shows (exhibitions); 3) rabbit as mammalian

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model used in many biological branches; 4) rabbit as an effective biotechnological tool used to produce many recombinant proteins and other biological active substances (antibodies, antigens, hormones, coagulation factors etc.); 5) rabbit as a wild resource and model for processes in population level mainly in their natural and new settled regions. In recent years increasing interest has been addressed to this species to understand the biological factors affecting traits that are important in their numerous applied fields.

There is a years-long and voluminous tradition of rabbit husbandry in Slovakia established already in the mid-19th century. At the turn of the 19th century, numerous breeder societies are founded, which brought significant social and economic contributions to the husbandry of the existing breeds and creation of new ones.

In Slovakia, rabbit husbandry was applied to the research programs at the end of the 1960s at the Department of Genetics and Experimental Biology of the Research Institute for Animal Production (RIAP) Nitra. This department, composed of a breeding hall with capacity of 400 full-metal cages, laboratory facilities equipped for biochemical and physiological analysis of the biological samples and administrative offices, was implemented in 1972.

The initiator of the rabbit research at the RIAP Nitra and in Slovakia as a whole, was Jaroslav Zelník (1925–1986), who was focused primarily on the use of rabbit as a meat production animal. He developed a system for evaluation of growth performance, yield at slaughter, reproduction performance of rabbits and elaborated the methods for meat production breeding. In a series of publications, he evaluated the meat production of the existing rabbit breeds (Zelník, 1970; Zelník *et al*., 1971; 1977; 1978; 1984; 1985) and he studied the influence of inbreeding (Zelník, 1970; 1974; 1975) and hybridization (Zelník *et al*., 1977a; 1978a) on production markers. The result of these activities was the development of rabbit meat lines used directly for production or as a part of the newly-established hybridization projects (Zelník, 1976; Zelník *et al*., 1983).

Long-term cooperation between J. Zelník and small-scale breeders resulted in the creation of a national breed – "Nitra rabbit". This breed has been introduced into The Book of Rabbit Breed Standards within Slovak Breeders Association in 1977. The creation of this breed has been preceded by five-year intense work on the development of inbred lines using Himalayan rabbit and Argente Champagne rabbit breeds. The complete methods, as well as the basic morphometric and production characteristics of Nitra rabbit were disseminated in a series of publications (Granát and Zelník, 1972; Zelník *et al*., 1977b; 1983; 1985). In this period, breeding efforts were focused to another rabbit population, which was accepted twenty years later as a national breed under the name "Zobor rabbit" (Parkányi and Rafay, 1990). From the genetic point of view, Zobor rabbit is an example of an intergenic interaction of the recessive epistasis type. Homozygous alleles determining acromelanism, which belong to the allelomorphic albino series, create together with the homozygous recessive allele constitution for the Dutch spottiness the phenotype coat color of incomplete albinism, expressed in the absence of pigment in mask and the occurrence of reduced coloring of limbs. Due to the influence of other alleles on the coloring, this phenotype is difficult to stabilize on genetic basis, which represents a challenge for many breeders.

At the end of the 70s, the broiler rabbit farm was established in Podhorany near Nitra. Their capacity was about 6,000 breeding does, and it was the largest broiler rabbit breeding farm in the former Czechoslovakia.

The results of the breeding efforts in the 70s and 80s were manifested in the early 90s, when the changing political and social situation caused an interest in broiler rabbit breeding as a sphere of agriculture with a great commercial potential. The previous theoretical research as well as the creation of synthetic population (Rafay *et al.*, 1984; Jakubec *et al*., 1986; Oravcová *et al*., 1988) resulted in the establishment of maternal (M91) and paternal (P91) line. Besides the use for experimental research, these populations were used in several newly-built broiler rabbit farms.

During the period of the mid-80s, an extensive study of the chromosomal polymorphism in the rabbit lymphocytes was published (Parkányi, 1983; 1986), which then confirmed the importance of variability at the cytogenetic level for the vitality of rabbits. Later the variability in chromosome number was used as a criterion for genomic analysis of other cell cultures (Parkányi *et al.*, 2004) and the variability of electrophoretic fractions of histones in the rabbit lymphocytes (Palyga *et al*., 1990). Rabbit was used as an animal model for certain monolocus and polygene-determined anomalies of anatomical, morphological, and physiological structures. Their hereditary types in rabbits and their characteristic phenotype (alopecia, buphthalmia, mandibular prognathism, luxation, cyclopism etc.) were described (Zelník, 1979; Parkányi and Rafay, 1982).

In the early 1970s, methods of genetic biochemical polymorphism evaluation were developed at the department. Based on the tendency of this era to use electrophoretic protein fractions as production markers, the reproductive, growth and carcass characteristics of various genotypes of rabbits were evaluated. Later the biochemical genetic polymorphism was used for the determination of the homozygosity of inbred lines. Likewise, studies focused on the evaluation of the activity of selected

enzymes in blood and the descriptions of their genetic determination as well as their role from the standpoint of utility parameters were realized. Research in the field of physiology is perhaps well-represented by the experiments focused on the evaluation of the mitochondrial metabolic activity manifested by oxygen consumption dependent on the genotype (Rafay *et al.*, 1990). Phenomenon of mitochondrial heterosis was described, which correlated with the heterosis phenomenon in the intensity of the live weight gain (Oravcová *et al*., 1992).

In the field of behavioural genetics, the rabbits were used to study genetic determination of behavioural characteristics related to mobility and escape reaction. Open field concept was used to estimate the factors determining behaviour of rabbits under predefined conditions (Rafay and Schumacher, 1995; Rafay *et al.*, 1998; Zelník *et al*., 1990) and evaluation of the biochemical profile of their blood under different stress conditions (Parkányi *et al*., 1985; Rafay and Parkányi, 1987; Parkányi and Rafay, 1989; Klusek *et al.*, 1995; Kolataj *et al*., 2002; Witek *et al*., 2004; Swiderka-Kolacz, 2006). Part of the results of these studies helped in the adjustment of rabbit stabling facilities from the welfare standpoint.

Due to its advantageous biological characteristics, rabbit was used at the department also as a model animal for experiments in the field of reproduction (Bavin and Rafay, 1990; Kish *et al*., 2001; Makarevich *et al.*, 1994; 1998; 2008; 2010; Švarcová *et al*., 2003; Parkányi *et al*., 2008). In these studies the results of research oriented on the optimization of the superovulation process, evaluation of spermatogenesis under different experimental conditions, defining of the conditions for early embryo culture, influencing the ovarian functions of females by changes to the breeding conditions, as well as sex detection through PCR-SRY on the X-chromatin were published. The processes of artificial insemination (Makarevich *et al*., 1998; 2008; Ondruška *et al*., 2008) were optimized and the knowledge on the processes of oogenesis, spermatogenesis and fertilization of rabbits were disseminated.

Nutrition and the gastrointestinal processes in rabbits attract systematic attention. In addition to studies evaluating various feedstuffs from the aspect of their contribution to increased yield (Chrastinová *et al*., 2000; 2002; 2003), a series of experiments evaluated the intake of probiotic microorganism from the standpoint of vitality and yield parameters of animals (Pogány-Simonová *et al.*, 2009). Nowadays, attention is paid also to the effects of genetically modified feed components (Chrenková *et al*., 2013; Chrenková, 2014). The examination of the possible replacing of antibiotics with plant extracts with bactericide effects has begun in advance.

Application of biotechnological methods in animal models meant a qualitative shift in the research at the RIAP. Using the method of DNA microinjection into the pronucleus (Chrenek *et al*., 1998; 2005), first transgenic rabbits with a gene for human coagulation factor VIII were generated. These transgenic animals were subjected to detailed analyses of endocrine profile (Sirotkin *et al*., 2007), fertilizing capacity (Chrenek *et al*., 2005), digestibility of nutrients (Ondruška *et al*., 2010) and meat quality (Chrenek *et al*., 2009).

CONCLUSION

Current research programs are focused on the investigation of proper markers of vitality and production efficiency of rabbits. The future research is focused on the preservation of genetic resources of the national breed rabbits. Years-long and successful cooperation with breeders is a prerequisite for solving the task of the preservation of genetic resources of rabbits in the Slovak Republic.

Rabbit department of RIAP successfully cooperates with domestic as well as foreign scientific and educational institutions; the results are joint publications and exchange of study stays of researchers. Up to date, several dozens of PhD theses prepared by students of Slovak as well as foreign universities have been completed and successfully defended in the field of rabbit research. Nowadays, the experimental and breeding facilities have undergone a significant reconstruction and modernization, which meets stricter requirements for breeding and exploitation of rabbits as farm and experimental animals.

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WHITE LUPINE IS A SUITABLE FEED COMPONENT IN RABBIT DIETS: A REVIEW

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ABSTRACT

The paper reports the recent results regarding a possible utilization of white lupine seeds (*Lupinus albus* cv. Amiga) for rabbit feeds. The white lupine based diets were compared with the diets containing a commonly used protein sources, i.e. soybean or sunflower meals. The range of white lupine dietary inclusion used in different experiments varied between 60 and 250 $g.kg^{-1}$. The main attention was focused on the growth performance, total tract apparent digestibility of diets, digestive health and meat quality of growing-fattening rabbits, as well as rabbit doe milk yield and milk composition, and the growth of their progeny. No adverse effect of feeding white lupine-based diets on the average daily weight gain, feed intake, final live weight, or feed conversion ratio of growing-fattening rabbits, as well as milk production was observed. Due to its fatty acid composition, the dietary inclusion of white lupine has the potential to change both meat and milk fatty acid profile in a beneficial way, without the need for the addition of dietary fat. Protein source did not affect the total tract apparent digestibility of crude protein and gross energy. From the point of view of chemical composition and performance, white lupine is the important component for rabbit feeds.

Key words: rabbit; protein source; white lupine; growth performance; milk yield; health

INTRODUCTION

Protein sources mostly used in rabbit diets in Europe are soybean (SBM) and sunflower (SFM) meals, with inclusion levels of 80-150 $g.kg^{-1}$ (Volek and Marounek, 2009; Villamide *et al.*, 2010; Volek and Marounek, 2011). As reported by Kelly *et al.* (1990), whole white lupine seeds (WLS) may represent an alternative protein source for rabbit feeds. In comparison with SFM and WLS, SBM contained higher concentrations of crude protein (CP) and most of limiting amino acids (AA), and lower content of fibre fractions (Volek and Marounek, 2009). The biological quality of WLS, however, can be improved by adding synthetic AA (Ballester *et al*., 1980). Cellulose is the predominant constituent of the structural polysaccharides of lupine hulls (Volek *et al.*, 2013). When compared with soybean hulls, white lupine hulls contain more neutral-detergent fibre (NDF) and acid-detergent fibre (García *et al*., 1997; García *et al.*, 1999; Volek *et al.*, 2013). The favourable effect of a higher dietary fibre intake on rabbit digestive health is well known (Gidenne, 2015); thus removed lupine hulls might represent an agro-industrial by-product suitable for use in rabbit feed. White lupine seeds have higher levels of ether extract (EE), water-insoluble pectin and oligosaccharides of the raffinose series than SBM and SFM (Volek and Marounek, 2009). As far as the fatty acid (FA) composition of SBM, SFM, or WLS is concerned, monounsaturated fatty acids are the main FA in WLS, whereas polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) are present at lower amounts than in SBM and SFM (Volek and Marounek, 2011; Volek *et al*., 2014). White lupine seeds contain less palmitic acid (C 16:0) and linoleic

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acid (C 18:2n-6) and more eicosanoid acid (C 20:1n-9), oleic acid (C18:1n-9) and α-linolenic acid (C 18:3n-3) than SBM and SFM. Oleic acid is the predominant FA in WLS (Volek and Marounek, 2009; Volek *et al*., 2014). A high PUFA n-3/PUFA n-6 ratio is typical for WLS (Boschin *et al*., 2008; Volek and Marounek, 2011; Volek *et al*., 2014). Apart from the chemical composition, leguminous seeds may be also important for a greater self-sufficiency regarding the supply of protein to balance the diets of animals (Carrouée *et al*., 2003), as well as for the increasing of the sustainability of European crop-livestock systems (Jensen and Hauggaard-Nielsen, 2003; Annicchiarico *et al*., 2010; Sulas *et al*., 2016). In this respect, lupine seed, as one of the grain legumes, may be a useful European-grown source of protein (Chiofalo *et al*., 2012).

The aim of this paper was to report the recent results regarding the effect of the WLS (*Lupinus albus* cv. Amiga) based diets on the growth performance, total tract apparent digestibility, digestive health and meat quality of fattening rabbits, as well as rabbit doe milk yield and milk yield composition and the growth of their progeny.

Growth performance and carcass traits of fattening rabbits

In comparison with the diets based on SBM or SFM, Volek and Marounek (2009) did not observe adverse effect of feeding WLS on rabbit performance. Regardless of rabbits' weaning age (between 30 and 37 days of age), also other authors confirmed that WLS can fully replace traditionally used protein sources (SBM and SFM) without an impairment of the average daily weight gain, feed intake, final live weight or feed conversion ratio (Volek and Marounek, 2011; Volek *et al*., 2014; Uhlířová *et al*., 2015b; Uhlířová *et al*., 2016). Volek and Marounek (2009) recorded the higher dressing-out percentage in rabbits fed the WLS diet in comparison with those fed the SFM or SBM diet. Uhlířová *et al.* (2015b) reported a higher chilled and reference carcass weight in rabbits fed with WLS diet than in rabbits fed with the SBM diet but the dressing-out percentage was not affected by dietary treatments. Similarly, Volek and Marounek (2011) did not observe any effect of dietary treatments (WLS vs. SFM diet) on the dressing-out percentage. These contradictory results regarding the dressing-out percentage may be related to a full digestive tract and skin weight, as well as slaughter weights of rabbits used for the determination of carcass characteristics in different experiments.

Total tract apparent digestibility

There is an agreement with the literature, that CP sources (SBM, SFM, WLS) did not affect the total tract apparent digestibility of CP and gross energy (Fekete and Gippert, 1986; Volek and Marounek, 2009; Volek and Marounek, 2011; Volek *et al*., 2014; Uhlířová *et al.*, 2015b).

Digestive health of weaned rabbits

Digestive disorders are the main pathological events affecting weaned or fattening rabbits (Rosell *et al.*, 2009). Volek and Marounek (2009) observed a higher incidence of diarrhoea in rabbits fed the SBM diet than in rabbits fed the WLS diet (8 vs. 2 rabbits; 30 rabbits per group; P = 0.083). Similarly, Volek *et al.* (2014) reported the lower sanitary risk index (as the sum of morbid and dead rabbits; Fernández-Carmona *et al.*, 2005) caused by digestive disease in rabbits fed the WLS diet, than in rabbits fed the SBM diet (3.0 vs. 16.7 %; 66 rabbits per group; P = 0.016). Uhlířová *et al.* (2015b) observed both lower morbidity (1 rabbit vs. 9 rabbits; P = 0.014) and the sanitary risk index (2 rabbits vs. 12 rabbits; 40 rabbits per group; P = 0.006) in the *ad libitum* fed WLS rabbits compared with the *ad libitum* fed SBM rabbits. On the contrary, Uhlířová *et al.* (2016) observed the non-significant effect of dietary treatments on digestive health, although a lower number of animals at sanitary risk was observed in the rabbits fed the diet based on WLS than in those fed with the SBM diet (5 vs. 13 rabbits; 130 rabbits per group; P = 0.085). The above mentioned findings suggest that feeding with the WLS diet is probably safer than with the SBM diet. However, the other experiments on a larger number of animals (Gidenne, 2015), and mainly under the conditions affected by Epizootic Rabbit Enteropathy (ERE, a high mortality disease of rabbits), are necessary.

Fatty acid profile and indexes related to human health in hind leg meat and perirenal fat

Volek and Marounek (2011) studied the effects of a diet supplemented with WLS on FA composition and characteristics of hind leg meat and perirenal fat of growing-fattening rabbits in relation to human health. A total of 20 weaned rabbit (10 animals per treatment) were fed one of the two diets included SFM or WLS as the main protein and FA source. The WLS diet significantly decreased SFA and PUFA content, as well as the PUFA n-6/PUFA n-3 ratio and saturation, atherogenic and thrombogenic indexes in hind leg meat. The FA profile and indexes related to human health in perirenal fat were similar to hind leg meat. These results are consistent with the findings of Laudadio and Tufarelli (2011), who observed in broiler chickens that feeding the lupine diet (*Lupinus albus* L. cv. Multitalia) resulted in lower SFA content in meat, as well as the PUFA n-6/PUFA n-3 ratio, and saturation, atherogenic and thrombogenic indexes.

Milk yield and milk composition

A short-term lactation experiment (one lactation period) revealed that WLS may be a perspective dietary CP source for lactating rabbit does, which can fully replace commonly used SBM (Volek *et al.*, 2014). Uhlířová *et al*. (2015a) studied the effect of the lactation and weaning diets based on WLS (in comparison with the diets based on SBM) on milk yield and milk composition of rabbit does, as well as on the growth of their progeny through the longer lasting experiment (over two lactation periods). Significant differences were observed in terms of the daily milk production. During the 1st lactation period, average milk yield was higher between day 22 and day 32 of lactation in does fed the WLS, whereas in the 2nd lactation period, milk yield was significantly higher over the whole lactation (35 days) in these does. Milk dry matter, protein, fat or ash contents were not affected by dietary treatments. When expressed per kg of metabolic weight, milk output and fat output were significantly higher in the does fed the WLS diet. These findings are related to the higher dietary EE content in the WLS diet (Pascual *et al*., 2003), due to higher EE content in WLS than in SBM, and are consistent with our previous results (Volek *et al.*, 2014). The milk of does fed the WLS diet contained significantly less caprylic acid, capric acid, lauric acid and C 18:2n-6 and more C 18:1n-9, C 18:3n-3 and eicosapentaenic acid. Different milk fatty acid profile of does fed the WLS diet corresponds with fatty acid profile of WLS, and confirmed our previous findings (Volek *et al*., 2014). Growth of litters was not affected by dietary treatments. The longer lasting experiment confirmed that the WLS is a suitable CP source for the lactation diet of rabbits in terms of milk yield and composition, feed efficiency and growth of litters.

Lupine hulls as a dietary ingredient in rabbit feed

Volek *et al.* (2013) studied the effect of the inclusion of lupine hulls (50 g of WLS hulls.kg^{-1}) in a rabbit diet on the digestibility on nutrients and growth performance. The results revealed that WLS hulls can serve as a suitable by-products for rabbit feed. Other experiments should be focused on determining the maximal dietary level of lupine hulls.

CONCLUSION

No adverse effect of feeding white lupine based diets on the average daily weight gain, feed intake, final live weight, or feed conversion ratio of growing-fattening rabbits, as well as milk production was observed. Due to its fatty acid composition, the dietary inclusion of white lupine has the potential to change both meat and milk fatty acid profile in a beneficial way, without the need for the addition of dietary fat. Protein source did not affect the total tract apparent digestibility of crude protein and gross energy.

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PREBIOTICS AND SYNBIOTICS IN BROILER CHICKEN PRODUCTION: *IN VIVO* PERFORMANCE AND MEAT QUALITY ASPECTS: A REVIEW

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ABSTRACT

A large amount of antibiotics has long been used to control pathogenic diseases and as growth promoters to improve performance in livestock. However, this approach had significant and unwanted side-effects, such as development of antimicrobial resistance and carry-over of the antibiotic residues to poultry products. In this light, the use of antibiotics as growth promoters (AGPs) was banned by the European Union since 2006, based on their possible negative consequences for animal health and food safety. This ban has led to animal performance problems and the increased incidence of enteric diseases in farms, with serious economic damage. In the post-antibiotics era, probiotics, prebiotics and synbiotics are proposed as alternatives to AGPs in poultry production. To be effective, these compounds have to be administered to the animals under fully controlled conditions and as early as possible. *In ovo* technology enables delivery of sustainable bioactives, such as pre-/probiotics and their combination, directly into the egg air chamber at day 12 of embryonic incubation. Previously, different types of prebiotics and the routes of delivery, as well as their synergistic combinations with probiotics, were tested in field and laboratory trials also by our research groups. Some of the obtained results (*in vivo* performance, slaughter and meat quality traits) are described hereinafter.

Key words: broiler chicken; prebiotic and synbiotic; *in ovo*; performance; meat quality

INTRODUCTION

Over the last fifty years the worlds' poultry production has almost quadrupled. Moreover, over the last eight years, the costs of poultry feed ingredients have increased considerably. This has been due to a greater global feed grain demand and an increased use of corn for ethanol production. Nowadays, the efficiency of poultry to convert the feed into meat plays a key role in economics in broiler industry. In fact, the 70 % of total cost of production is contributed by feed (Willems *et al.*, 2013). Therefore, improvement of feed conversion ratio (FCR) will considerably increase the margin of profit. Between 1950 and 2000, the majority of poultry feeds contained antibiotic growth promoters (AGPs) used as a tool for the control of pathogenic diseases and for the efficient livestock production. AGPs act by modifying the intestinal microflora, especially against Gram-positive bacteria, which are associated with animals' poorer health performance. However, this approach had significant and unwanted side-effects, such as development of antibiotic-resistant pathogens and carry-over of the antibiotic residues to poultry products, such as meat and eggs. Therefore, the role of AGPs in the emergence of antibiotic resistance in humans has been questioned, and on the basis of the 'precautionary principle' (Turndige, 2004) the European Commission decided to ban AGPs. The last phase of the EU-wide ban on AGPs in animal feed took effect some years ago (EC Regulation No. 1831/2003).

The ban of antibiotics at sub-therapeutic level contributed to increased incidence of enteric diseases

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in farms, with serious economic consequences. Many alternatives have been investigated to replace antimicrobials without any loss of productivity or negative influence on health. Probiotics, prebiotics and synbiotics are one of the proposed solution, as alternatives to AGPs, to prevent enteric disease and increase performance in poultry. As claimed by some authors, alternative for AGPs are of practical significance, when they improve animal performance at levels comparable to AGPs. There is a growing interest in the use of a variety of probiotics and prebiotics in several feeding trials in broiler chickens to promote animal health by altering the intestinal microbial community (Awad *et al*., 2008).

Probiotics are live microorganisms which, when administered in adequate amounts, exhibit a health benefits on the host, including: regulation of bacterial homeostasis, stabilization of gastrointestinal barrier function (Salminen *et al.*, 1996; Gaggìa *et al*., 2010), expression of bacteriocins (Mazmanian *et al*., 2008; Gaggìa *et al*., 2010), immunomodulatory effects (Salzman *et al.*, 2003; Gaggìa *et al*., 2010). Prebiotic (fructooligosaccharides, inulin, galactooligosaccharides, transgalacto-oligosacchiarides, raffinose family oligosaccharides) has been defined as "non-digestible food ingredients that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" (Gibson and Roberfroid, 1995), especially bifidobacteria and lactobacteria (Baffoni *et al*., 2012). They are not hydrolysed or absorbed in the upper tract of digestive system. Prebiotics are a source of carbon and energy for the friendly strains of bacteria already inhabiting in the colon, where bacterial fermentation processes of some nutrients occurs (Dankowiakowska *et al.*, 2013). Some studies have suggested that synbiotics, a mixture of probiotics and prebiotics, is the best option to active the metabolism of one or a limited number of health promoting bacteria and/or by selectively stimulating their growth improving the host's welfare and thus the growth (Gibson and Roberfroid, 1995; Slawińska *et al*., 2014; Kamel and Mohamed, 2016). The main importance of this form of synergism is that a probiotic alone, i.e. without a source of nourishment, which can be represented by a prebiotic, cannot survive well in the digestive system. Some researchers reported the importance and benefits of this kind of synergy between probiotics and prebiotics and the effectiveness in helping young animals to achieve better growth performance (Patterson and Burkholder, 2003). There are different ways to deliver these bioactive substances into avian gastrointestinal tract. Conventionally, in-feed or in-water supplementation has been used at first hours/days post-hatching. This approach relies on amount of feed and/or water intake, the quality of water (chlorinated) and other experimental factors (Bednarczyk *et al*., 2016). As a consequence, consumed dose of prebiotics varies in the first hours/days after hatching. Furthermore, during early post-hatching period, infection of chicks by detrimental bacteria is also possible. Therefore, to be effective, these compounds have to be administered to the animals under fully controlled conditions and as early as possible. In fact, some recent research tends to exclude the unwanted effects of several factors that may affect the action of supplements.

In ovo technology enables delivery of sustainable bioactives, such as pre-/probiotics and their combination, directly into the egg air chamber at day 12 of embryonic incubation; it allows for a precise delivery of the bioactive substance to all embryos, which equalizes the effects across the flock and assures proper development of the gut microflora in all chicks. Previously, different types of prebiotics and their synergistic combinations with probiotics were tested in field and laboratory trials also by our research groups.

Bioactive substances used and results obtained

During the last years, different prebiotics and their synergistic combinations with probiotics were tested in field and laboratory trials by our research groups:

a) commercial prebiotics, as DN (DiNovo®, BioAtlantis Ltd, Tralee, Co., Kerry, Ireland) a *Laminaria* spp. seaweed extract containing laminarin and fucoidan, and BI (Bi²tos, Clasado Ltd, Sliema, Malta) a non-digestive trans-galactooligosaccharides (GOS) from milk lactose digested with *Bifidobacterium bifidum* NCIMB 41171; RFO (raffinose family oligosaccharides) in-house extracted from lupin (*Lupinus luteus*) seeds (Gulewicz *et al*., 2000).
b) different synbiotic preparations (SYN1: BI + *Lactobacillus salivarius*; SYN2: RFO + *Lactobacillus plantarum*).

Some of the obtained results (*in vivo* performance, slaughter and meat quality traits) in different trials are reported below.

Trial 1

A trial was performed to evaluate the effect of different prebiotics (DN, BI and RFO) and mode of their administration on *in vivo* performance, carcass and meat quality traits in Ross 308 broiler chickens (Bednarczyk *et al*., 2016). The prebiotics were used for comparison between different routes of delivery: *in ovo* injection (T1), *in ovo* injection combined with in-water delivery (T2) and in-water delivery (T3). Control group (C) was injected *in ovo* with physiological saline only and did not receive any prebiotic in-water. Hatching eggs were collected from the same breeder flock and incubated in the commercial broiler hatchery. At day 12

of incubation 1500 eggs, containing viable embryos, were randomly allotted into four experimental groups (375 eggs per group). Eggs were injected *in ovo* with 0.2 mL solution containing: 3.5 mg.embryo^{-1} BI, 0.88 mg.embryo^{-1} DN and 1.9 mg.embryo^{-1} RFO. The C group was injected *in ovo* with physiological saline only and did not receive any prebiotic in-water. Following injection, each hole was sealed with hot glue and the egg incubation was continued until hatching. Solutions of prebiotics were injected *in ovo* using dedicated automatic system (Bednarczyk *et al*., 2011). After hatching chicks were sexed and 600 males (42.0 g average weight) were randomly assigned to ten experimental groups (60 males per group): T1 (DN, BI and RFO), T2 (DN, BI and RFO), T3 (DN, BI and RFO) and C. Chicks from T1 and C groups were raised without any additional supplementation with prebiotic. T2 and T3 groups were supplemented in-water with respective prebiotic (DN, BI or RFO) for first seven days of life. Those animals received 12 ml of the prebiotics dissolved in water per pen (20 mg of prebiotic.ml^{-1}). Birds were grown up to 42 days of age in collective cages (n = 6 replicate cages, 10 birds in each cage). Broilers were fed commercial diets *ad libitum* according to age. Amounts of feed offered to each cage were recorded. Feed intake (FI) and feed conversion ratio (FCR) were calculated on a cage basis. The prebiotics increased body weight gain (BWG), especially during the first 21 days of life, irrespective of route of delivery (T1, T2 or T3), as compared with the C group ($P < 0.05$). These results provide further support for the hypothesis concerning well-established growth promoting effect of dietary prebiotics, attributed to their ability to strongly bind the pathogenic bacteria and decoy pathogens away from the intestinal lining. Prebiotic-treated chickens showed trend for increased FI and FCR; this could be due to the stimulation of the intestinal microbiota expansion in the chicken guts by the injection of prebiotics during the *in ovo* development. In fact, it has been suggested that the effect of prebiotics on chicken growth performance could be related to metabolism modification linked to an increase in the digestive enzymes activity (Pruszynska-Oszmalek *et al*., 2015), the decrease in bacterial enzymes activity and ammonia production along with the improved feed intake and digestion (Kabir, 2009). Our results indicate a positive stimulation of the broiler body weight (BW) expressed as soon as in the starter period (1-21 days), which might be explained by early supplementation of chicken embryos with prebiotics using *in ovo* method. However, injection of prebiotics in ovo combined with in-water supplementation did not express synergistic effects on broiler performance compared to *in ovo* injection only. These results confirm that single *in ovo* prebiotic injection into the chicken embryo can successfully replace prolonged in-water supplementation post-hatching.

Carcass weight and yield were unaffected by prebiotics. However, *in ovo* administration significantly increased carcass weight and yield compared to in-water administration. On the contrary, pectoral muscle (PM) weight was significantly higher in all prebiotic groups, regardless of the mode of administration, compared to the C group. All prebiotics increased significantly fiber diameter (μm) when compared with the untreated control. No differences were observed with respect to mode of application. The histological observations showed a trend towards intramuscular fat infiltration in the DN group when compared with the C group ($P = 0.07$), whilst no differences were found among the other groups and the different methods of administration. These differences in the intramuscular fat content could be related to different growth rate and feed conversion efficiency. Cholesterol levels in PM were unaffected by prebiotics or methods of application. The total saturated fatty acid (SFA) and monounsaturated fatty acids (MUFA) amount was affected neither by prebiotics nor by mode of administration. The obtained results on SFA content are consistent with the study of Rule *et al*. (2002) conducted on broiler chicken. The total polyunsaturated fatty acid (PUFA) content was similar in all experimental groups, however, prebiotic groups had a slightly higher ($P = 0.082$) amount of PUFA compared to C group. Regarding the selected fatty acid ratios, only the ratio of PUFA to SFA (P/S) was significantly different among experimental groups with higher ($P < 0.01$) value for DN group compared to the control one. The obtained value of P/S ratio is a little bit higher than the recommended value of 0.4 –0.7, even if it is lower than values of other meat species (Wood *et al*., 2003). Anyway, the obtained data showed a particularly lower n-6/n-3 ratio due to the higher incidence of n-3 fatty acids, probably due to the inclusion of n-3 fatty acids into the diet administered to the birds. This is a positive aspect for a nutritional point of view, because the obtained value is assigned between the ideal value of 1 and the maximum value of 4. In addition, meat from all experimental groups is characterized by low values of atherogenic index (AI) and the thrombogenic index (TI), even though are similar among groups. These indices, calculated according to the formulas suggested by Ulbricht and Southgate (1991), take into account the different effects, which the single fatty acid might have on human health and, in particular, on probability of increasing the incidence of pathogenic phenomena, such as atheroma and/or thrombus formation (Ulbricht and Southgate, 1991).

In conclusion, *in ovo* administration of prebiotics was associated with the improvement of body weight, PM weight and PM fiber diameter, which are relevant

for commercial poultry production. Prebiotics significantly improved fatty acid profile and nutritional ratios of meat. Delivery *in ovo* combined with in-water supplementation of prebiotics did not show synergistic effects on broilers performance compared to *in ovo* injection only. These results confirm that a single injection of prebiotics into the chicken embryo can successfully replace prolonged in-water supplementation post-hatch. At the same time, the amount of the prebiotic used was at least ten times lower in case on *in ovo* method (3.5 mg BI.embryo^{-1} *in ovo* vs. 40 mg BI.chick^{-1} in-water). As such, *in ovo* method should be further recommended to the poultry industry.

Trial 2

The study was carried out to evaluate effect of two different synbiotics (SYN1 and SYN2) on *in vivo* performance and meat quality traits in broiler chickens (Cobb 500FF). Hatching eggs were collected from the same breeder flock and incubated in the commercial broiler hatchery. On day 12 of incubation, 5850 eggs were divided into 3 experimental groups treated with different bioactives, *in ovo* injected: SYN1, the group injected with 0.2 ml of a synbiotic formulation containing 2 mg.embryo^{-1} of Bi^{2}tos (ClasadoBioSciences Ltd.), trans-galactooligosaccharides enriched with 105cfu/embryo of *Lactobacillus salivarius* IBB3154; SYN2, group injected with 0.2 ml of a synbiotic formulation containing 2 mg.embryo^{-1} of raffinose family oligosaccharides (RFO) enriched with 105 cfu. embryo^{-1} of *Lactobacillus plantarum* IBB3036; C, control group injected with 0.2 ml of physiological saline solution. The injection hole was covered with a drop of organic glue and the incubation was continued until hatching. Among the hatched chickens, 2040 males (680 per each group) were randomly chosen and reared in a commercial poultry house (PiastPasze Sp. z.o.o., Olszowa, Poland). Chickens were raised in pens (n = 75 per pen) with 8 pen replicates per treatment for effect on performance. Moreover, separate pens for sampling (n = 10 birds per pen: 8 replications per each experimental group) were included in the experimental design. Animals were fed *ad libitum* with commercial diets according to their age and had free access to water. The FI and FCR were calculated on a pen basis. At 42 days of age, two birds per pen (16 birds per treatment) were randomly chosen from the separate pens for sampling and slaughtered. At slaughter, hot carcass weight was recorded and carcass yield was calculated. The PM was removed from each carcass and weighed; its percentage was calculated basing on hot carcass weight. At 24 hours *post-mortem*, pH, colour and water holding capacity (WHC) were recorded on the right PM. The left PM was vacuum packaged and frozen until chemical analysis for total lipids, cholesterol and fatty acids.

In ovo synbiotic administration had no significant effect on mortality, growth performance and slaughter traits (carcass weight and yield, breast weight and yield). Similarly, physicochemical characteristics (pH, color, WHC), intramuscular collagen content and the degree of collagen maturation (hydroxylysylpyridinoline crosslink/collagen) of PM were not significantly affected by synbiotics. Differently, synbiotic administration had a significant effect on total lipid and fatty acid composition, but it depended on the kind of bioactivities administered. SYN2 lowered ($P = 0.06$) the muscle lipid content. The results on fatty acid (FA) composition showed a marked difference in the the proportion of several FA among the experimental groups. Meat from SYN1 group, compared with that of C and SYN2 groups, displayed an unfavorable FA profile due to: i) higher ($P < 0.01$) content of total saturated fatty acids (SFA); ii) lower monounsaturated fatty acids (MUFA) ($P < 0.05$ compared to SYN2); iii) lower ($P < 0.01$) polyunsaturated fatty acids (PUFA); and iv) lower n-6 PUFA ($P < 0.01$) and n-3 PUFA ($P < 0.01$ and $P < 0.05$ compared to C and SYN2, respectively). From the nutritional point of view, a higher P/S ratio is recommended; indeed it should be increased over 0.4. Atherogenic and thrombogenic indexes were significantly lower in SYN2 and C groups compared to SYN1. Total cholesterol content was similar among groups (41.10 ± 1.70 mg.100 g^{-1}).

In conclusion, the results of this study indicate that *in ovo* administration of synbiotics did not negatively affect productive performance and physiochemical properties of meat. However, the meat from C and SYN2 birds showed a preferable fatty acid profile, with a positive effect on nutritional properties of the chicken meat.

CONCLUSION

Thanks to the experience and the new knowledge acquired by our team over the years, during field and laboratory studies, we are able to give a fairly complete application of the innovative *in ovo* technology of bioactive compound delivery for improvement of the multiple production and health traits in broiler chickens, including growth rate, feed intake and nutrient digestibility, as well as meat quality. Nevertheless, future studies need to delve more into the mode of action of these bioactive substances in order to promote the use of pre-/synbiotics, which are consumer- and environment-friendly and contribute to the reduction of antibiotic use for therapeutic treatment in poultry production. This will open *in ovo* injection for a large scale application in different production systems.

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SOME ASPECTS OF POULTRY BIOTECHNOLOGY: A REVIEW

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ABSTRACT

Animal biotechnology is the application of scientific and engineering principles to the processing or production of materials by animals to provide goods and services. Sometimes the animal biotechnology has been limited to genetic-based biotechnology only. However, the animal biotechnology uses the other, different techniques, such as artificial insemination, embryo transfer, *in vitro* fertilization, embryo culture, cloning by nuclear transfer from embryonic or adult somatic cell, etc. Due to the specificity of embryonic development in birds, which occurs inside the egg, the growing embryo can be directly manipulated via a window that is cut on the eggshell at a very early embryonic stage. This fact was used to develop *in ovo* technology – direct administration of a bioactive substance suspended in a solution to the incubating egg. This review integrates recent progress and new insights into methods of transgenic bird production (A), and possibility to modify the avian development (B).

Key words: chicken; biotechnology; transgenesis; *in ovo*

INTRODUCTION

A. Avian transgenesis

The production of transgenic birds has been hampered by the yolk-laden structure of the ovum and their unique reproductive system. The transgenic chickens have been produced by two different procedures, in general. The first is based on the viral transfection systems and the second non-viral, uses the genetically modified embryonic cells, transferred directly into the recipient embryo. Although viral transfection systems allows for efficient introduction and expression of transgenes in chicken dividing and non-dividing cells also (McGrew *et al.*, 2004), they have some important limitations: (i) restriction in the size of the vector genome to less than 8 to 10 kb (Byun *et al*., 2011), (ii) vector insertion can cause the disruption of endogenous genes by insertional mutagenesis or the transactivation of neighboring endogenous genes (Li and Lu, 2010), (iii) integrated lentiviral vectors are subject to positional effects (Yi *et al.*, 2011). However, the much more important limitation is a public concern, which has questioned the safety of lentivirus-based technology. In this situation some alternative strategies were developed, and the idea of generation of transgenic chicken through chimeric intermediates was described (Raynaud, 1976; Petitte *et al.*, 1990). The generation of transgenic chickens has been attempted through chimeric intermediates produced by the transfer of blastodermal cells. The same idea was proposed in many other experiments, however in this case primordial germ cells (PGC), precursors of gonads, were proposed as the vehicle for introduction the transgene into the chicken genome. PGCs are especially increasingly being used in research on the development of chicken bioreactor. Chicken bioreactors provided, among others, human erythropoietin (Koo *et al*., 2010), interferon alpha- 2b (Rapp *et al*., 2003), interferon beta-1a (Lillico *et al*., 2007), monoclonal antibodies (Kamihira *et al*., 2005), granulocyte colony stimulating factor (G-CSF) (Kwon *et al*., 2008). Genetic modifications may also be used in reducing the negative impact of poultry

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production on environment condition. Introduction of salivary phytase transgenes into chicken can solve the problem of environmental pollution with phosphorus, by forcing its distribution in the body of animal (Sang, 2003).

Now, the novel, promising strategy, allowing efficacious enrichment of manipulated chicken PGCs on the basis of genome editing, has been proposed (Park *et al.*, 2014; Oishi *et al*., 2016). Highly efficient and precise genome editing tools are actively adapted in poultry species, and in the near future will create the new bioindustry in poultry (Han *et al*., 2016).

B. Modification of avian development

Generally, *in ovo* method enables the administration of a bioactive substance: carbohydrates, fatty acids, amino acids, minerals, vitamins, nanoparticles, prebiotics, probiotics or synbiotics directly to the incubating egg. As a consequence, *in ovo* delivery of bioactives not only have improved performance traits, such as the growth rate, feed intake, nutrient digestibility (Ohta *et al*., 1999; Bednarczyk *et al*., 2011) and meat quality (Maiorano *et al*., 2012), but also significantly increased activity of some enzymes (Liu *et al*., 2013; Pruszynska-Oszmalek *et al.*, 2015) and influenced immune system development and function (Bhanja and Mandal, 2005; Bakyaraj *et al*., 2012; Slawinska *et al.*, 2014; Madej and Bednarczyk, 2016; Madej *et al.*, 2015; Plowiec *et al*., 2015).

CONCLUSION

Thanks to these new techniques of cells isolation, manipulation and modification, as well as thanks to *in ovo* embryogenesis modification, bird biotechnology has had, and will also certainly have in future, important place in the improvement of animal health and productivity.

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SECTION I.: Laboratory and Small Farm Animals – Rabbit

PHENOTYPIC ANALYSIS OF RABBIT MESENCHYMAL STEM CELLS USING FLOW CYTOMETRY AND RT-PCR

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The aim of this preliminary study was to analyze the phenotype of rabbit mesenchymal stem cells (MSCs) derived from bone marrow using immunological method (flow cytometry) and molecular technique (RT-PCR). Four young (6 months old) and clinically healthy rabbits of New Zealand White (NZW) line reared in a partially air-conditioned hall of a local rabbit farm at APRC Nitra were used in the experiment. Mononuclear cells were isolated using Biocoll solution from the rabbit bone marrow, resuspended in MEM-Alpha medium with supplements and then plated on a T75 tissue culture flasks. Cells were cultured for 3 weeks until the passage 3. MSCs were then dissociated using 0.05 % Trypsin-EDTA. Cell aliquots were afterwards divided into prepared tubes with antibodies against CD29, CD44, CD73, CD105, CD166, CD45 and CD34 and stained according to manufacturer's instructions. Stained cells were counted using flow cytometer FACS Calibur. The same CD markers were evaluated using RT-PCR. Total RNA from 4 samples were extracted using TRI Reagent, and then cDNA was synthesized from 2 µg of RNA using Moloney murine leukemia virus (M-MLV) - reverse transcriptase according to manufacturer's instructions. The rabbit GAPDH gene served as an internal amplification control. The flow cytometry revealed that rabbit MSCs were highly positive for CD29 and CD44 (85.4 ± 2.0 % and 86.7 ± 5.1 %, respectively), dim positive for CD166 (14.5 ± 2.0 %) and negative for CD73, CD105, CD45 and CD34 (1.9 ± 1.3 %, 2.1 ± 0.3 %, 6.8 ± 1.2 % and 1.2 ± 0.2 %, respectively). However, the molecular analysis showed that beside CD29, CD44 and CD166, rabbit MSCs expressed also CD73 and CD105. On the other hand, they were negative for CD45 and CD34. As a positive control for these unexpressed CD markers, an RNA sample from fresh PBMCs was used. This discrepancy in observed results might be caused by the non-specificity of the antibodies (CD105 and CD166) used for flow cytometry. In conclusion, although the flow cytometry is a quite objective and efficient method for the cell phenotype determination, molecular analysis, such as RT-PCR, also should be used in order to characterize properly the typical CD markers expressed by the specific type of stem cells.

Key words: rabbit; MSCs; flow cytometry; RT-PCR

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TYROSINASE ASSOCIATION WITH PIGMENTATION OF THE RABBIT BANDING COAT

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The study was aimed at identifying the association of the tyrosinase gene polymorphism with changes in pigmentation in the banding coat of rabbits. We analysed tyrosinase DNA sequences in selected groups of rabbits with banding hair: 6 animals of Big Chinchilla, 3 animals of Wild rabbit, 4 animals of Vienna Wild, 3 animals of Dwarf Chestnut Rex and 38 animals of Marten Blue rabbit. Comparing obtained sequences of these rabbit breeds with tyrosinase sequences available in GenBank (AF210660 and NC_013669), within c.881A>G position in the second exon of tyrosinase gene, we found an association of this position with the pigmentation of banding hair. The AA genotype for c.881A>G position has band with dark pigment (all the above rabbit breeds besides Big Chinchilla), while the GG genotype (only in Big Chinchilla) has snow-white band, completely without pigment. This GG genotype without pigment in band corresponds exactly with the GG genotype on c.881A>G position of acromelanic albino with Himalayan characters (typical for Russian, Californian rabbit and Rabbit of Nitra). There is a difference among these specific acromelanic albinos and Big Chinchilla rabbits in the tyrosinase exon 3 in c.1073C>T position. Big Chinchilla rabbits have TT genotype, whilst acromelanic albinos as well as two colour phenotypes of Marten Blue rabbit (dark and light standard) have CC genotype in c.1073C>T position.

Key words: rabbit banding coat colour; tyrosinase

C-REACTIVE PROTEIN LEVELS IN RABBITS WITH DIFFERENT CRP GENOTYPE AFTER VACCINATION

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The aim of this study was evaluation of the acute phase reaction and immune response of rabbits triggered by vaccination with two standard vaccines. The C-reactive protein (CRP) is one of the acute phase proteins, plasma proteins, which change their concentration in response to an inflammatory or infectious process, regardless of the causative agent. In the experiments, 20 juvenile (at the age of 42 days) crossbreed rabbits (the New Zealand-based line of white rabbits) were used. Rabbits were genotyped for CRP promoter by PCR-HRM analysis. Samples of rabbit peripheral blood were collected from *vena auricularis centralis* to heparinized tubes before vaccination, and 48 hrs or 96 hrs after vaccination. Animals with known CRP promoter genotype (AC/TT at positions -119 and -222, respectively) were divided into two groups: one group (MXT; n = 10) was vaccinated by subcutaneous injection (0.5 ml) with

a live attenuated myxoma virus vaccine (Pharmavac MXT, Pharmagal Bio, Slovak Republic), second group (RHDV; n = 10) was vaccinated by subcutaneous injection (0.5 ml) with an inactivated RHDV vaccine (Castorex, Pharmagal Bio, Slovak Republic). The blood plasma C-reactive protein (CRP) level was measured by ELISA kit using a double-antibody sandwich. Analysis of the results was performed by t-test with the level of significance at $P < 0.05$. The results are expressed as means ± standard deviation. In the MXT group the plasma CRP level was significantly higher following 48 and 96 hrs after MXT vaccination. The initial (0 hrs) CRP plasma level was 6.09 ± 0.93 mg.l^{-1}, following 48 hrs - 7.23 ± 1.39 mg.l^{-1} ($P < 0.05$), and following 96 hrs it was 7.44 ± 1.76 mg.l^{-1} ($P < 0.05$). From the individual point of view, 4 animals in this group had higher concentration of CRP at 48 hrs than the mean value for the group (5.2, 7.5, 5.8, 5.48 mg.l^{-1} before vaccination and 9.24, 9.57, 7.21, and 7.53 mg.l^{-1} at 48 hrs, respectively). Due to faster and higher increase in CRP plasma level, these individuals are probably more suitable for use in vaccination programs. On the other hand, we found no significant increase in the plasma CRP level after RHDV vaccination. The animals in RHDV group showed CRP plasma level 6.38 ± 1.36 mg.l^{-1} at 0 hrs, 6.72 ± 1.37 mg.l^{-1} ($P < 0.5$) at 48 hrs, and 6.36 ± 2.09 mg.l^{-1} at 96 hrs. One animal in this group showed faster and higher increase in CRP plasma level at 48 hrs after RHDV vaccination than the mean value for the group (from 5.01 to 9.55 mg.l^{-1}). The immune response of the juvenile rabbits to attenuated myxoma virus was different than the immune response to inactivated RHDV. These finding suggests that virus-specific contribution to pathogenesis by interacting with inhibiting host proteins is involved in the regulation of inflammation.

Key words: rabbit; vaccination; C-reactive protein

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EFFECT OF VERBASCOSIDE AND LYCOPENE DIETARY SUPPLEMENTATION ON SEVERAL PLASMA OXIDATIVE MARKERS IN WEANING RABBITS

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Supplementation with plant extracts rich in polyphenols and carotenoids reduces plasma oxidative stress in farm animals and, at the same time, improves animal welfare. The active additives that were used, lycopene and verbascoside, are considered powerful antioxidants that act both with a directly scavenger activity on reactive oxygen species, and by preventing the chain reactions caused by free radicals. The aim of the study was to evaluate the effect of feed addition with verbascoside and lycopene extracts on several plasma oxidative parameters in growing rabbits. In the experiment, 120 New Zealand white rabbits were used for a period of 60 days, and divided into 4 groups of 30 animals per each, fed the following dietary treatment: one group received control feed (CON) and the other three experimental groups received 5 mg of lycopene per kg of diet (LIC group), 5 mg of verbascoside per kg of diet (VB group) and the third received combination of both extracts with the same dose (MIX group). From blood samples, taken at 0 d, 30 d and 60 d, the following plasma oxidative markers were determined: ROMs, TBARS, retinol and alpha-tocopherol. The results showed positive effect of lycopene and verbascoside dietary treatment resulting in a marked improvement ($P < 0.05$) of plasma oxidative profile in rabbits. In particular, the experimental groups manifested lower values of ROMs (LIC group -29.5 %, VB and MIX group -37.0 %) and TBARS (LIC group - 42.2 %, VB group - 46.8 % and MIX group - 48.5 %) compared to the control group. The use of plant extracts caused also an increase in the content of plasma fat-soluble vitamins, such as retinol and alpha-tocopherol. In this case, LIC and VB groups presented an increase of 18.4 % and 32.3 % of retinol, and 106.3 % and 96.9 % of alpha-tocopherol, respectively. The use of plant lycopene and verbascoside dietary extracts improved the plasma oxidative markers, manifesting a protective activity at the cellular level counteracting free radicals.

Key words: feed additives; verbascoside, lycopene, oxidative markers, rabbit

EFFECTS OF ENTEROCIN M AND DURANCIN ED26/E7 APPLIED TO BROILER RABBITS ON MICROBIOTA AND SELECTED PARAMETERS

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Bacteriocins are natural proteinaceous substances with anti-microbial effect, but (known from our previous studies) they can stimulate also phagocytic activity (PA) or weight gain of animals. Most bacteriocins produced by enterococci are represented by enterocins; they are mostly related to the species *Enterococcus faecium*. However, enterocins produced also by the species *E. mundtii*, *E. hirae* or *E. durans* were found. The species *E. faecium* and *E. durans* belong to *E. faecium* group of enterococci. Enterocin M used in our study is produced by the environmental strain *E. faecium* AL41/CCM8558 and durancin ED26E/7 is produced by *E. durans* ED26E/7 (from ewes lump cheese). Strains and enterocins were characterized at our laboratory. Among food-producing animals, rabbits are frequently bred for the nutritional aspect of their meat. Health status of the animals can be beneficially influenced by natural substances. Following our previous studies using the beneficial properties of enterocins in poultry but also in rabbits, this study has been focused on comparison of effect of Ent M and durancin ED26E/7 in rabbits on their microbiota and other parameters related to their health status. Rabbits of meat line M91 were used. Rabbits (72, housed individually in cages) were divided into 3 groups; experimental (E1-Ent M, E2-durancin ED26E/7) and control (C), 24 rabbits in each. Common breeding regimen was a cycle 16 h of light, 8 h of dark; temperature 16 ± 4 ºC, humidity 70 ± 5 %. Animals fed commercial granulated diet for growing rabbits (dry matter 847.49g.kg^{-1}, crude fibre 146.97 g.kg^{-1}, N-substances 177.99 g.kg^{-1}) *ad libitum* with free access to water. Enterocin M and durancin (50µl.animal^{-1}.day^{-1},

activity 12 800 arbitrary units per ml) were applied into water of E1, E2 rabbits for 21 days. Sampling was done at the start of the experiment (faeces, blood), on day 21 and 42 (end of the experiment). On day 21 and 42, animals were slaughtered, faeces, caeca, appendix, blood and *Musculus longissimus dorsi* were sampled. In faeces of E2 the counts of bacteria were not influenced by enterocins. In caecum and appendix, the reduction of coliforms on day 21 was found (difference 2.67 log cycle; 1.80 in E2 compared to C). In appendix clostridia were also reduced (1.33 log cycle, 0.42). By Ent M (E1) pseudomonads were reduced in faeces and caecum. Moreover, significant reduction of coliforms in faeces of E1 was noted on day 42. PA of both groups E1, E2 was significantly increased on days 21 ($P < 0.001$) and 42 ($P < 0.01$) compared to C. Glutathion-peroxidase values, biochemical parameters (total proteins, cholesterol, triglycerides, alaninaminotranspherase, calcium), *Eimeria* oocysts, meat quality and morphometry were not negatively influenced by both enterocins. In conclusion, Ent M and Durancin ED26E/7 seem to be suitable additives in rabbit husbandry.

Key words: rabbit; beneficial effect; enterocin; durancin

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SEX-DEPENDENT EFFECT OF QUERCETIN ON COMPACT BONE MICROSTRUCTURE IN ADULT RABBITS

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Quercetin, the most abundant bioflavonoid in the human diet, has a beneficial effect on human health. It has cardioprotective and anti-carcinogenic properties. Some studies suggest its protective effects against the bone atrophy. Nevertheless, the effect of quercetin on bone microstructure is not sufficiently described. Therefore, the purpose of our study was to investigate the impact of quercetin on femoral bone microstructure in 5-month-old rabbits of either sex. Animals from experimental groups (group E♀, n = 3; group E♂, n = 2) received quercetin intramuscularly at dose of 100 $\mu g.kg^{-1}$ body weight for 90 days, three times per week. Five rabbits without quercetin administration served as a control group (group C♀, n = 3; group C♂, n = 2). Qualitative histological characteristics of the compact bone in rabbits exposed to quercetin were different as compared to the control group. In rabbits from the E♀ and E♂ groups primary vascular longitudinal bone tissue was not observed in some areas near endosteal surfaces. In these rabbits, evident changes were present in the middle part of the substantia compacta, where primary vascular longitudinal bone tissue was identified and expanded from the periosteum. Concurrently, in male rabbits from the group E♂, a lower number of secondary osteons was recorded in the middle part of the compact bone. From the histomorphometrical point of view, a significantly increased ($P < 0.05$) sizes of primary osteons' vascular canals were observed in female rabbits from the C♀ in comparison with the C♂, E♂ and E♀ groups. On the contrary, males from the E♂ group have significantly increased ($P < 0.05$) area and perimeter of primary osteon vascular canals as compared to females (E♀). Haversian canals showed greater values ($P < 0.05$) in females from the C♀ and E♀ groups when compared to the E♂ group. Similarly, the sizes of secondary osteons were significantly increased ($P < 0.05$) in males from the E♂ compared to the C♂, E♀ and C♀ groups. On the other hand, no significant differences in trabecular bone microstructure (including bone volume, trabecular number, trabecular thickness, trabecular separation and bone surface) and cortical bone thickness were found among all analysed groups of rabbits. It can be concluded that intramuscular application of quercetin at the dose used in this study has sex-dependent effect only on the compact bone microstructure.

Key words: compact bone; microstructure; quercetin; rabbit; trabecular bone

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SECTION II.: Erasmus PhD students – Welfare, Biotechnology and Animal Food quality

EFFECT OF DIFFERENT CRYOPROTECTANTS ON ROOSTER SPERMATOZOA CRYOPRESERVATION

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Sperm cryopreservation is the only effective method for the *ex situ* management of genetic diversity in birds ensuring the creation of semen cryobanks, but remains challenging obstacle affecting the survivability of rooster sperm. Therefore, effective freezing protocols are needed. This study aims to determine the effect of different cryoprotectants on the motility of Ross 908 rooster sperm. Semen was collected from ten roosters, pooled and divided into five aliquots. After primary dilution (1:1 v/v) with Kobidil+ extender at room temperature, semen was cooled down to 5 °C for 30 min and diluted (1:1 v/v) with the same extender enriched with various cryoprotectants as follows: 6 % dymethylacetamide (DMA), 7.5 % dymethylformamide (DMF), 9 % methylacetamide (MA) or 8 % ethylene glycol (EG) at final concentrations. *Kobidil*+ extender without cryoprotectant was used as control. Each sample was packaged into 0.25 ml straws, equilibrated at 5 °C for 45 min and frozen for 15 min in

liquid nitrogen vapor (5 cm above) followed by direct plunging in LN_2 at -196 °C. Frozen semen was thawed at 5 °C for 2 min and analyzed by Computer Assisted Sperm Analysis (CASA) to determine total motility and progressive movement. Although the quality of frozen semen was lower ($P < 0.05$) than that of fresh one (46.6 % vs 60.7 %), the semen cryopreserved with 8 % EG exhibited the best motility values ($P < 0.05$). These are preliminary results of a more extensive project focused on the best freezing protocol for rooster spermatozoa in Slovakia.

Key words: rooster; frozen semen; cryoprotectant

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EFFECTS OF REDUCED GLUTATHIONE UPON INTRACELLULAR PEROXIDE AND SUPEROXIDE LEVELS IN BOAR SPERM DURING *IN VITRO* CAPACITATION AND PROGESTERONE-INDUCED ACROSOME EXOCYTOSIS

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The main aim of this study was to determine the putative effects of reduced glutathione (GSH) on the intracellular superoxide (O_2^-) and peroxide levels (H_2O_2) in boar sperm following *in vitro* capacitation (IVC) and progesterone-induced acrosome exocytosis. A total of 7 ejaculates, collected from 4 healthy Pietrain boars aged 2 to 3 years, were used in this study. The sperm-rich fractions were collected manually, diluted to a final sperm concentration of 2×10^7 sperm.mL^{-1} in a commercial extender (Androstar Plus®; Minitub Ibérica SL, Tarragona, Spain), and cooled down to 16–17 °C. Afterwards, each ejaculate was divided into five samples in accordance with the experimental treatments (C-; C+; 1mM GSH; 2mM GSH; 5mM GSH). Cells were incubated in a specific medium designed to induce IVC in boar sperm (CM medium; groups C+, 1mM GSH. 2 mM GSH and 5mM GSH) or in a medium in which boar cells did not become capacitated (NCM medium; group C-). Cells were incubated in these media for 4 h and intracellular levels of O_2^- and H_2O_2 were determined by flow cytometry using two different oxidation-sensitive fluorescent probes: hydroethidine (HE) and 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA). At 4 h progesterone was added to a final concentration of 10 µg.mL^{-1} to induce *in vitro* acrosome exocytosis (IVAE). The cells were incubated for 1 h and aliquots were taken after 1 min, 5 min and 60 min of progesterone addition. Under these conditions, percentages of viable sperm with high intracellular superoxide and peroxide levels significantly ($P < 0.05$) increased after 4h of incubation in CM (O_2^-: from 11.6 % ± 1.3 % at 0 h to 17.2 % ± 1.9 % at 4 h; H_2O_2: from 2.4 % ± 0.5 % at 0 h to 18.6 % ± 2.0 % at 4 h). Interestingly, the subsequent addition of progesterone induced a different behaviour with regard to ROS generation. Thus, there was a temporary decrease in the percentage of viable spermatozoa with high O_2^- levels, which was counteracted after 5 min of the hormone addition, reaching maximum after 60 min of incubation. In contrast, the percentage of viable spermatozoa with high H_2O_2 levels was roughly maintained following progesterone addition. The addition of GSH at 0 h of incubation counteracted the observed increase in the rate of sperm cells with high O_2^- and H_2O_2 levels at all GSH concentrations. This counteracting effect of GSH progressively diminished after the addition of progesterone, showing no differences between C+ and treatments after 60 min of incubation with the hormone. Our results indicate that GSH prevents the increase of ROS levels during IVC. This work also suggests that the IVC-concomitant changes in ROS levels could underlie several of the molecular phenomena linked with the sperm capacitation. However, more research is still needed to elucidate the putative role of ROS in launching sperm capacitation.

Key words: boar sperm; flow cytometry; reduced glutathione GSH; ROS levels

FLOW CYTOMETRIC ANALYSIS OF CHO-K1 CELLS AFTER LIPOFECTION WITH NEW GENETIC CONSTRUCTS

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Transfection efficiency of animal cells by synthetic DNA carriers has been usually low and transgene was gradually lost during embryonic development. Recently, the transposon elements have been proposed to create a more versatile method to target germ line stem cells. Transposons are genetic elements that can relocate between different genomic sites, and the enzyme transposase can excise unique DNA sites and recombine transposons into targeted sites in the genome. The preferred host cells for the production of therapeutic proteins *in vitro* is Chinese hamster ovary (CHO-K1)- derived cell line. This study aimed to optimize a method of CHO-K1 cell line lipofection with transposon constructs carrying a gene expression cassette and integrating it into the host genome by transposase. We compared effect of cells density (1 x 10^5 cells.ml^{-1} and 5 x 10^5 cells.ml^{-1}), amount of lipofectant Xtreme HP DNA (1-8µl) and plasmid constructions (co-transfection - pCMV-Tol2 with pL-miniTol2-OVA5IFN, pCMV-Tol2 with pL-miniTol2-OVA5Egfp, transfection- pL-OG-OVAIFNEnh-Egfp, pEGFP-N1; Promega) on viability of the cells. To select transfected cells we used G418 at 400µg.ml^{-1}, which was determined based on antibiotic resistance curve. The viability of cells was the marker of transfection efficiency and was examined by flow cytometry FACS Aria after propidium iodide (PI) staining. Regardless of lipofectant amount and plasmid constructions, the highest transfection efficiency was obtained for concentration of 1 x 10^5 cells.ml^{-1}. Compared to control (32 %), the highest lipofection efficiency (60-61 %) was achieved for co-transfected cells with 0.5µg pCMV-Tol2 and 1µg pL-miniTol2-OVA5IFN/pL-miniTol2-OVA5Egfp and 1µl Xtreme HP DNA. The viability and EGFP expression after transfection with pL-OG-OVAIFNEnh-Egfp was 67.3 % and

32,3 % and for pEGFP-N1 was 66.9 % and 31 %, respectively. For 5 x 10^5 cells.ml^{-1} application of 0.5µg pCMV-Tol2 and 1µg pL-miniTol2-OVA5IFN/pL-miniTol2-OVA5Egfp and 2µl Xtreme HP DNA resulted in lower viability (33.1 and 29.5 %, respectively). Our results show that transfection efficiency for CHO-K1 cell line depends on proper selection of three factors: cell density, concentration of DNA plasmid and the amount of transfection reagent.

Key words: flow cytometry; lipofection; viability; antibiotic kill curve

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EFFECT OF *IN OVO* ADMINISTRATION OF DIFFERENT SYNBIOTICS ON PERFORMANCE AND MEAT QUALITY TRAITS IN BROILER CHICKENS

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The effect of *in ovo* administration of two different synbiotics on performance and meat quality traits was studied in broiler chickens. On day 12 of incubation, 5850 eggs (Cobb 500FF) were randomly divided into 3 experimental groups and *in ovo* injected with either: 0.2 ml of a saline solution (Control, C); 0.2 ml of a synbiotic containing 2 mg.embryo^{-1} of Bi^{2}tos (Clasado BioSciences Ltd.), trans-galactooligosaccharides enriched with 10^5 cfu.embryo^{-1} of *Lactobacillus salivarius* IBB3154 (SYN1) or 0.2 ml of a synbiotic containing 2 mg.embryo^{-1} of raffinose family oligosaccharides (RFO) enriched with 10^5 cfu.embryo^{-1} of *Lactobacillus plantarum* IBB3036 (SYN2). After the injection, the injection hole was covered with a drop of organic glue and the incubation was continued until hatching. Among the hatched chickens, 2040 males (680 per each group) were randomly chosen and reared in a commercial poultry house (PiastPasze Sp. z.o.o., Olszowa, Poland). Chickens were raised in pens (n = 75 per pen) with 8 pen replicates per treatment for the effect on performance. Moreover, separate pens for sampling (n = 10 birds per pen: 8 replications per each experimental group) were included in the experimental design. Animals were fed *ad libitum* with commercial diets according to their age and had free access to water. Feed intake (FI) and feed conversion ratio (FCR) were calculated on a pen basis. At 42 days of age, two birds per pen (16 birds per treatment) were randomly chosen from the separate pens for sampling and slaughtered. At slaughter, hot carcass weight was recorded and carcass yield was calculated. The pectoral muscle (PM) was removed from each carcass and weighed; its percentage was calculated based on hot carcass weight. At 24 hours *post-mortem*, pH, colour and water holding capacity (WHC) were recorded on the right PM. The left PM was vacuum packaged and frozen until chemical analysis for total lipids, cholesterol and fatty acids. Data were analyzed by a one-way ANOVA. Mortality rate, FI and FCR were similar ($P > 0.05$) between experimental groups. Synbiotics did not affect slaughter weight, weight and yield of carcass, PM weight and its yield percentage, or pH, colour, WHC and cholesterol content of PM. SYN2 group displayed a lower lipid content ($P < 0.05$) compared to C group. Meat from SYN1 birds displayed: i) a higher content of total saturated fatty acids (SFA) ($P < 0.01$); ii) lower monounsaturated fatty acids (MUFA) ($P < 0.05$ compared to SYN2); iii) lower polyunsaturated fatty acids (PUFA) ($P<0.01$); and iv) lower n-6 PUFA ($P < 0.01$) and n-3 PUFA ($P < 0.01$ and $P < 0.05$ compared to C and SYN2, respectively). Regarding the nutritional ratios, the n-6/n-3 ratio was affected by the synbiotic administration ($P = 0.039$). However, C and SYN2 groups had a higher PUFA/SFA ratio compared to SYN1 ($P<0.01$). Atherogenic and thrombogenic indexes were lower in the SYN2 and C groups ($P<0.01$) compared to SYN1. Low atherogenic and thrombogenic indexes characterize pro-health status of a meat in terms of fatty acids composition. In conclusion, the results of the present study indicate that *in ovo* administration of synbiotics did not negatively affect productive performance and physicochemical properties of meat. However, the meat from C and SYN2 birds showed a preferable fatty acid profile with a positive effect on nutritional properties of the chicken meat.

Key words: broiler chicken; synbiotics; *in ovo* administration; performance; meat quality

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THE EFFECTS OF REARING SYSTEM AND INTRAMUSCULAR VITAMIN E INJECTION ON GROWTH PERFORMANCE AND MEAT QUALITY OF BROILER CHICKENS

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In the last decade, alternative and more environmentally friendly poultry production systems have been proposed to satisfy the increasing consumer demands for healthy and natural meat products. In general, the outdoor rearing system could affect the quality of fat, increasing the PUFA content. Vitamin E, which has a protective effect on muscle membrane lipid peroxidation, can extend the shelf-life of meat and improve its nutritional quality. The objective of the study was to evaluate the impact of production system (indoor vs. outdoor) and i.m. vitamin E (DL-α-tocopheryl acetate) injection on meat quality of fast-growing genotype (Ross 308) raised for 42 days. Birds were randomly assigned to indoor pens and pens with outdoor access. Thirty chicks from each outdoor and indoor groups were randomly allotted to three sub-groups: vitamin E-treated with 50 or 100 IU of DL-α-tocopheryl acetate, respectively, i.m. injected (left pectoral muscle, PM) 14 days before slaughter, and control group i.m. injected with a saline. Animals were fed commercial diets according to their age *ad libitum*. Birds of outdoor group were allowed access to

grassy yards during daytime. At 42 days of age, all birds were slaughtered, hot carcass weight was recorded, and carcass yield was calculated. The main cuts were weighed and their percentage was calculated based on hot carcass weight. At 24 hours *post-mortem*, pH and color were recorded on right PM. The left PM was vacuum-packaged and stored frozen until fatty acids determination. Data were evaluated by factorial ANOVA, where rearing system and vitamin E were the main factors. Slaughter weight was not affected by rearing system or vitamin E treatment. However, indoor rearing raised carcass weight and yield ($P < 0.01$), as well as breast, leg and wing weights ($P < 0.01$). Vitamin E treatment did not affect ($P > 0.05$) slaughter traits. Meat pH was not significantly ($P > 0.05$) affected by rearing system and vitamin E treatment, and the detected values were within the acceptable range (from 5.6 to 5.9). Birds from the outdoor group displayed darker meat (lower L*; $P < 0.01$) with higher redness (a*, $P < 0.05$) and yellowness (b*, $P = 0.06$) compared to indoor chickens. The i.m. injection of 100IU and 50IU of vitamin E caused a drop ($P < 0.05$) in lightness (L*) and yellowness (b*) of meat, respectively. Significant interactions were found between factors for L* ($P < 0.01$) and a* ($P < 0.05$). Fatty acid profile was not affected by rearing systems and was slightly affected by the vitamin E treatment; however, significant interaction between rearing system and vitamin E in several fatty acids was found. In conclusion, the results suggest that rearing system greatly affected slaughter traits and meat color of broiler chickens, but had no significant influences on meat fatty acid profile. Vitamin E treatment showed no significant effect on growth performance, but affected meat color and level of a few fatty acids. Nevertheless, future studies are required to define these interactions in order to improve the quality of the meat.

Key words: broiler chicken; rearing system; intramuscular vitamin E; growth; meat quality

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GENETIC DISTANCE IN RELATION TO SPERM AND PGC COMPETITION IN CHICKEN

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Sperm competition is an evolutionary mechanism which occurs when spermatozoa from two or more males vie for fertilization of oocytes derived from one female. Experiments with different animal species have shown that this mechanism favors males with features that ensure successful fertilization. But the connection between sperm competition and functional qualities of primordial germ cells (PGCs) was not studied earlier. PGCs competition can be defined as a contest between PGCs derived from two or more donors to migrate to recipient`s gonads and their ability to form germline chimeras. The aim of the study was to determine the relationship between genetic similarity of birds and sperm and PGCs competition results. The research material consisted of roosters from 4 breeds - the donors of semen and PGCs: Transylvanian Naked Neck Black (TNB), Transylvanian Naked Neck White (TNW), Hungarian Speckled (HS) and Green-legged Partridgelike (ZK) and laying hens Tetra SL – recipients of semen and PGCs. Genetic distance between these groups of birds was determined based on 8 microsatellite markers recommended by FAO/MoDAD Advisory group (http://www.fao.org/dad-is). Subsequently, 6 roosters of each donor breed and 12 hens Tetra SL were selected. At first, the sperm competition was performed by collecting a semen from selected roosters and after the equalization of sperm concentration and semen mix selected females were inseminated. Afterwards, the PGCs competition was conducted by creating germline chimeras through injection of pooled PGCs from 6-day-old embryo donors to 3-day-old embryo recipients. The origin of resulting chicks was analyzed using the same 8 microsatellite markers. The results of genetic distance showed that the greatest distance to the Tetra SL hens was characterized by ZK roosters (0.5) and the lowest by TNB roosters (0.25). Whereas, evaluation of the origin of offspring both from sperm and PGCs competition indicated that most of the chicks were obtained from TNB males (45 % and 56 %) and the least from ZK roosters (8 and 25 %). A positive correlation between genetic similarity of sperm/PGCs donors and recipients and sperm and PGCs competition results has been demonstrated.

Key words: test competition; germline chimera; genetic distance; microsatellite markers

STABLE TRANSGENE INTEGRATION INTO THE CHICKEN GENOME USING PRIMORDIAL GERM CELLS

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Primordial germ cells (PGCs) are precursors of germline cells and are the only cells in the developing embryo, which have the ability to transmit genetic information to the subsequent generations. PGCs can be isolated from the stage 7 germinal crescents, 2.5-day-old chicken embryos or from gonads of 6-day-old embryos. Modified chicken PGCs were used for production of chicken chimera. First generation of transgenic chickens demands intercrossing of F1 germplasm chimeras. Transgenic chickens have a huge opportunities to be used as bioreactors for the production of valuable pharmaceutical proteins, which can be produced in the oviduct and deposited in the egg. The aim of the study was to reveal the presence of the HBsAg surface antigen of hepatitis B virus in F1 chicken generation using molecular tools as PCR and qPCR. PGCs were isolated from embryonic blood of Ross 308 chickens

at the 14-16 HH stage of embryonic development, purified by Percoll density gradient centrifugation and transfected by electroporation with pC-OVAHBV plasmid containing human hepatitis B virus surface antigen gene (HBsAg, GenBank accession no. Z35717) under ovalbumin promoter. Transfected cells were cultured in OPTI MEM with G418 and injected into the dorsal aorta of recipient embryo at stages 14-16 HH to produce chicken chimeras. Chicken chimeras carrying transgene were intercrossed to produce a next generation of transgenic birds. F0 chimeras after PCR-positive screening for HBsAg gene were mated to obtain F1 generation. HBsAg transgene detection was performed on DNA isolated from blood using PCR. Estimation of copy numbers was carried out by qPCR (ddCt method of relative gene expression), and DNA from positively verified individuals are devoted for further transgene location analysis by Genome Walking. PCR revealed 67 transgenic chickens with integrated HBsAg gene. qPCR revealed the presence of one copy of the transgene in 22 individuals, two copies in 6 individuals, 3 copies in 4 individuals, 4 copies in 1 individual and in one transgenic chicken was the carrier of 11 HBsAg copies. Analysis demonstrated the presence of HBsAg surface antigen gene of hepatitis B virus in the chicken genome.

Key words: chicken chimeras; PGCs; transgene; qPCR

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EFFECT OF DIETARY BEE POLLEN SUPPLEMENTATION ON FATTY ACID COMPOSITION OF CHICKEN MEAT

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The experiment was conducted to evaluate the effect of bee pollen on fatty acid (FA) composition of chicken meat. Broiler chickens (n = 180) were raised for a 42-d feeding period and were assigned into 2 groups: a basal diet without supplementation, as a control (C) and a basal diet, supplemented with ethanol extract of bee pollen (400 mg.kg^{-1} of feed mixture; E). Chickens were reared under the same conditions, fed the same diets of identical nutritional content and allowed to consume feed and water on an *ad libitum* basis. The FA composition of breast and thigh meat was determined by a direct method for fatty acid methyl ester (FAME) synthesis using a Gas Chromatograph (Agilent, 7890A series, USA). By supplementing diet with bee pollen, total saturated fatty acid (SFA) and total polyunsaturated fatty acid (PUFA) ratios in the breast muscle were found to be quite low, whereas total monounsaturated fatty acid (MUFA) ratio was found to be high when compared to C. In particular, contents of pentadecanoic, palmitic, heptadecanoic, and stearic acid (0.02, 24.50, 0.04, and 6.65 g.100 g^{-1} in E vs. 0.07, 26.72, 0.11, and 6.85 g.100 g^{-1} in C, respectively) were decreased ($P > 0.05$). Dietary bee pollen supplementation affected FA composition of thigh muscle resulting in lower ($P \leq 0.05$) total SFA ratio and stearic acid (C18:0) content (33.00 and 5.44 g.100 g^{-1} in E vs. 33.89 and 5.94 g.100 g^{-1} in C, respectively), whereas total MUFA ratio and myristoleic acid (C14:1) content increased ($P \leq 0.05$; 50.57 and 0.24 g.100 g^{-1} in E vs. 49.57 and 0.22 g.100 g^{-1} in C, respectively). In addition, the ratio of n-3 to n-6 PUFA was increased, whereas the ratio of n-6 to n-3 PUFA was decreased in the thigh muscle in E. Although bee pollen does not appear to markedly affect the FA composition of chicken meat, it could be included into chicken diet as potential dietary supplement to improve chicken meat quality.

Key words: broiler chicken; bee pollen; breast; thigh, fatty acid

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DOSE-DEPENDENT EFFECT OF SULFORAPHANE ON CACO-2 CELLS GROWTH

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Sulforaphane (SFN) belongs to the isothiocyanate family and it is a biologically active metabolite of glucoraphanin, which is found at the highest concentrations in broccoli, but also in other cruciferous vegetables (cauliflower, kale, cabbage, radish, arugula, etc.). Experimental studies have shown that SFN has anti-inflammatory, anti-microbial, anti-diabetic and anti-carcinogenic effects. In the present study we have focused on monitoring the changes in cell numbers after application of SFN on the human epithelial colorectal adenocarcinoma Caco-2 cell line. The cells (European Collection of Cell Cultures (ECACC 86010202)) were cultured in DMEM (Dulbecco's modified Eagles medium) supplemented with 10 % fetal calf serum (FCS), 2 mM L-glutamine and mixture of penicillin (100 U.mL^{-1}) streptomycin (100 μg.mL^{-1}) and amphotericin B (250 ng.mL^{-1}) and maintained at 37 °C in a humidified atmosphere of 5 % CO_2. The cells were seeded into 24-well plates (approximately 10 000 cells per well) and incubated with SFN at different concentrations (1, 5, 10, 20 and 30 μM) for 48 h. After culture, the cells were washed in PBS (phosphate buffered saline), fixed with 3.7 % buffered formaldehyde solution and stained with a 0.5 % crystal violet solution. Subsequently, after incubation with destaining solution, 100 μL of supernatant were transferred to 96-well microtitration plate and absorbance was measured at 540 nm on Microplate reader (Bio-Rad, Model 680). The data were analysed using Dunnett's test by one-way analysis of variance (ANOVA) in GraphPad Prism 5 software (GraphPad Software, Inc, La Jolla, CA, USA). Differences from controls at $P < 0.05$ were considered as significant. Lower concentrations of SFN (1 and 5 μM) caused a significant ($P < 0.05$) increase in Caco-2 cell number in comparison to control group, which indicates undesirable growth stimulatory effect. Application of 10 μM SFN did not affect the cell numbers as compared to the control. However, we observed significant ($P < 0.05$) decrease in the number of cells in experimental groups with addition of 20 or 30 μM of

SFN. Therefore, we assume that the SFN doses higher than 20 μM may cause slow down or block of Caco-2 proliferation.

Key words: Caco-2 cells; crystal violet; sulforaphane

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IMPACT OF PUNICALAGIN ON PORCINE OVARIAN GRANULOSA CELLS *IN VITRO*

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Punicalagin is a major antioxidant of pomegranate. Punicalagin is represented in pericarp, husk or seeds of pomegranate. It belongs to the group of hydrolysable tannins - polyphenolic compounds. Punicalagin is metabolised to ellagic acid (antioxidant) and microorganisms present in colon can metabolize ellagic acid to urolithins. The aim of our *in vitro* study was to investigate the effect of punicalagin on the cell cycle and secretion of steroid hormones by porcine granulosa cells from ovaries of pre-pubertal pigs. The granulosa cells were cultured during 24 h without (control group) or with various doses (0.01, 0.1, 1, 10 and 100 $\mu g.ml^{-1}$) of punicalagin. Punicalagin (at 1 $\mu g.ml^{-1}$) had stimulatory effect on the mitochondrial activity of the cells (MTT assay). Markers of proliferation (cyclin B1, proliferating cell nuclear antigen - PCNA) and apoptosis (p53, caspase-3) were detected by immunocytochemistry. Punicalagin significantly increased ($P \leq 0.05$) cyclin B1, while decreased PCNA content, when given at 1 $\mu g.ml^{-1}$ but not at other concentrations tested. Apoptotic markers were not significantly affected by any punicalagin concentration. The secretion of progesterone and 17 β-estradiol by ovarian granulosa cells was not significantly influenced by punicalagin. Effects of punicalagin on porcine ovarian granulosa cells are questionable. Our findings suggest possible involvement of punicalagin in the process of proliferation of porcine ovarian granulosa cells. Further elucidation of possible role of punicalagin is required.

Key words: apoptosis; proliferation; punicalagin; steroid hormones

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PHYSICOCHEMICAL PROPERTIES AND FATTY ACID CONTENT IN MEAT OF MANGALITSA BREED AND THEIR CROSSBREEDS

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Nowadays, there is a trend in the pork market to make products based on traditional specialities, which require a specific quality of meat with emphasis on dry matter, intramuscular fat content and fatty acid composition with higher share of unsaturated fatty acids and essential fatty acids. The aim of this study was to compare the physicochemical properties and fatty acid content in *Musculus Longissimus Dorsi* (MLD) of Mangalitsa breed, Mangalica x Duroc crossbreeds and Slovak Large White (SLW) pig meat breed. The experimental material consisted of 28 pigs reared under the same intensive conditions and fed complete feed mixtures for fatteners *ad libitum*. The fattening period lasted from 30 kg to 100 kg of body weight. In this study, the Mangalitsa x Duroc crossbreeds had significantly lighter, redder and more yellow meat compared to Mangalitsa and SLW breeds ($p < 0.05$). From the point of meat texture, the more tender meat was from the Mangalitsa x Duroc crossbreeds and the stiffest meat was from SLW pigs ($p < 0.05$). The Mangalitsa x Duroc crossbreeds had the lowest water content but the highest intramuscular fat and cholesterol content in MLD ($p < 0.05$). On the contrary, SLW had the highest water content and the lowest intramuscular fat and cholesterol content ($p < 0.05$). In MLD, SLW pigs had the highest percentage of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) compared to Mangalitsa breed and Mangalitsa x Duroc crossbreeds, while the Mangalitsa breed had the lowest percentages of SFA and MUFA ($p < 0.05$). The ratio of polyunsaturated fatty acids was the highest in the MLD of Mangalitsa and the lowest in SLW ($p < 0.05$). In conclusion, the meat from Mangalitsa breed and their crossbreeds has higher quality traits and nutrition quality for special meat products compared to SLW pig breed. However, economical efficiency of the Mangalitsa breed rearing under intensive conditions is debatable.

Key words: Mangalitsa; Slovak Large White; meat; SFA; MUFA; PUFA

DIFFERENCES IN RUMEN TEMPERATURE OF DAIRY COWS AT THE START AND PEAK OF LACTATION

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The aim of the study was to examine the effect of daily diet change from the start of lactation to the peak of lactation on ruminal temperature in relation to the lactation number (2nd lactation and 3rd lactation group) using continuous monitoring of ruminal pH and temperature with e-bolus at the university´s experimental farm in Oponice. Holstein cows (n = 7) were fed Total Mix Ratio *ad libitum* once (between 4:00 and 5:00) and milked 3 times per day (6:00, 12:00 and 18:00). The bolus introduced via Esophagus measured pH and temperature values every 15 minutes (96 measurements per day) with an accuracy ± 0.1 pH. Data were downloaded and collected with HathorHBClient v. 1.8.1 and statistically evaluated with IBM SPSS v. 20.0 software (one-way ANOVA, Independent Samples t-test). There was no difference in the daily temperature mean between diets at the start (38.66 ± 1.30 °C) and the peak of lactation (38.69 ± 1.26 °C). The average daily temperature of dairy cows at the start of 2nd lactation was 38.68 ± 1.29 °C and at the peak of lactation - 38.60 ± 1.25 °C. The average daily temperature of dairy cows at the start of the

3rd lactation was 38.64 ± 1.30 °C and at the peak of lactation it was 38.77 ± 1.26 °C. The difference between lactations was statistically significant at the peak of lactation (0.45 %; $p < 0.01$) but not at the start of lactation. In conclusion, daily diet change from start of lactation to the peak of lactation did not influence rumen temperature but it was dependent on the number of lactations.

Key words: bolus; rumen monitoring; temperature; diet change; lactation differences

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THE RELATIONSHIP AMONG AGEING, FUNGIFORM PAPILLAE DENSITY AND DETECTION THRESHOLD FOR CAFFEINE

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Ageing is accompanied by potential reduction in the fungiform papillae (FP) density on the tongue, which has been associated with taste disorders. We examined whether FP density is associated with detection threshold for caffeine and ageing. Two groups of participants that differed significantly from each other ($p < 0.01$; $\alpha = 0.05$) were recruited in the study: the group A – juniors (n = 51, age: 19 – 26, mean 21.12 ± 1.28) and the group B – seniors (n = 49, age: 51 – 82, mean: 66.82 ± 6.47). Quantification of FP has been done following a protocol of Nuessle *et al.* (2015). The detection thresholds for caffeine were measured in accordance with ISO 3972:2011. Differences between groups were evaluated by non-parametric test (Mann-Whitney) and relationships revealed by correlations (Spearman) using XLSTAT (Addinsoft, 2014) package program. The mean FP density value within the group A was 35.84 ± 13.47 per cm^2. The lowest and the highest densities were 10.44 ± 0.57 per cm^2 and 71.56 ± 1.66 per cm^2, resp. On the other hand, the mean FP density value within the group B was 8.73 ± 7.03, with minimum of 0.25 ± 0.57 per cm^2 and maximum of 31.07 ± 0.70 per cm^2. The mean detection threshold for caffeine was 0.94 ± 0.55 $mmol.L^{-1}$ and 1.46 ± 0.81 $mmol.L^{-1}$ in groups A and B, respectively. The groups significantly differed from each other by values of the FP densities ($p < 0.01$; $\alpha = 0.05$) and the detection thresholds as well ($p < 0.01$; $\alpha = 0.05$). The relationship between FP density and age had almost strong negative statistically significant correlation ($\rho = -0.76$; $R^2 = 0.58$; $p < 0.01$; $\alpha = 0.05$). Detection threshold for caffeine was dependent moderately on the age of the subject ($\rho = 0.35$; $R^2 = 0.13$; $p < 0.01$; $\alpha = 0.05$) rather than on FP density ($\rho = -0.30$; $R^2 = 0.09$; $p < 0.01$; $\alpha = 0.05$), which points to high inter-individual variability in the FP number.

Key words: tongue; fungiform papillae; caffeine

Acknowledgements: This work was financially supported by the grant APVV-0629-12.

SECTION III.: Laboratory and Small Farm Animals – Poultry, bee, mouse

THE JAPANESE QUAIL CHORIO-ALLANTOIC MEMBRANE AS AN EXPERIMENTAL *IN VIVO* MODEL FOR ANGIOGENESIS RESEARCH AND CANCER DIAGNOSIS AND TREATMENT

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An outstanding alternative of the use of experimental animals for *in vivo* testing is avian chorio-allantoic membrane (CAM). The chorioallantoic membrane (CAM) is a simple, highly vascularized extra-embryonic membrane visible from day 4 of incubation, which performs many functions during avian embryo development. CAM is structurally similar to the retina, buccal mucosa, lungs, placenta and blood-brain barrier. The CAM model fulfils the 3Rs concept (Reduction, Refinement and Replacement), is economically feasible and has a potential to be used in testing of new drugs and biomaterials, toxicity, cancer diagnosis and treatment. Most frequently used is chicken CAM, however, Japanese quail CAM can be used with similar results. The use of the quail CAM model has advantages, such as short embryonic development, impressive egg production, smaller space for farming and experiments and lower total cost. In our experimental work with quail embryos we mostly used the *ex ovo* technique. Fertilized eggs were incubated in a forced draught incubator, and at embryonal day 3 (ED 3) the eggs were opened, the embryos transferred into the six-well tissue culture plates and cultivated until ED 7, when the experiments started. Tested substances can be applied systematically, or topically on CAM surface. We examined angiogenic effect of several compounds (e.g. endogenous peptide leptin, heparin, fraxiparine) administered *in ovo* or in *ex ovo* culture. Changes in blood vessel density were quantified by the fractal analysis. Results clearly showed stimulating effect of leptin on blood vessel development. In another series of experiments we explored possible use of quail CAM for photodynamic diagnosis and therapy of cancer. Photodynamic therapy (PDT) is a promising and innovative treatment for small localized tumours. We have demonstrated the effects of photodynamically active drug Hypericin (HYP). HYP was applied by topical application onto the quail CAM surface to study its effects on vascular damage. HYP applied on tumour implanted on CAM surface better visualized location of tumour under the fluorescent light. Addition of low density lipoproteins, as a delivery molecule, even more improved detectability of tumour position. Our results indicate that Japanese quail CAM model is a useful tool for the study of anti-vascular therapy and tumour angiogenesis, development of new biophotonic techniques as well as novel drug testing.

Key words: Japanese quail; chorio-allantoic membrane; angiogenesis; photodynamic diagnosis

Acknowledgements: This research was supported by grants VEGA 2/0102/15 and APVV-15-0485.

ENTEROCIN M – PRODUCING STRAIN *ENTEROCOCCUS FAECIUM* AL41 CAN BENEFICIALLY AFFECT INTESTINAL MICROFLORA OF OSTRICHES

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Untraditional poultry species with high quality meat, such as ostriches, are also farmed in Slovakia. Although ostriches have well-developed immune system in adult age, ostrich chickens are susceptible to various diseases. To prevent diseases or to protect farming, probiotic bacteria are frequently used to stimulate the immune system of the animals. Probiotic bacteria have beneficial impact on the host. Best studied are lactic acid bacteria. Enterococci belong to lactic acid bacteria; they can produce ribosomal synthesized antimicrobial peptides – enterocins. The aim of this study was to detect enterococcal species in faeces of ostriches, to test their sensitivity to enterocins and to test antimicrobial effect of enterocin M-producing probiotic strain *Enterococcus faecium* AL41 in the digestive tract of ostriches. Forty-six enterococci, which belong to three species *Enterococcus hirae*, *E. faecium* and *E. mundtii*, were isolated from faecal samples of ostriches. Isolated enterococci were sensitive to seven enterocins (semi-purified in our laboratory) produced by *E. faecium* strains of environmental, ruminal and animal origin with inhibition activity 200–25600 $AU.ml^{-1}$. In the model experiment, 86 animals at the age of 1–3 weeks (46 in the experimental group; 40 in the control group) were used. The model experiment lasted 42 days; rifampicin marked *E. faecium* AL 41/CCM8558 was applied for 21 days (dose of 400 µl/day/ostrich; concentration 10^9 $CFU.ml^{-1}$). Faeces were sampled at the start of the experiment (0. – 1. day), 1 week of application (day 7), 3 weeks of application (day 21) and 3 weeks after cessation of application (day 42). Faecal samples were treated by the standard microbial method (ISO). *E. faecium* AL 41 sufficiently colonized intestinal tract of ostriches. Antimicrobial effect of AL 41 strain was noted by significant decrease of coagulase––negative, coagulase–positive staphylococci, coliform bacteria, *Pseudomonas* spp. and other enterobacteria ($p < 0.001$) at day 21. *E. faecium* AL 41 strain is probably used as an additive also in untraditional poultry. We can recommend the use of the strain with enterocin production for prevention or elimination of potential pathogens from the digestive tract of ostriches.

Key words: ostriches; enterocins; benefit; antimicrobial effect

Acknowledgements: The study was supported by the project VEGA 2/0004/14 "Bacteriocins produced by probiotic strains of Firmicutes and their use to improve the health of food animals".

CAMPYLOBACTERS FROM POULTRY IN RELATION TO BIOACTIVE SUBSTANCES

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Bacteria from the Campylobacter genus in terms of zoonotic aspect are considered to be the most frequent undesirable agents in poultry. Although these bacteria belong to the obligatory microflora of the digestive tract of poultry, many strains have pathogenic effect. Campylobacteriosis belongs to the most commonly reported zoonosis in European Union as well as in Slovakia. Campylobacter increasing resistance to certain antibiotics has been recorded. Therefore, the aim of the study was to show alternative ways of reducing and/or preventing infections caused by these bacteria. Campylobacters were isolated from different sources of poultry. Sensitivity to antibiotics and bioactive substances with anti-microbial effect (bacteriocins/enterocins, sage, oregano, gallidermin) was tested by disc and quantitative agar diffusion method. Enterocins (produced by mostly animal origin strains of *E. faecium*) are natural bioactive substances with antimicrobial effect which belong to bacteriocins. From the 69 tested strains of Campylobacters, all of them were resistant to at least 2 of 10 antibiotics. In the strains from Slovak poultry (SK strains), the highest resistance was recorded to nalidixic acid and ciprofloxacin. In strains isolated from Italian poultry (IT strains), resistance to nalidixic acid and cefotaxim was also recorded. The control strain *Campylobacter jejuni* CCM 6191 was resistant to 7 antibiotics, but it was also sensitive to 7 enterocins from 8. A total sensitivity to at least 1 enterocin was observed in 83 % SK strains and 85 % IT strains. SK strains were sensitive mainly to Ent 131; IT strains to Ent 9296. The growth of 57 % SK strains and 44 % IT strains was influenced by effect of sage. The oregano effect was higher; 67 % SK strains and 56 % IT strains were inhibited. Sensitivity to gallidermin was observed in 35 % SK strains and 13 % IT strains. Our preliminary results indicate that *Campylobacter* spp. have a tendency to be sensitive to bioactive additives; it has impact on the basic research and prevention. An important result is, that most of the resistant strains were susceptible to enterocins.

Key words: poultry; campylobacters; enterocins; bioactive substances

Acknowledgements: The study was supported by the project VEGA 2/0004/14 "Bacteriocins produced by probiotic strains of Firmicutes and their use to improve the health of food animals".

MATERNAL STRESS DURING THE PREIMPLANTATION DEVELOPMENTAL PERIOD: THE CONTEXT OF MATERNAL METABOLIC STATUS

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Stressful environment can affect reproductive functions in humans as well as in animals. We investigated whether maternal stress can influence the embryo at the earliest preimplantation stages of development. We demonstrated that early embryos are equipped by cell receptors capable to "feel" stress hormones. Using the mouse model, we showed that maternal stress can significantly affect preimplantation embryo development and that some negative effects of stress, acting during the preimplantation period, can retain even in the adult period. We also investigated the response of early embryos to

maternal stress with respect to the mother physiological status. Female mice differing significantly in somatic parameters (body weight, body fat amount) were used in our experiments. We found differences in endocrine profiles of mothers differing in their somatic parameters, and we also detected various responses to stress in these females. Moreover, we found that maternal stress influenced the preimplantation embryo development differently, depending on the mother´s physiological status. In summary, our results indicate that early embryos can "feel" maternal stress and that the stress mediators can directly affect the early embryo development. Our results also indicate that besides the stress strength and duration, maternal physiological status can significantly influence the impact of stress on the early embryo as well.

Key words: mouse; embryo; maternal stress; endocrine profile

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SECTION IV.: PhD students

ANTERIOR PITUITARY HORMONES OF FEMALE RABBITS AFTER APRICOT SEED ADMINISTRATION *IN VIVO*

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Fruits and vegetables are universally applied as healthy food. *Rosaceae* species represents a diverse group of plant foods that vary greatly in content of energy and nutrients. The apricot (*Prunus armeniaca* L.) is a member of the *Rosaceae* family. Apricots are a source of many bioactive substances in the human diet. Apricot seeds are used especially in medicine, cosmetic and oil production. They are particularly rich in lipids, proteins, fibres and, depending on the variety, seeds contain toxic cyanogenic glycoside - amygdalin. Female reproductive system is regulated through the hypothalamus-pituitary-gonadal axis. The axis controls development, reproduction including folliculogenesis, and aging in animals. The anterior portion of the pituitary gland produces follicle-stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL). The aim of our *in vivo* study was to assess the effects of apricot seeds on hormone profile - plasma levels of anterior pituitary hormones in female rabbits (age of 128 days). Concentrations of FSH, LH and PRL were determined by ELISA method. Overall 32 rabbits were divided into four groups: control group without apricot seeds and 3 experimental groups with apricot seeds mixed to feed (at doses of 60, 300 and 420 mg.kg^{-1} b.w.) during 12 weeks. Significant ($P < 0.05$) inhibition of FSH release induced by apricot seeds was found at the doses of 300, 420 mg.kg^{-1} but not at 60 mg.kg^{-1} b.w. The application of apricot seeds did not affect PRL and LH plasma levels. Our study suggests that biological active substances present in apricot seeds could influence ovarian folliculogenesis.

Key words: follicle stimulating hormone; luteinizing hormone; prolactin; folliculogenesis; apricot seeds

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EFFECT OF DMSO ON THE VIABILITY OF FROZEN-THAWED CHICKEN BLASTODERMAL CELLS

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At present, two strategies for poultry biodiversity conservation are used: *in situ* and *ex situ*. The latter includes the storage of frozen genetic material in liquid nitrogen (LN2). In birds, due to the large size and structure of female gametes the cryopreservation technique is not applied. Germ cells used for cryopreservation include blastodermal cells (BCs) and primordial germ cells (PGCs). The aim of our study was to evaluate the effect of different concentrations of dimethylsulfoxide (DMSO) in the cryopreservation medium on the viability of blastodermal cells in chickens. Blastodermal cells were collected from the germinal disc at X stage embryos of ROSS 308 breed. Blastoderms were isolated from yolk and washed several times in calcium/magnesium-free PBS (CMF-PBS; Applichem, Darmstadt, Germany) to remove as much yolk as possible. Then, the cells were washed and centrifuged in CMF-PBS for 4 min at 400 x g and supernatant was discarded. The cell suspension (100 µl) was diluted in a freezing medium composed of various concentrations of dimethylsulfoxide (DMSO: 5 %, 8 %, 10 %, 12 % and 15 %, resp.), frozen in cryovials using freezing box Bicell (Nihon Freezer, Tokyo) to - 80 °C (cooling rate: 1 °C/min) and placed into LN2 (-196 °C) for one week. The cell suspension was thawed by taking samples out of LN2 and placing them to the water bath (20 °C) for 4 min. The viability of blastodermal cells was determined using Trypan blue exclusion test of cell viability. Our preliminary results showed that the highest viability of blastodermal cells was observed in the presence of 12 % DMSO (90 %) compared to the other

tested concentrations: 5 % (70.7 %), 8 % (80.1 %), 10 % (82.5 %) and 15 % (59.6 %), resp. We can conclude that the addition of 12 % DMSO to the freezing medium provides the highest survivability of frozen/thawed blastodermal cells and is suitable for cryopreservation of chicken genetic material for storage in the gene bank.

Key words: chicken; blastodermal cells; cryopreservation

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MOLECULAR ANALYSIS OF THE RABBIT AMNIOTIC FLUID-DERIVED STEM CELLS

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The aim of this study was the isolation and molecular analysis of stem cells derived from rabbit amniotic fluid (AFSCs). Three pregnant rabbit does of the New Zealand White line were used in this study. Rabbits were humanely slaughtered at the 23rd day of gravidity and the amniotic fluid was collected, mixed with EGM-2 culture medium at the ratio of 5:6 and cultured in T25 culture flasks. Stem cells were isolated using method of plastic adherence. mRNA was isolated from stem cell suspension using TRI reagent. Consequently, cDNA was synthesized and RT-PCR techniques were applied to detect CD surface markers typical for stem cells derived from amniotic fluid. Expression of seven markers - CD29, CD44, CD73, CD105, CD166, CD34, CD45 was evaluated in this experiment. GAPDH (housekeeping gene) was used as an input control for each sample. AFSCs were positive for CD29, CD44, CD73, CD105 and CD166 markers, but negative for CD34 and CD45 markers. As a positive control for these markers, rabbit blood mononuclear cells were used. In conclusion, the AFSCs are undifferentiated cells with the potential to the high specialization that may find use in the treatment of various animal or human diseases and in tissue engineering. Contribution of the amniotic stem cells to agriculture arises from the possibility of their cryopreservation in order to preserve valuable genetic information and thus to maintain the animal biodiversity.

Key words: rabbit; amniotic fluid stem cells; PCR; CD molecules

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GENETIC CHARACTERIZATION OF SLOVAK CARNIOLAN HONEYBEE USING MICROSATELLITE MARKERS

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Carniolan honeybee (*Apis mellifera carnica*) is considered to be an endangered sub-species of *Apis mellifera* in Slovakia due to its illegal crossbreeding with allochtonous breeds. The aim of our study was to develop a molecular approach to differentiate Carniolan honeybee from other common European honeybee sub-species. The genetic variability of honeybees from 19 regions of Slovakia was investigated using microsatellite analyses of 80 *Apis mellifera carnica* samples together with 61 reference samples of *Apis mellifera mellifera*, *Apis mellifera macedonica* and *Apis mellifera* (*Buckfast*). Ten microsatellite markers running in two multiplex PCR reactions were used. All markers were highly informative (PIC > 0.5). The highest number of observed alleles was 28 on the C1602 locus, the lowest number was 7 on the AC011 locus. We used Bayesian and frequencies-based methods in self-assignment test of 'GeneClass' software (v 2.0). In actual sample size, more than 90 % individuals were assigned correctly; quality index was higher than 90 %. One hundred thousand samples were simulated using Bayesian Markov chain Monte-Carlo resampling (Cornuet *et al*., 1999). Simulated samples were self-assigned again using the same methods with more than 85 % correctly assigned individuals. Population genetic structure was inferred through the Bayesian clustering method incorporated in the 'Structure' software (v 2.3.4). All three reference populations can be distinctly identified and the Slovak population appears to be admixed between smaller populations, probably due to high number of A.m. carnica lines in Slovakia. Overall, genetic analyses have shown clear differentiation of Slovak Carniolan honeybee among three European sub-species of *Apis mellifera*.

Key words: carniolan honeybee; genetic diversity; individual population assignment; nuclear DNA markers

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POSTERS

EFFECT OF QUERCETIN APPLICATION ON SOME ANTIOXIDANT PARAMETERS OF RABBIT BLOOD *IN VIVO*

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The goal of the present study was to determine the effect of the chronic application of quercetin at various doses on selected antioxidant parameters of rabbit blood. Rabbits (n = 40) were divided into the control group (C) and 3 experimental groups (E1-E3). Experimental groups received quercetin (Sigma Aldrich, Saint Louis, USA) in injectable form (intramuscularly) at 10 $\mu g.kg^{-1}$ in the E1 group, 100 $\mu g.kg^{-1}$ in the E2 and 1000 $\mu g.kg^{-1}$ in the E3 group three times per week for 90 days. The control group received water injection (Imuna Pharm a.s., Šarišské Michaľany, Slovak Republic). Significant ($P < 0.05$) increase in the superoxide dismutase (SOD) level in the E3 group was observed in comparison with the control group. Significant ($P < 0.05$) decrease in the glutathione peroxidase (GPx) level in E2 and E3 groups was observed when compared to the control. Chronic application of different doses of quercetin caused slight decrease in the glutathione reductase (GR) level in the E1 group and E3 group in comparison to the control group. Serum iron content and total iron binding capacity (TIBC) did not show any statistical differences. Our findings demonstrate that quercetin may have some effect on activity of antioxidant enzymes. More experiments with various doses of quercetin should be performed.

Key words: quercetin; antioxidants; rabbits; blood

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ISOLATION AND CRYOPRESERVATION OF GERM CELLS OF FISH

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Artificial spawning is dominant in intensive fishing, which further implies the need for conservation of royal jelly and eggs. Germ cells possess the ability to differentiate into functional gametes of both sexes. In the early phase they are suitable for cryopreservation, and because of a high level of plasticity they provide secure maintenance of genetic resources. Germ cells, or primordial germ cells (PGCs), in their subsequent stages (spermatogonia / oogonia SG / OG), have the potential to undergo proliferation – differentiation into functional gametes and thus transfer the genetic information to the next generation. The main difference between the manipulation of the PGC and SG is a relatively simple method of isolation of a large number of SG, with respect to the isolation efficiency of only tens of PGCs. Deficiency in these early stages of cryopreservation of germ cells (SG / OG and their subsequent phases), is a limited activity shifts to the desired position in the gonads when these cells are transplanted.

In order to preserve germ cells it is necessary to prepare Percoll gradient and separate them from somatic cells. Cryopreservation protocol for early stage germ cells by cooling to -80 °C (1 °C/min) includes addition of an extender (0.5 % BSA, phosphate buffered saline (pH 8), d-glucose) and standard cryoprotectant. Research on cryopreservation of germ cells in fish has multiple significances for both science and practice. The fish is a species suitable for research due to its specific embryology. Also, as a vertebrate, fish shares many features with other vertebrates, mammals and with humans. Finally, fish are a large group of vertebrates with different species and types, which provides a wide field of work. For these reasons, fish are the ideal candidate to conduct research and all in terms of defining the cryopreservation as a powerful tool in understanding reproduction, and as a part of the research into infertility. Results show that relevant viability and fertility rates of germ cells after cryopreservation can be obtained with no negative influence on spawning process, which indicates good stability of GC in cryoprotectants. A large number of fish species in the world today are protected and/or endangered and cryopreservation, as a method of improving reproduction, plays a major role in their preservation.

Key words: cryopreservation; germ cells; fish

RAMAN SPECTROSCOPY – USEFUL TOOL FOR METABOLOMICS PROFILING OF SPENT MEDIA COLLECTED AFTER *IN VITRO* CULTURE OF MICE PREIMPLANTATION EMBRYOS

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Since a robust assay of embryonic health is unlikely to arise from an analysis of a small number of molecules, various methods, such as Raman spectroscopy, for analyzing wider spectrum of interactions between an embryo and culture medium are being tested at present time. Results of our recent research showed that somatic condition of female mice affected the quality of naturally produced oocytes, zygotes and natural development of preimplantation embryos. The aim of the present study was to compare overall patterns of metabolic activity of *in vitro* cultured preimplantation embryos isolated from control and fat mouse dams by means of non-invasive profiling of spent culture media using Raman spectroscopy. To produce females with two different types of body condition (control and fat), a previously established two-generation model was used, based on overfeeding of a portion of laboratory mice during prenatal and early postnatal development. When reaching adulthood, spontaneously

ovulating females were naturally fertilized. Embryos were isolated at the 2-cell stage of development and cultured to the blastocyst stage in synthetic oviductal medium KSOMaa. Embryos from fat mice (displaying significantly elevated body weight and fat) showed similar developmental capabilities *in vitro* as embryos isolated from normal control dams (displaying physiological body weight and fat). The results show that alterations in the composition of culture medium caused by the presence of developing mouse preimplantation embryos can be detected using Raman spectroscopy. Metabolic activity of embryos was reflected in evident changes in numerous band intensities in the 1620-1690 cm-1 (amide I) region and in the 1020-1140 cm-1 region of the Raman spectrum for KSOMaa. Moreover, multivariate analysis of spectral data proved that the composition of proteins and other organic compounds in spent samples obtained after the culture of embryos isolated from fat dams was different from that in spent samples obtained after the culture of embryos from control dams. This study demonstrates that metabolic activity of cultured preimplantation embryos might depend on the body condition of their donors, and that Raman spectroscopy is able to detect differences in metabolic activity between mouse embryos obtained from fat and control dams, even when they show similar developmental capacities.

Key words: preimplantation embryo; obesity; *in vitro* culture; Raman spectroscopy

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ANALYSIS OF SEVERAL TRAITS OF SARIS GIGANTIC RABBIT

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The aim of this work was to analyze several production traits in the population of Saris gigantic rabbit, a new breed originated from the Zobor rabbit breed. The experiment started in 2011, and after 5 years of reproduction a small herd consisting of 182 females and 73 males was formed in March 2015. The average number of all born pups in the litter was 9.09 ± 4.14 (35 females, 98 litters). The average weight of the litter was 359.5 ± 199.20 grams (min - 184; max - 506). The weight of the growing rabbits was determined once a month during the age from 60 to 240 days. The following weight of rabbits was recorded: at the age of 60 days -1102.03 ± 129.14 g (n = 45), at the age of 90 days - 2245.65 ± 193.10 g (n = 43), at the age of 120 days - 3048.68 ± 292.24 g (n = 38), at the age of 150 days - 4186.08 ± 329.18 g (n = 22), at the age of 180 days - 4897.03 ± 382.14 g (n = 18), at the age of 210 days - 5211.35 ± 413.16 g (n = 15) and at the age of 240 days - 5642.03 ± 456.20 g (n = 13). The average weight of slaughtered rabbits (at the age of 150 days) was 3146.10 ± 325.89 g for males (n = 20) and 2989.90 ± 225.78 g for females (n = 18). Slaughter yield for males was 57.01 % with the head and 51.32 % without head; for females it was 56.43 % with the head and 50.39 % without head. Based on these results, we can state that rabbits in the tested population manifested very good growth ability.

Key words: Saris rabbit; fertility; weight, slaughter yield

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EFFECT OF GOJI (*LYCIUM CHINENSE*) ON THE RABBIT MALE REPRODUCTION

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Goji or *Lycium chinense* (*Solanaceae* family) is a berry containing specified polysaccharide-protein complex which has biological impacts which may be beneficial for human health. Goji contains two basic compounds, carotenoids and polysaccharides. Goji is a powerful antioxidant containing 7-times more vitamin C than oranges and numerous other vitamins such as A, B1, B2, B6 and E. The aim of this study was to evaluate the effect of *Lycium chinense* on the rabbit reproduction parameters. Only sexually mature rabbit bucks (n = 8) were used in this experiment. Animals were fed a commercially available diet KV (C; n = 4) or a diet enriched with 1 g of *Lycium chinense* berries per buck daily (E; n = 4). The rabbits were fed for 65 days. Semen quality of rabbit bucks was assessed using the SpermVision CASA system (Minitüb, Tiefenbach, Germany). Our preliminary results showed no effect of Goji on semen concentration (1.39 ± 0.23 vs. 1.05 ± 0.48 mld.ml^{-1}), semen total motility (74.39 ± 0.28 vs. 81.43 ± 1.99 %) and progressive movement (58.18 ± 2.40 vs. 69.23 ± 4.12 %), for experimental group and control, respectively. In conclusion, Goji showed no beneficial effect on rabbit sperm quality parameters measured *in vitro*.

Key words: rabbit; *Lycium chinense* berries; semen concentration; motility

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GROWTH ABILITY OF ORAVKA CHICKEN

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The aim of this study was to analyse growth ability of the Oravka chicken. The weight of 5-, 12-, and 20-week old birds of both sexes, which were born in five successive years (2011 to 2015), was analysed. The experiment was performed in *in-situ* conservation flock (NAFC, RIAP Nitra). Birds were descendants of the roosters, who have been replaced every year. Because the birds weighed during one year had the same father, it was difficult to distinguish between variance influenced by a year and variance influenced by a father, therefore the effects "father" or "year" could be overlapping. Moreover,

fixed effects of sex, age, interaction of sex x father/year x age and random effect of bird were included in the mixed model, which was used to study chicken growth ability up to 12 weeks of age. All fixed effects significantly affected weight of the birds; repeatability accounted for 35 % of variance. Least square means were compared using Scheffe's multiple-range tests; the older birds were, the higher differences between weights in favour of males were occurred. Differences between 5-week old males and females (499.5 to 572.0 g vs. 343.3 to 384.1 g) were insignificant, whereas differences between 12-week old males and females (1299.9 to 1459.4 g vs. 1071.9 to 1104.8 g) tended to be or were significant and differences between 20-week old males and females (2236.5 to 2605.0 g vs. 1632.0 to 2054.7 g) were significant. Further research focused on more frequent weighing (e.g. once a week) is needed to enable more detailed analyzes of growth curves of the Oravka chicken.

Key words: Oravka; bird, growth ability; weight; sex; age

STIMULATION OF MITOCHONDRIA DURING MATURATION INFLUENCES DEVELOPMENT AND GENE EXPRESSION IN BOVINE EMBRYOS DERIVED FROM OOCYTES WITH DIFFERENT MEIOTIC COMPETENCE

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Supplementation of maturation media by metabolic regulators improves development and influences expression of metabolism regulating genes in mammalian embryos. Although positive effect of mitochondrial stimulator L-carnitine on mammalian oocytes and embryos was described, there is no information about its specific impact on oocytes with different meiotic and developmental competence. The study was designed to characterize the effect of L-carnitine during maturation on development and gene expression in bovine embryos derived from oocytes with different meiotic competence. Ovaries of slaughtered cows from the growth to stagnation phase of folliculogenesis were used. Meiotically more competent (MMC) and meiotically less competent (MLC) oocytes were collected separately either from medium or small follicles. The oocyte subpopulations matured with or without 2.5 mM L-carnitine were fertilized and cultured up to the blastocyst stage. The blastocyst formation rate and kinetics of blastocyst expansion were assessed. To analyse the expression of some genes playing roles in mitochondria biogenesis, apoptosis and embryonal development, a real RT-PCR was carried out using D8 expanded blastocysts. Significantly more MLC oocytes matured with L-carnitine developed into D7 early blastocysts and D8 expanded blastocysts in comparison with the control oocytes (31.7 % vs. 23.1 % and 33.3 % vs. 25.8 %, respectively). On the other hand, a significantly higher proportion of D8 expanded blastocysts was obtained in MMC oocytes matured with L-carnitine compared to the control oocytes (72.7 % vs. 59.3 %). The significant differences in ATP5C1 transcript level were found between blastocysts developed from MMC or MLC oocytes matured as a controls. The ATP5C1 transcript level significantly increased in the blastocysts from MMC oocytes, while its level decreased significantly in the blastocysts from MLC oocytes after both the oocyte categories were matured with L-carnitine. The GJA1 and GJB5 transcript levels were elevated in blastocysts from MLC oocytes matured with L-carnitine, but no differences in GJA1 and GJB5 transcript levels were found in the blastocysts from MMC oocytes matured with L-carnitine in comparison to the blastocysts from control oocytes. It can be concluded that L-carnitine presence during maturation enhances production of bovine embryos from less competent oocytes, accelerates blastocyst expansion from more competent oocytes and modulates expression of some genes in blastocysts derived from both oocyte sub-populations.

Key words: Bovine oocytes; meiotic competence; embryos; development; gene expression

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BIOCHEMICAL PARAMETERS IN BLOOD SERUM AND BREAST MUSCLE OF JAPANESE QUAILS AFTER FEEDING COMPLETE FEED MIXTURES WITH GM MAIZE

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The objective of this study was to determine the effect of selected maize varieties in diets on chosen biochemical parameters in the blood serum and meat quality of Japanese quails (*Coturnix coturnix japonica*). We evaluated the concentration of total protein, glucose, triglycerides, cholesterol, ALT, AST, Ca, P, Mg, Na, K in the blood serum of Japanese quails after feeding complete feed mixtures with 40 % of GM maize and isogenic maize. Live weight growth, feed conversion and health of quails after feeding of *Bacillus thuringiensis* (Bt) transgenic maize were recorded. Transgenic maize containing the Cry3Bb1 protein expressed in MON 88017 provides protection against the western corn rootworm (*Diabrotica vigifera*) and northern corn rootworm (*Diabrotica barberi*). GM maize MON 88017 was tested on two lines of quails (meat type and laying type of Japanese quails). In blood serum of quails following values were measured: total protein 32.75 – 35.25 $g.L^{-1}$, glucose 13.82 – 14.85 $mmol.L^{-1}$, triglycerides 1.25 – 1.49 $mmol.L^{-1}$, cholesterol 6.41 – 7.02 $mmol.L^{-1}$, ALT 0.13 – 0.16 $\mu mol.L^{-1}$, AST 4.08 – 5.07 $\mu mol.L^{-1}$, Ca 1.95 – 2.23 $mmol.L^{-1}$, P 2.2 – 2.7 $mmol.L^{-1}$, Mg 1.18 – 1.33 $mmol.L^{-1}$, Na 145.75 – 149.86 $mmol.L^{-1}$, K 2.98 – – 5.2 $mmol.L^{-1}$. In the experiment Bt maize was tested on 184 one-day-old Japanese quails (*Coturnix coturnix japonica*). Bt transgenic maize deteriorates neither health of animals,

nor the production of animal proteins valuable for human nutrition compared to conventional maize. The influence of Bt transgenic maize was significant in the group of Japanese quails of the meat type with the highest concentration of AST and potassium in blood serum. The breast muscles contained: total protein at 227 – 229 g, fat 16 – 22 g, Ca 0.20 – 0.26 g, P 2.5 – 2.6 g, Mg 0.28 – 0.30 g, Na 0.37 – 0.40 g, K 4.01 – 4.16 g, Fe 15.5 – 21.6 mg, Mn 0.3 – 0.5 mg, Zn 18.5 – 25.7 mg, Cu 1.4 – 2.2 mg per kg of original matter. Feeding of transgenic maize MON 88017 and isogenic maize to Japanese quails had no negative influence on the biochemical parameters of blood or on the biochemical parameters of meat. Similarly, it had no negative influence on body weight and egg quality parameters during the laying period in Japanese quails.

Key words: GM maize; Japanese quail; blood serum; meat quality; macro- and microelement

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POTENTIAL EFFECTS OF 4-OCTYLPHENOL ON HORMONE SECRETION BY MICE LEYDIG CELLS

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Hazardous chemical emission to the environment by a number of anthropogenic activities may cause adverse effects on human health and the environment. The most hazardous substances are endocrine disruptors. Prominent endocrine disruptors are alkylphenols, especially nonylphenol-ethoxylates and octylphenol-ethoxylates. They are used as surfactants in consumer products such as pesticides and detergents and as stabilizers for many other synthetic products. Major pathways of exposure to these substances have not been described, and may include dermal absorption from products, inhalation or dietary ingestion. Octylphenol is identified as substance of high concern. According to some experimental studies, exposure to octylphenol is associated with developmental and reproductive anomalies. Leydig cells produce the male steroid hormones and are considered the primary target of disruption by endocrine disruptors. Leydig cells express estrogen receptors and are subjects of estrogen action. Octylphenol is considered a typical endocrine disruptor with estrogenic action. In adult rats, repeated subcutaneous treatments with high doses of octylphenol induced serious disruption of the male reproductive tract through its estrogenic actions. In the present *in vitro* study Leydig cells were isolated from the testes of ICR mice. The aim of our study was to determine potential effect of 4-octylphenol on androstenedione and testosterone production by mice Leydig cells. The cells were cultured in the presence of human chorionic gonadotropin (hCG) and 4-octylphenol (at 0.04, 0.2, 1.0, 2.5 and 5.0 $\mu g.mL^{-1}$) for 44 h. Concentrations of steroid hormones in media were determined using enzyme-linked immunosorbent assay (ELISA). Exposure to different doses of 4-octylphenol showed possible influence on hCG-stimulated hormone secretion. Results of our *in vitro* study indicate slight decrease in androstenedione production. However, no significant differences were observed for any of the tested concentrations. On the other hand, the lowest dose of 4-octylphenol (0.04 $\mu g.mL^{-1}$) significantly ($P < 0.05$) increased testosterone secretion compared to the control group. Other experimental groups responded to 4-octylphenol by slight insignificant increase in testosterone output. More detailed and systematic research in endocrine toxicology is, however, required for a better understanding of risks associated with endocrine disruption in humans and wildlife.

Key words: mice; 4-octylphenol, testosterone, androstenedione

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BLOOD MINERAL PROFILE AFTER APPLICATION OF PATULIN IN RABBITS AND ITS MODULATION BY EPICATECHIN

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The aim of the present study was to investigate patulin-induced changes and protective effect of epicatechin in rabbit blood. Adult female rabbits (n = 25) were randomly divided into five groups: control group (C) and experimental groups (E1, E2, E3 and E4). Patulin was applied intramuscularly in groups E1, E2, E3 and E4 (10 $\mu g.kg^{-1}$ of body weight BW) twice a week, and animals in groups E2, E3 and E4 received also epicatechin three times a week for 15 days at doses of 10 $\mu g.kg^{-1}$ (E2), 100 $\mu g.kg^{-1}$ (E3) and 1000 $\mu g.kg^{-1}$ (E4). The blood serum was used for the analysis of following parameters: calcium, phosphorus, magnesium (Ecoline kits using RX Monza, United Kingdom), potassium, sodium and chloride (EasyLite Plus; Medica Corporation, USA) according to manufacturer instructions. We found that patulin had no effect on serum mineral parameters (phosphorus, magnesium, potassium, sodium chloride) as the differences among the groups remained insignificant ($P < 0.05$). Epicatechin also had no effect on tested parameters. Further studies with various doses of these compounds are needed.

Key words: rabbit; epicatechin; patulin; blood serum; minerals

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PROGRESS IN METHODOLOGY OF GENETIC DIVERSITY MONITORING IN PINZGAU CATTLE

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The aim of this study was to present progressive methodologies based on genomic data with an effect on future management of animal genetic resources in Slovakia. A genome-wide scan of Slovak Pinzgau cattle, as endangered autochthonous population, was carried out in relation to estimate the state of genetic diversity at a more precise level compared to previously published studies. All animals stored in the national Gene Bank have been genotyped by using Illumina BovineSNP50 v2 Bead chip. Alongside traditional parameters, such as allele and genotype frequencies or heterozygosity, estimated basing on single SNPs. The detailed analysis of genomic data enables a more realistic estimation of genetic diversity, population structure as well as level of admixture. It provides also an opportunity for calculation of genomic inbreeding coefficient from runs of homozygosity (ROH), linkage disequilibrium (LD) and effective population size (N_e). Moreover, the high-throughput data allow identification of the genomic regions affected by selection that can be unique for any autochthonous population. The analysis of SNPs associated with selection pressure focused mainly on the extreme values of F_{ST} index or integrated Haplotype Homozygosity Score (iHS). The analysis of ROH segments with different lengths allows for the knowledge of the age of inbreeding. Our results showed that the ROH segments >4 Mb covered in average 1.91 % of the genome and the ROH >16 Mb reached 0.43 %, which signalize inbreeding in recent generations. The identified level of inbreeding was higher compared to previously published results based on pedigree data. According to the available pedigrees only four animals were inbred, whereas based on $F_{ROH>8Mb}$ up to 13 animals can be regarded as inbred. On the other hand, the patterns of LD values provided information of both ancient population history (50 kb ≈ 1500 generations ago) and more recent events (4000 kb ≈ 12.5 generations ago) through calculation of N_e. As expected, due to the limited number of Pinzgau bulls used in breeding praxis, the observed values of recent N_e was on the limit characterizing the endangerment status of population (N_e = 80). The analysis of the selective sweeps through iHS score with connection to Austrian Pinzgau population identified several genomic regions that reflected the impact of different breeding goals of Slovak and Austrian herds. Similarly, based on the F_{ST} approach it was possible to find a threshold value showing genomic differences between them. Most affected SNPs were located on BTA 6, 7, 12, 16, 23 and 29 near the genomic regions containing the candidate genes like *CSNK1G3*, *CYP21*, *CSNK2B*, *PRL* and *PRP* that were previously associated with muscle formation or milk production. The results of this study can provide valuable data for increase of animal selection strategy efficiency. All of the applied methods are universal and wide-spectrum approaches that should be applied to other population of AnGR in Slovakia and represent the manual for effective monitoring of diversity to maintain the genetic potential of animal genetic resources for further generations.

Key words: Bovine SNP50 array; genetic variability; genome-wide scan; Pinzgau cattle

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RABBIT PRONUCLEAR ZYGOTE VITRIFICATION USING TWO DISTINCT CARRIERS

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Although vitrification is less time-consuming procedure than slow-freezing and does not require expensive equipment, relatively high-cost vitrification devices might be limiting in animal embryo vitrification practice. Therefore, in our study we compared the efficiency of the cryotop and low-cost calibrated plastic inoculation loop (CPIL) devices for vitrification of rabbit pronuclear zygotes in term of their post-thaw *in vitro* development. Presumptive pronuclear stage zygotes were recovered from the oviducts of superovulated New Zealand White rabbit females 19 hours after artificial insemination. Zygotes were vitrified, using minimum essential volume procedure in the vitrification solution composed of 15 % EG + 15 % DMSO + 0.5 M sucrose + 20 % FBS in TCM 199, then were placed to a cryotop or CPIL and plunged directly into liquid nitrogen within one minute. After warming, only zygotes with intact *zona pellucida* were selected for culture. Cleavage rate of the zygotes and development to the morula or blastocyst stage was examined after 24, 72 and 120 h of culture, respectively. No difference in cleavage rate (48.9 ± 4.6 vs. 48.5 ± 24.8) as well as in morula (39.8 ± 3.5 vs. 35.4 ± 15.9) or blastocyst (20.2 ± 7.0 vs. 19.5 ± 8.5) rates was found between the cryotop and CPIL, resp., although it was significantly lower ($P \leq 0.05$) than in fresh control (cleavage: 92.7 ± 12.6; morula: 92.7 ± 12.6; blastocyst: 90.9 ± 12.7). Therefore, our results demonstrate the efficiency of substantially cheaper device (CPIL) as a carrier for zygotes during vitrification.

Key words: zygote; vitrification; carrier; development

Acknowledgements: This study was supported by the grant APVV-14-0043.

THE EFFECTS OF DAILY MILK YIELD, MILK FAT AND PROTEIN CONTENT AND SCC ON THE MILK ANTIOXIDANT CAPACITY IN HOLSTEIN-FRIESIAN CATTLE

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Recently, an increased interest in food products containing natural antioxidants has been observed. Milk and dairy products are rich sources of natural antioxidants. Vitamins A, C and E, carotenoids, enzymes (such as glutathione peroxidase, superoxide dismutase), some proteins and fatty acids are the main antioxidants in milk. The main role of antioxidants in the body

is the protection from damage caused by free radicals. Increased amount of free radicals in organism is observed during the oxidative stress. Consumption of foods rich in natural antioxidants may have an impact on attenuation of oxidative stress. The aim of the study was to analyze the effects of daily milk yield, milk fat and protein content as well as SCC (somatic cell count) on the antioxidant capacity of milk. The investigated material was represented by 127 milk samples from Polish Holstein-Friesian cows of the Black-and-White variety (HO). The total antioxidant capacity of milk samples was measured by the Trolox Equivalent Antioxidant Capacity (TEAC) method. The data on milk performance (daily milk yield, fat and protein content and SCC) were obtained from the reports of monthly milk recording. It was found that milk samples from high-producing cows (> 30 kg) were characterized by lower antioxidant activity than milk samples collected from cows with lower yield (< 20 kg). High-yield cows often suffer from negative energy balance, which may cause a reduction in endogenous antioxidant precursors, thus the antioxidant activity of milk can be reduced. There were no statistical differences in the antioxidant activity of milk depending either on milk fat and protein content or SCC.

Key words: dairy cow; milk; antioxidant activity; protein; fat; somatic cell count

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EFFECT OF THE FIRST AND SECOND FARROWING ON NUMBER OF PIGLETS BORN ALIVE IN LARGE WHITE AND LANDRACE SOWS

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The primary requirement of intensive pig production is to obtain the maximum number of pigs with high genetic potential for productive properties per sow per year. High level of reproductive efficiency of pigs is one of the basic preconditions for successful animal production. Reproductive efficiency is measured by the number of live, healthy offspring of high genetic quality, per sow per year. This condition is possible to provide by a maximum intensity of propagation of parents with superior genetic traits. One of the main factors that help us achieve successful animal production conditions is the farrowing structure of the herd. On the basis of number of sows and the percentage of participation we can develop a plan of new exclusions for the next group. This paper analyzes the characteristics, number of piglets born alive, gilts and sows at first and second farrowing in Large White (LW) and Landrace (L) race. The analysis included 2200 sows from one farm in Vojvodina, which were farrowed in the period since 2009 to 2015. The analysis also included gilts from their firs farrowing, which were in the period of the first farrowing at the age of 364 days. From total of 2200 sows in the herd 11.24 % were sows at the first farrowing (LW 50.49 %, 49.13 % L) and 18.22 % at the second farrowing (50.49 % LW; 49.13 % L). The number of piglets born alive from sows at first farrowing was 9.9 (9.7 LW; 9.9 L) and the number of piglets born alive from sows at second farrowing was 10.1 (9.9 LW; 10 L). Greater fertility of sows can be achieved by working on improving the genetic base of pure breeds from their own herd. Costs of pig production can be reduced by increasing the born piglets per sow per year, and, therefore, good fertility is a basic assumption for economical operations. The paper demonstrates a higher number of piglets born alive at the second farrowing of sows compared to the first farrowing of sows. The largest number of piglets born alive was recorded in the Landrace race sows at the second farrowing.

Key words: born alive; sows; parity structure

DETERMINATION OF OOCYTE QUALITY USING BRILLIANT CRESYL BLUE STAINING

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Determination of oocyte quality is an important part in experiments for embryo production. The most frequent way to analyze the quality of porcine oocytes is characterization of morphology, which includes number of cumulus cell layers and granulation of the ooplasm. Despite this assessment, *in vitro* production (IVP) of porcine embryos still remains limited. Recently, a biochemical method based on analysis of the glucose-6-phosphate dehydrogenase (G6PDH) enzyme activity, named Brilliant cresyl blue (BCB) test, has been developed to assess the quality of oocytes. G6PDH is synthesized in growing oocytes (BCB negative, BCB-) and is inactivated in fully-grown oocytes (BCB positive, BCB+). BCB- oocytes remain colourless and BCB+ oocytes stain blue. Therefore, BCB test allows to distinguish fully-grown oocytes with higher quality from the growing oocytes. Another method of the oocyte quality assessment is measurement of their diameter. The goal of our study was to improve selection of porcine oocytes *in vitro*. We selected porcine oocytes according to morphology as described above. Afterwards, the oocytes were stained with 13 μM BCB diluted in mPBS (0.4 % BSA, bovine serum albumin, 0.34 mM glucose, 5.5 mM pyruvate, 50 $IU.ml^{-1}$ penicillin and 50 $\mu g.ml^{-1}$ streptomycin) at 38.5 °C in humidified air for 1.5 hours. Control group was cultured in mPBS without BCB under the same conditions. Then, the average diameter (the mean of two perpendicular diameters; Zen blue, magnification 200x) was measured in three groups of oocytes: BCB+, BCB- and control, resp. The experiment was done in 3 replicates. Data are expressed as mean ± S.E.Ms. Differences in oocyte diameters were analyzed by one-way ANOVA with Tukey's post-test (Sigmaplot 12.0, London, UK). We found significant differences between BCB+ (122.21 ± 0.92 μm) and BCB- group (117.99 ±1.02 μm; $P < 0.01$) and between the control (123.03 ± 0.65μm) and BCB- group (117.99 ± 1.02 μm; $P < 0.001$). There was no significant difference between BCB+ (122.21 ± 0.92 μm) and control group (123.03 ± 0.65μm). In conclusion, measurement of the oocyte diameter combined with BCB test are effective tools to analyze the quality of oocytes used for IVP.

Key words: brilliant cresyl blue; diameter; oocyte; pig

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ULTRASTRUCTURE OF RABBIT VITRIFIED PRONUCLEAR STAGE ZYGOTES

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In the present study, ultrastructure of rabbit vitrified pronuclear stage embryos was evaluated. Presumptive zygotes were recovered from the oviducts of superovulated rabbit females 19 hours after artificial insemination by flushing the oviduct. Zygotes were equilibrated (20 % ethylene glycol; 3 min), and transferred into the vitrification solution (40 % EG (v/v), 18.0 % (w/v) Ficoll 70 and 0.3M sucrose). Zygotes were placed onto a cryotop in 1-2 µl of vitrification solution and plunged directly into liquid nitrogen within one minute. After storage (2 hours), zygotes were warmed by immersing the cryotop into the warming solution (0.5 M sucrose) for 3 min, washed three times (0.25 M sucrose) and finally equilibrated in K-DMEM containing 20 % FBS for 10 min. Embryos were then fixed in Karnovsky´ fixative solution (2 % paraformaldehyde and 2.5 % glutaraldehyde in 0.15 M sodium cacodylate buffer, pH 7.1–7.3), individually embedded into 2 % agar and post-fixed in 1 % osmium tetraoxide. Samples were then embedded into Durcupan ACM (Fluka). Ultrathin sections (70 nm) were cut by Leica UC6 ultramicrotome and placed on nickel grids, contrasted with uranyl acetate and lead citrate and examined under a transmission electron microscope (JEM100 CXII, Jeol, Japan) operating at 80 kV. In zygote sections mainly mitochondria, vacuoles, lipid droplets and Golgi complex cisterns in the partially disintegrated ooplasma were observed. The ultrastructural images showed also pronuclei with nuclear precursor bodies and disintegrated nuclear envelope. Microvilli of cytoplasmic membrane were disrupted. In the perivitelline space, abundant cell debris were present. The structure of the *zona pellucida* was also affected by vitrification.

In conclusion, ultrastructural analysis of rabbit vitrified pronuclear stage zygotes revealed several cellular damages, mainly in the structure of the cytoplasmic membrane, nuclear envelope as well as the ooplasma disintegration.

Key words: rabbit; zygote; ultrastructure; organelle; vitrification

Acknowledgements: This work was supported from the grant of Slovak Research and Developmental Agency APVV-14-0043.

POLYMORPHISM OF 5-HYDROXYTRYPTAMINE (SEROTONIN) RECEPTOR 2A (5HTR2A) GENE IN POLISH RED CATTLE – PRELIMINARY STUDY

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Serotonin or 5-hydroxytryptamine (5-HT) is a tissue hormone, an important neurotransmitter in the central and peripheral nervous system. Serotonin plays many important biological functions in organism; among others it is a potent vasoconstrictor activator, an inhibitor of pain pathway in spinal cord, and is essential in the processes of sleeping and blood coagulation. 5-HT acts through the specific receptors - 5-HTR. It has been shown that the polymorphisms of the genes encoding the serotonin and its receptors were associated with human behavior (e.g. hyperactivity, apathy, susceptibility to depression, etc). In dairy and beef cattle herds, the breeders have been focusing on animal temperament, which has a direct impact on the handling of animals and thus the efficiency of animal production. The bovine serotonin receptor 2A (5HTR2A) gene is located on the chromosome 12 and consists of 4 exons and 3 introns. Many SNP (single nucleotide polymorphism) mutations have been mapped in encoding sequences of this gene. So far, there are no literature data on the association between the SNPs of 5HTR2A and cattle behavior. The aim of the study was to analyze the polymorphism of second exon of 5HTR2A gene in Polish Red cattle. Research material consisted of 56 milk samples from which DNA was isolated. Analysis of the polymorphism was performed by SBT method.

Key words: cattle; behavior; serotonin; receptor; SNP

Acknowledgements: This study was supported by the grant DS-3259/ZHB/2016.

IMPROVEMENT OF HIGH FAT DIET-INDUCED HYPERLIPIDEMIA AND HYPERGLYCEMIA BY AN AQUEOUS EXTRACT OF LISOSAN® REDUCTION IN MICE

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Lisosan® Reduction is a cereal and herb mixture produced from powdered *Triticum aestivum* (Lisosan® G), *Desmodium adscendens*, *Malus domestica*, extract of rhizoma of *Picrorhiza kurroa* and seeds of *Hordeum vulgare*. The aim of this study was to evaluate the effects of aqueous extract of Lisosan® Reduction in mice fed a high fat diet. Thirty two C57BL/6J male mice were divided into 4 groups and fed different diets for 11 weeks: control diet (CTR), control diet with 60 $mg.kg^{-1}$ b.w. of Lisosan® Reduction extract (CTR+Red), high fat diet (HFD) and high fat diet with 60 $mg.kg^{-1}$ b.w. of Lisosan® Reduction extract (HFD+Red). Serum AST, ALT, cholesterol, glucose and triglyceride content was measured with standard enzymatic techniques. Lipids were extracted from hepatic tissue adopting a gravimetric method, while histological examination of liver section was performed by haematoxylin and eosin staining. The supplementation of aqueous Lisosan® Reduction extract in CTR+Red mice did not cause any alteration of serum AST, ALT, cholesterol, glucose and triglycerides level, hepatic lipid content and histological results, compared to the CTR group. Otherwise, the supplementation of Lisosan® Reduction in HFD+Red group showed a significant decrease ($P < 0.01$) in serum cholesterol (-27.6 %), glucose (-22.5 %) and triglyceride (-30.8 %) level, while no changes in serum AST and ALT concentrations were detected when compared to the HFD group. Moreover,

HFD+Red mice did not display any improvement of hepatic lipid accumulation compared to HFD, confirmed by histological results. In conclusion, Lisosan® Reduction showed a hypocholesterolemic, hypoglycemic and hypotriglyceridemic effect, but did not protect against steatosis in mice fed a high fat diet.

Key words: Lisosan® Reduction; hypercholesterolemia; hyperglycemia; high fat diet; mice

DISTRIBUTION OF L-SELECTIN (CD62L) ON CATTLE CELLS AND TISSUES

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L-selectin, also known as SELL or CD62L, is a cell adhesion molecule that belongs to the selectin family of proteins. L-selectin acts as a "homing receptor" for lymphocytes to enter secondary lymphoid tissues via high endothelial venules, facilitating entry of lymphocytes into the secondary lymphoid organs. L-selectin is also present on the surface (trophoblast) of human embryo. L-selectin facilitates the adhesion of the embryo, prior to the implantation into the uterine endometrium. Presence of L-selectin on human spermatozoa and possible role in sperm adhesion to oocytes were also examined. The CD62L molecule was identified on the head of human spermatozoa and the anti L-selectin antibody inhibited the sperm-*zona pellucida* binding. The aim of this study was to identify the distribution of bovine CD62L molecule focusing on the gametes and the tissues of reproductive organs. Tissues for histochemical staining were obtained at local slaughterhouses. Ejaculated spermatozoa were received from Slovak Breeding Services Inc. The eggs at different developmental stages were prepared by *in vitro* culture of ovarian eggs. The CD62L molecule was detected by FACS analysis, indirect immunoperoxidase and immunofluorescence tests using the anti-CD62L monoclonal antibody IVA-94 (the specificity verified by Third Workshop on Ruminant Leukocyte Antigens, Davis-USA, 1995). In the blood, most leukocytes (granulocytes 100 %, monocytes 98 %, and lymphocytes 75 %), 46 % of platelets and none of erythrocytes expressed the CD62L molecule. The indirect immunoperoxidase test gave clear staining of sections in the cattle spleen and lymph node. L-selectin was present on spleen mantle zone B Lymphocytes. Strong staining was found in the T cell region of PALS especially round the central artery. Positive cells were also seen in the interfollicular region and medullary cords. Very weak reactions were found in the Peyer's patches of the small intestine. In the liver, kidney and lung no reaction was found. The reaction pattern of anti-CD62L antibody (IVA-94) was similar to those observed in human lymphoid tissue with exception of the weak reaction in the bovine intestine Peyer's patches. L-selectin is present in the tissue of bull reproductive tract. The most intensive staining was found in the interstitial tissue with circular muscle fibres of epididymis. CD62L molecule was detected on the developing spermatozoa in the lumen of the tubules of testis and epididymis. Anti-CD62L monoclonal antibody was reactive with majority of ejaculated and fixed (with methanol or acetone) spermatozoa on the anterior part of the head. Expression of CD62L molecule in the cow reproductive tissues (vagina, ovary, oviduct, uterus) was recognized by immunofluorescence assay. Positive reaction of IVA-94 was observed on mature oocytes and two day-old embryos.

Key words: molecule CD62L; MSCs; bovine tissue; histochemistry

Acknowledgements: VEGA-2/0037/16; APVV-15-0196 and bilateral project SAV-AV ČR 15-05.

EFFECT OF HYPERTHERMIA ON SEVERAL MOTION PARAMETERS OF RABBIT SPERMATOZOA

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The aim of our study was to examine the impact of hyperthermia on several motion parameters of rabbit spermatozoa (VAP - average path velocity, VCL - curvilinear velocity and VSL - straight-line velocity). Six male rabbits from M91 and P91 meat rabbit lines were used in the study. Experimental hyperthermia (temperature 36 ± 3 °C) was simulated in a closed breeding area with an installed heat aggregate and temperature sensor. The environmental conditions were set in specialized halls used for rabbit breeding for 21 days; food was supplied *ad libitum* in form of KKV1 feed. Semen samples were collected using an artificial vagina once a week during the period of the experiment (3 collections). The control semen collection was performed prior to exposure of animals to high ambient temperature. After the collection the samples were transferred to the laboratory at 22-25 °C, and the analysis of spermatozoa motion parameters was carried out using CASA–SpermVision system (MiniTüb, Tiefenbach, Germany). The following parameters were evaluated: VAP (μm.s^{-1}), VCL (μm.s^{-1}) and VSL (μm.s^{-1}). Analysis of the effects of hyperthermia on the mentioned parameters showed that the lowest VAP (24.62 ± 24.05 μm.s^{-1}), VCL (47.24 ± 46.19 μm.s^{-1}) and VSL (21.45 ± 21.05 μm.s^{-1}) of the sperm in ejaculates was recorded in the control collection (collection 0) compared to the first (45.30 ± 5.51 μm.s^{-1}; 91.93 ± 11.21 μm.s^{-1}; 34.54 ± 6.49 μm.s^{-1}), second (28.06 ± 33.66 μm.s^{-1}; 52.97 ± 63.55 μm.s^{-1}; 21.01 ± 25.24 μm.s^{-1}) and third (41.65 ± 28.30 μm.s^{-1}; 93.86 ± 64.76 μm.s^{-1}; 34.77 ± 25.85 μm.s^{-1}) collections. Significant differences ($P < 0.05$) in the VCL values were recorded among the control, the first and the third collections. Our results showed that hyperthermic stress can alter motion parameters of rabbit spermatozoa.

Key words: CASA; hyperthermia; rabbit sperm; VAP; VCL; VSL

REPRODUCTION PARAMETERS OF BROWN HARE FARMED IN SLOVAKIA

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The farming of brown hares is important for eventual revitalization of wild populations. The aim of our work was to analyse and compare the reproductive parameters of brown hare in selected farms in Slovakia. In 2016, 63 breeding pairs were involved in breeding on four farms. The animals were kept under similar conditions and fed granular compound feed. Totally, five couples (7.9 %) were without offspring, and in six couples one adult hare was died during the breeding period. These couples were excluded from further analyzes. The average number of litters per a fertile female was 4.29, while the highest recorded number of litters was 6 and the lowest was 1. Large differences in reproductive parameters between farms were found. Totally 570 leverets were born in 223 litters. The average number of leverets in the litter was 2.56. A difference was observed in the average number of leverets born per fertile female between individual farms (from 10.2 to 15.3). The average number of born leverets was 9.63 and 7.67 leverets per 1 fertile female were weaned. The highest number of leverets born per female was 19 and the highest number of weaned leverets was 18. The highest number of leverets per one litter was 6. The average mortality was 21.05 % of leverets, of which 1.58 % were born dead and 19.47 % were dead before weaning. Mortality was highest during the first 7 days of life (16.5 %). Frequency of litters during the year was balanced rather from February to August (from 11.2 to 15.5 %). Litter in January was recorded only in one case (0.45 %) and six litters were recorded in September (2.69 %). Most leverets (119, 19.1 %) were born in May. Despite the differences in reproduction, a farm breeding of hares may be a convenient source of subjects for the revitalization of wild populations. However, the issue of their adaptation needs to be resolved before their release into the wild.

Key words: brown hare; *Lepus europaeus*; reproduction; farming

Acknowledgements: This work was supported by the Slovak Research and Development Agency under the contract no. APVV-0368-10 and APVV-15-0474.

FREQUENCY OF REMOVAL REASONS OF SOWS IN NORTH SERBIA, VOJVODINA

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Sow longevity is a large and often overlooked component of profitability for comercial swine farms. The aim of this study was to describe the reasons for removal in pig farms under climate conditions for North Serbia. We studied the records of one herd with an average inventory of 2200 sows and 4840 farrowings. The data collection was based on a PC herd monitoring program. The average annual removal was 48.5 %. Among the removed sows, 83.7 % were slaughter, 13.8 % were found dead and 2.5 % were euthanized. Reproductive failure was the most common cause of culling. The reasons for removal included locomotor problems and bad body conditions with a responsibility for 13.17 % of all removals, of which lameness was the main problem (60 %). Reproductive disorders as a reason had 30 % frequency; it included anoestrus (7.76 %), negative pregnancy check (17.15 %), abortion (7.04 %), failure to conceive (10.29 %) and failure to farrow (5.54 %). Other reasons were grouped into the category "not farrow". Diseases were responsible for 13.8 % of all removals, of which the main reasons were respiratory problem and diarrhea and also death just after farrowing. Not only the sows intended for slaughter are included in this study, but also euthanized and found dead. Our results indicate also that the season as well as lower and higher parities affect the culling of sows. It was concluded that sows on many farms in North Serbia are being culled prior to reaching their reproductive problems.

Key words: sow; culling; removal; reproduction

SOMATIC CELL COUNTS IN MILK OF SUCKLED NATIVE VALACHIAN EWES DURING LACTATION

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Somatic cells counts (SCC) in milk are considered a main indicator of the udder health. Udder health is influenced by many internal and external factors. Machine milk removal is considered a factor negatively influencing udder health, especially due to milking more ewes by the same teat cup. Milking more ewes by the same teat cup spreads the bacteria among ewes if one of the ewes is infected. Different conditions of milk removal could be observed when ewes are suckled by own lambs, especially in the native breed of ewes with developed strong mother-young relationship. Such maternal bond limits possible contamination of the udder by suckling foster lambs. Though SCC is in close relationship to udder health, there are still no legislative acceptable limits for SCC, as it is in dairy cows. The aim of the work was to monitor SCC in milk of suckled native Valachian ewes throughout lactation. The milk samples were collected from 39 suckled Valachian ewes during lactation (April, May, June and July). Before each sampling of milk the ewes were separated from their lambs and after i.v. 4 IU oxytocin injection the ewes were machine milked. Obtained milk was discarded. At approximately 5 hours after last milking ewes were milked out again after i.v. 4 IU oxytocin injection. The samples (n = 152) were collected from obtained milk for SCC analysis. On the basis of SCC in milk, fifth classes of SCC were assigned to study the frequency of distribution of animals with health problems of udder: SCC $<200\times10^3$ cells.ml^{-1}, SCC between 200-400$\times10^3$ cells.ml^{-1}, SCC between 400-700$\times10^3$ cells.ml^{-1}, SCC between 700-1000$\times10^3$ cells.ml^{-1} and SCC $>10^6$ cells.ml^{-1}.

The frequency of distribution of ewes into different SCC classes was 70 %, 20 %, 3 %, 0 % and 7 %, respectively. The worst distribution of ewes into different SCC classes was found out in May: 36 %, 49 %, 3 %, 0 % and 13 %, respectively. In all others months, more than 79 % of animals were assigned to the first SCC class. Obtained data point out that suckled Valachian ewes had low SCC in milk throughout lactation indicating a good udder health. These results could contribute to the possible validation of limits for SCC in a future legislative.

Key words: Valachian ewes; suckling; milk; somatic cells; lactation

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PHENOMENON ACROMELANISM IN RABBITS

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This phenomenon is described as the production of pigment on the peripheral parts of the body as a result of thermolability of tyrosinase, the enzyme which plays an important role in the pigment production. Tyrosinase gene was analyzed at the molecular level in five Marten Blue rabbit families with a total of 38 animals. The aim was to determine an association of tyrosinase polymorphism with different phenotypes with three colours of this breed (dark phenotype, standard light and acromelanic albinos). After High Resolution Melting analysis of all five tyrosinase gene exons, the differences between these colour phenotypes have been shown in the second exon of the tyrosinase gene. By comparing the tyrosinase DNA sequences of analysed rabbits with available tyrosinase sequences in GenBank (AF210660 and NC_013669) we found two positions in the second exon of the tyrosinase gene related to the three different colour phenotypes of blue marten rabbit breed, position c.860G>C and position c.881A>G, each position associated with a slightly different drawing by a pigment. Individual genotypes for these positions are: for the c.860G>C position dark marten rabbit has CC genotype, marten light standard rabbit has CG genotype and acromelanic albino has GG genotype. In the position c.881A>G, rabbits with dark marten phenotype have AA genotype, standard light marten rabbits have AG genotype and acromelanic albino rabbits have GG genotype. The difference between these two positions is that the standard phenotype with GC genotype for position c.860G>C has weaker pigmented face, making lighter area between eyes (so-called mirror) compared to colour phenotype bearing AG genotype for position c.881A>G in the tyrosinase gene.

Key words: rabbit coat colour; acromelanism; tyrosinase

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