

**SLOVAK AGRICULTURAL  
UNIVERSITY IN NITRA**

**FACULTY OF BIOTECHNOLOGY AND FOOD  
SCIENCES**

**DEPARTMENT OF ANIMAL PHYSIOLOGY**

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**VII. SLOVAK CONFERENCE OF ANIMAL  
PHYSIOLOGY**

May, 23 – 24<sup>th</sup> 2007  
Topolčianky, Nitra, Slovak Republic

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**ABSTRACTS**

of the international conference of the

**VII. SLOVAK CONFERENCE OF ANIMAL  
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**Dear participant, colleagues, friend,**

we are cordially pleased to welcome you on the 7-th Slovak Seminar on Animal Physiology with international participation. As in the previous meeting the seminar is organized by the Department of Animal Physiology, Faculty of Biotechnology and Food Sciences at Slovak Agricultural University in Nitra.

Aim of our seminar is a presentation of actual information's about animal physiology but also a meeting of researchers in a same field of research, to find new contacts and discussion of joint project in close future.

We appreciate acceptance of collegeague from various universities and research institutes at this seminar. Specially we would like to express our acknowledgement to foreign participants.

We would like to wish you creative atmosphere for presentation of many interesting presentations, stimulating ideas and pleasant stay at the seminar.

Finally we would like to express our thanks to members of scientific and organizing committee for their activities and all active participants for perfect course of seminar.

Nitra, May 23-th 2007

Organizing committee

## **Effect of hypodynamy on the kidneys of Japanese quails (*Coturnix coturnix japonica*)**

Almášiová, V., Cigánková, V., Holovská, K., Zibrín, M.

*University of Veterinary Medicine, Košice, Slovak Republic*

The aim of present study was to observe the influence of simulated weightlessness on the structure and ultrastructure of kidneys of Japanese quails reared under the conditions of experimental long-term hypodynamy. Hypodynamy is a method to simulate weightlessness in the earth laboratory conditions. Two days after hatching the quails were suspended in special shirts below the ceiling of a cage so their feet did not touch the floor. They could consume food and water ad libitum. Experimental animals were sacrificed after 7, 14, 21, 28, 35, 42, 49, 56, and 63 d of hypodynamy. Animals of the same age, hatched at the same time, and fed the same diet were used as controls. The samples of kidney were fixed and routinely processed for light (LM) and transmission electron microscopy (TEM). It has been found that short-term hypodynamy (7–21 days) does not induce visible damage to kidney structure. Due to long-term hypodynamy (28–63 days) there are some morphological changes in the proximal and distal tubular cells as well as in collecting ducts. However, the structure of renal corpuscles was not damaged.

## **The effect of including faba bean in the diet and hen's age on eggs albumin quality**

AL-Sardary Sardar Y. T., Abas. Kamaran Abduljalil

*Salabaddin University, Iraq*

The aim of this experiment was to study the influence of field beans (FB) and also birds' age on some albumin parameters of commercial layers. Seven different experimental diets (Raw Faba Beans "RFB" 10%, 20% and 30% compared with Roasted Faba Beans "RoFB" 10%, 20% and 30%) and both groups compared with commercial layers' diet labeled control (C). The experiment was performed in a seven group laying test with 3 replicates, with a total number of 630 birds aged 43 weeks. The control group and six trial groups were enclosed in 3 floor

caged batteries. To test the effect of the birds' age on the albumin quality, 28 days during the experiment period (P) were observed. For the feeding we used a feed mixture of wheat, barley, soybean meal, RFB or RoFB, vegetable fat with an addition of minerals, vitamins and enzymes. The influence of the diet and ages on Haugh Unit (HU), Albumin Index (AI) and Albumin Percentage (AP) were studied. HU increased significantly with including RFB and RoFB in the diet compared with C. And the values were increased linear with increasing RoFB levels. AI also increased generally, and the values were significant at the level of 10% and 20% for both RFB and RoFB groups compared with C. But was insignificant at the level of 30% for both RFB and RoFB groups. HU decreased with the increasing of the bird's age but AI and AP increased significantly by advanced ages of the bird.

### **Effect of experimental administration of nickel on egg albumen quality and egg weight of market eggs**

Arpášová, H., Massányi, P., Capcarová, M., Kolesárová, A., Kalafová, A.

*Slovak Agricultural University, Nitra, Slovak Republic*

The experiment was implemented on the efficient Isabrown laying hens producing brown shell eggs. We used 53 weeks old hens. The hens were fed by the standard feed mixture HYD – 10. Animals received fodder ad libitum. Hens (n=20) were divided into four groups (K, E1, E2, E3). Experimental hens (5 in each group) received nickel (NiCl<sub>2</sub>) per os in drinking water for 28 days. In control group K hens received drinking water without additions. In experimental groups supplement in the drinking water nickel (NiCl<sub>2</sub>) in different concentrations was tested. The nickel concentration was in experimental group E1 0.02g.l<sup>-1</sup>, in experimental group E2 0.2g.l<sup>-1</sup> and in experimental group E3 2g.l<sup>-1</sup>. In all groups laying hens received drinking water ad libitum. The experiment lasted 28 days. The following efficiency indexes were monitored – egg weight, albumen weight, index of albumen, percentage and the Haugh units of the albumen. The greatest differences during experiment were achieved between control group C and experimental group E3 with the highest concentration 2 g.l<sup>-1</sup> NiCl<sub>2</sub>. Different values in the other groups with low concentrations NiCl<sub>2</sub> were not so bold in comparison with control group. The egg weight was in order groups:

62.27; 59.11; 64.90; 50.97g. Statistically significant differences were achieved among the following combinations of groups: very high significant difference ( $P \leq 0.001$ ) between control group and experimental group E3; between experimental groups E3 and E2 respectively E3 and E1; high significant difference ( $P \leq 0.01$ ) between experimental groups E1 and E2. Difference between control group and experimental group E3; between experimental groups E3 and E2 respectively E3 and E1 were statistically very high significant ( $P \leq 0.001$ ) for albumen weight. Statistically high significant difference ( $P \leq 0.01$ ) was observed between experimental groups E2 and E1 and significant difference ( $P \leq 0.05$ ) was observed between control group and experimental group E2. Differences between control group and experimental group E2 respectively experimental groups E3 and E2 were statistically significant ( $P \leq 0.05$ ) for percentage albumen from egg weight. By evaluation of Index albumen and Haugh units were found no significant differences between control and experimental groups ( $P > 0.05$ ).

### **Distribution of nickel in the organism of rats after an experimental administration.**

Bábiková, L., Toman, R., Golian, J., Massányi, P., Hluchý, S., Lukáč, N., Šiška, B.

*Slovak Agricultural University, Nitra, Slovak Republic*

The aim of this study was to evaluate the distribution of nickel chloride in the organism of rats after an experimental administration. The rats were given nickel chloride in a different concentrations. The first part of the study (group A and group P) was carried out on male rats ( $n=10$ ) which were injected (i.p.) with nickel chloride at a single dose of 25  $\text{mg.kg}^{-1}$  and 35  $\text{mg.kg}^{-1}$  b.wt., respectively, and killed 48 hours after the nickel administration. In the second part of research (group B) 10 rats males were orally dosed with 100  $\text{mg.l}^{-1}$  in drinking water during three months. Ten males served as the control group (K) without the nickel administration. The samples of kidney, muscle (m. quadriceps femoris), liver and testis were taken for the nickel analysis. The highest concentration of nickel was detected in the kidney after an intraperitoneal administration of 35  $\text{mg.kg}^{-1}$  of nickel chloride. The difference between the group P and the control one was significant

( $p < 0.001$ ). The nickel level in the kidney were significantly higher in group A when compared to the group B ( $2.48 \pm 0.83 \text{ mg.kg}^{-1}$  and  $0.24 \pm 0.14 \text{ mg.kg}^{-1}$ , respectively). The differences in liver nickel concentration in all experimental groups were insignificant. The analysis of nickel concentration in the muscle tissue showed the significant decrease from  $1.18 \pm 0.79 \text{ mg.kg}^{-1}$  in group K to  $0.20 \pm 0.12 \text{ mg.kg}^{-1}$  in group B. In the testis, the nickel concentration was highest in group P ( $0.61 \pm 0.19 \text{ mg.kg}^{-1}$ ) in comparison to the control (K) and experimental group B ( $0.22 \pm 0.19 \text{ mg.kg}^{-1}$  and  $0.22 \pm 0.17 \text{ mg.kg}^{-1}$ , respectively). These differences were statistically significant ( $p < 0.05$ ). These findings indicate that the acute administration of nickel chloride causes the highest increase in the level of the metal in kidney.

### **The role of transcription factor p53 and hormone ghrelin in control of porcine ovarian cell functions**

Benčo A., Sirotkin A.V.<sup>1</sup>

*Constantin the Philosopher University, Nitra; <sup>1</sup>Slovak Agricultural Research Centre, Nitra, Slovak Republic*

The aim of our in vitro experiments was to examine (1) the role of transcription factor p53 in control of apoptosis, proliferation and secretory activity of porcine ovarian cells, (2) the role of ghrelin in control of these processes in porcine ovarian cells, (3) the role of p53 in mediating ghrelin effects on porcine ovary. Transfection of porcine cells with cDNA construct for transcription factor p53 decreased ASK-1, Bax, PGF and increased PCNA, OT and PGE<sub>2</sub>. We have studied (1) effect of additions of hormone ghrelin on occurrence of apoptosis (expression ASK-1, Bax) proliferation (expression of PCNA) and release of hormones (prostaglandin F, oxytocin and prostaglandin E<sub>2</sub>) in cultured porcine ovarian granulosa cells, (2) effects of transfection of porcine ovarian granulosa cells with cDNA construct on these processes. It was observed, that ghrelin inhibited expression of ASK-1, Bax did not affect expression of PCNA, increased prostaglandin F, oxytocin and PGE<sub>2</sub> release by porcine cells. Moreover, transfection of porcine cells with p53 cDNA construct reversed effect of ghrelin on ASK-1 from inhibitory to stimulatory, did not affect influence of ghrelin on Bax, converted ghrelin influence on PCNA from zero to inhibitory effect, did not affect influence of ghrelin on prostaglandin F,

changed the effect of ghrelin on oxytocin from stimulatory to inhibitory in porcine ovaries. The obtained results suggest that p53 is regulator of apoptosis, proliferation and secretion. Hormone ghrelin is involved in control of porcine ovarian functions; it can be inhibitor of apoptosis, and stimulator of hormone secretion. The opposite effects of ghrelin on normal cells and cells with overexpression of p53 indicate that this transcription factor could be potential mediator of ghrelin action on the porcine ovary.

### **The effect of the probiotic Bonvital on production parameters in fattening pigs**

Bindas, L., Vajda, V., Maskařová, I.

*University of Veterinary Medicine, Košice, Slovak Republic*

Results of the feeding experiment conducted on 94 fattening pigs, based on supplementation of their diet with probiotic preparation Bonvital at a dose of  $4.4 \times 10^5$  CFU/kg mixed feed ( $3.27\text{-}5.68 \times 10^5$  CFU/kg) during 103-day fattening period up to the slaughter grade, confirmed positive influence of this preparation on growth intensity, feed conversion and significantly higher slaughter weight. The fattening pigs in experimental group reached slaughter weight of  $108.6 \pm 10.3$  kg while those in the control group weighed  $101.9 \pm 11.3$  kg, the difference 6.7 kg ( $P < 0.01$ ). The experimental group reaching mean weight gain 707.8 g/day and control group 639.8 g/day, the difference 68 g, i.e. 10.6%. The feed consumption per kilogram weight was in the experimental group 3.39 kg compared to the control 3.75 kg. The feed consumption per kg weight gain in the control group was higher by 0.36 kg, which amounts to 11.6%.

### **The use of genomics in the research of physiological background of economically important farm animals traits**

Bulla, J.

*Slovak Agricultural University, Nitra, Slovak Republic*

Genomics in livestock has been concentrating on the construction of genome maps and their use for the mapping of monogenic and quantitative trait loci. Most traits of economic importance in farm

animals are complex in that they are polygenic and influenced by non-genetic environmental factors. Large single gene effects can be detected as segregating QTLs (quantitative trait loci) with genetic markers. In recent years, subsequent positional cloning efforts have led to the elucidation of the causal mutation for several genetic diseases, metabolic variation but also monogenic factors affecting muscle mass, meat quality and ovulation rate in different livestock species. In several cases, unexpected or previously unknown genes have been detected. Physiological evidence is however very preliminary especially for the elucidation of pleiotropic effect and for quantitative traits. Experiments studying metabolic control of production characteristics can be designed to capitalize on the existence of genes and its functional variants.

### **Effect of hypodynamy on structure and function of duodenal enterocytes of Japanese quails**

Cigánková, V., Almášiová, V., Holovská, K., Lenhardt L., Škrobánek P.<sup>1</sup>, Zibrín, M.

*University of Veterinary Medicine, Košice, <sup>1</sup>SAS, Ivanka pri Dunaji, Slovak Republic*

The effect of hypodynamy on the structure, ultrastructure, and enzymatic activity of the duodenal enterocytes of Japanese quail (*Coturnix coturnix japonica*) was studied. On the second day after hatching, 42 chicks were placed in individual slings suspended by a flexible metal device in such a manner that their legs could not touch the floor. Feed and water was provided ad libitum. Experimental animals were killed at 5, 7, 14, 21, 28, 35, and 42 d of age. The birds of control group were kept under standard conditions on the floor. The samples of the duodenum were routinely processed for light microscopy (LM) and transmission electron microscopy (TEM), and alkaline phosphatase (AP) activity was determined. Morphological changes in enterocytes of chicks reared under hypodynamy from day 2 after hatching were observed on days 5 and 7 of age. LM showed that the apical ends of intestinal villi contained clumps of necrotizing enterocytes which were more numerous compared to the control. TEM revealed loss of microvilli and changes in both the nucleus and cytoplasm in these cells. Besides considerable damage to mitochondria,

they exhibited also typical vesiculation of cytoplasm and damaged intercellular junctions. From day 14 to 42 of age morphological changes were less pronounced. On day 7 of age, the activity of AP in the microvilli of duodenal enterocytes of experimental chicks was increased in comparison with the control ( $P < 0.001$ ). On day 14 of the experiment, AP in the investigated structures showed only a slight increase ( $P < 0.05$ ) and on day 21 was also slightly increased ( $P < 0.01$ ). On days 28, 35, and 42 of age, no significant differences in the activity of AP were observed. Hypodynamy acted as a stress factor on Japanese quail, which was stronger at the beginning of the experiment. Animals gradually adapted to the respective conditions. Our results show that Japanese quail chicks are capable of feed consumption and feed conversion under conditions of hypodynamy.

### **Quantitative analysis of kidneys of *Apodemus flavicolis* from ecosystems in Novaky**

Drábeková, J., Jančová, A.<sup>1</sup>, Massányi, P., Lukáč, N.

*Slovak Agricultural University, Nitra; <sup>1</sup>Constantin the Philosopher University, Nitra, Slovak Republic*

This work presents histological analysis of kidney tissue. We evaluated microscopical kidney structures of species *Apodemus flavicolis* (Muridae, Rodentia). Samples for histological preparations were taken from adult individuals, which were in a very good physical condition and sexual active. They come from ecosystems that are situated close to thermal power station Novaky. For quantitative analysis morphometrical image analyzer softwer (Micro Image ver. 4.0) was used according to micromorphological criteria. The diameter of renal corpuscle, glomeruli, Bowman's capsule, tubules as well as height of tubular epithelium, surface of tubules and lumen, perimeter of tubules and lumen were evaluated. The differences between male and female in quantitative analysis were statistical significant ( $P < 0.05$ ).

## **Effect of graded levels of mycotoxin on clinical plasma characteristics in chicks**

Faixová, Z., Faix, Š.<sup>1</sup>

*University of Veterinary Medicine, Košice; <sup>1</sup>Slovak Academy of Sciences, Košice, Slovak Republic*

An experiment was conducted to investigate the effect of different doses of deoxynivalenol (DON) on plasma indices of broiler chickens. Forty-two, 1-d old male broiler chicks were fed 1 of 3 diets containing DON for 42 d. The diets included (1) control (0.2 ppm DON); (2) low level of DON (1 ppm DON) and high level of DON (3 ppm DON). The administration of 1 ppm DON to the diet altered total protein, triglycerides, free glycerol and potassium levels. Dietary inclusion of 3 ppm DON resulted in altered calcium, potassium, total protein, triglycerides, free glycerol levels and AST activity. No biochemical parameter, however, responded to increased DON concentration in the diet. The feeding of DON-containing diets did not significantly alter plasma chloride, cholesterol and albumin levels or AST, ALP and LDH activities. It was concluded that both levels of DON in the diets tested significantly affected protein and lipid metabolism in broiler chicks.

## **Leptin as a regulator of bovine oviductal functions in vitro**

Fazekašová, J.<sup>1,2</sup>, Makarevič, A.V.<sup>1</sup>, Sirotkin, A.V.<sup>1</sup>, Bulla, J.<sup>1,2</sup>

<sup>1</sup>Slovak Agricultural Research Centre, Nitra; <sup>2</sup>Slovak Agricultural University, Nitra, Slovak Republic

The aim of this study was to examine effects of hormone leptin on apoptosis and proliferation and secretory activity of cultured fragments of bovine oviducts, as well as compare it with effects of well-known mitogen – insulin-like growth factor I (IGF-I). Effects of leptin on the expression of pro-apoptotic peptides (Bax, caspase-3), anti-apoptotic peptide (Bcl 2) and proliferation peptide (PCNA) were analyzed using Western-blotting, and the release of IGF-I by cultured oviductal fragments was measured using radioimmunoassay (RIA). Leptin stimulated IGF-I release at all doses added. Leptin when given at low dose (1ng/ml) stimulated the expression of anti-apoptotic peptide Bcl2. Leptin at higher (10, 100 ng/ml) concentrations decreased PCNA-

content and stimulated the expression of pro-apoptotic peptides – Bax and caspase-3. IGF-I has an opposite effect on these substances. The opposite effects of leptin and IGF-I on apoptosis and proliferation assume an existence of feedback interrelationships between these substances. It is probable, that leptin and IGF-I are antagonists in control of oviductal functions. This finding suggests different biological roles of leptin and IGF-I in the oviduct.

### **Carotenoid absorption in laying hens**

Gregosits, B.; Kerti, Annamária; Szabó, Cs.; Bárdos, L.

*Szent István University, Gödöllő, Hungary*

In the present study the effects following a large dose (20ppm) of  $\beta$ -carotene (BC), lutein (LU) and lycopene (LY) supplementations (192.3 mg  $\beta$ -carotene 10.4%; 357 mg Lutein CWS/S-TG 5.6%; 384.6 mg Redivivo lycopene 5% DSM Nutritional Products) both when given separately (20ppm) and together (20-20-20ppm,  $\Sigma$ 20ppm) on LU+zeaxanthin,  $\beta$ -cryptoxanthin, LY, BC, tocopherol, retinol and retinyl palmitate absorption were investigated in laying hens. Serial blood samples were taken at 6 hourly intervals before the supplementation and for up to 48h post-dosing and analyzed with rpHPLC for the carotenoid and retinoid levels. In the cases of all the three applied carotenoids (BC, LU and LY) supplemented both separately and combined the concentration of the respective carotenoid significantly increased in response to the high-carotenoid intake. The response curves occurred maximally over the 6-12 hours after doses. The plasma appearance of LY in order of magnitude was weaker (near the baseline) although the dosages were identical (20ppm), in contrast with BC and LU. When the combined carotenoid doses were ( $\Sigma$ 20ppm) applied, the smaller individual supplementation (6.6ppm/each) resulted in greater increase in the plasma concentrations of all three carotenoids. Our data confirm an interactions between LU, LY and BC when given in a combined dose in the concentrations of plasma carotenoids, namely the non polar carotenoid (LY) worsened the effectiveness of the other two oxycarotenoids (LU and zeaxanthin).

## **Evaluation of serum concentrations of selected biochemical markers of health and nutrition to the level of lifestyle of Slovak population**

Harasník, V., Slivková, J., Marcinková, M., Čižmárová, M.

*Slovak Agricultural University, Nitra, Slovak Republic*

This study focused on identification of biochemical indicators of nutrition and health status of selected groups in Slovak population. This project was carried out in all Slovak areas in July, 2004. 687 respondents took part in it (46.90% men and 53.10% women, respectively). Their average age was  $45.2 \pm 9.2$  years. The most important life-style factors are insufficient physical activity, some bad aspects of nutrition and smoking. The high incidence of obesity, unfavourable blood pressure values in men and worse lipid metabolism indicators in men are the most severe factors from the objective examinations. Values of all these biochemical indicators worsen with age. Only women and men from youngest groups had all their examination results within normal limits. The worst results from objective examinations with the highest average number of risk factors were found in persons with elementary education only and respondents who come from countryside, in smokers, and in physically non-active and obese persons.

## **Assessment of nutritional status and chosen biomarkers in relation to cardiovascular risks among selected population in the Slovak Republic**

Harasník, V., Marcinková, M., Slivková, J., Čižmárová, M.

*Slovak Agricultural University, Nitra, Slovak Republic*

This work analysed nutritional habits and some health biomarkers of cardiovascular prevention among selected groups of Slovak population. The examined group was constituted of 817 respondents (41.90% men and 58.10% women respectively). Their average age was  $48.70 \pm 9.91$  years. The „nutritional” health is entirely dependent on cardio-protective foods. Orientation on this intake of optimal composition, is associated with significantly reduced values of risk parameters for atherosclerosis in nutrition- rationalist group (total cholesterol, LDL-

cholesterol, triacylglycerols, atherogenic index, saturated fatty acids, high glucose) and significantly higher values of antiatherogenic substances (fatty acids from n-3 range, HDL-cholesterol, vitamins C and E). Thus, rational diet is very necessary to fully support human health and primary prevention of nutrition associated cardiovascular diseases. The worth results from objective examinations were found in persons with the elementary education and subjects, who lived in cities, in non-smokers, physically active and apparently lustily lived persons.

### **Effect of probiotic preparation on production of fat in body of chickens**

Haščík, P., Bobko, M., Kačániová, M., Čuboň, J., Kulíšek, V., Pavličová, S.

*Slovak Agricultural University, Nitra, Slovak Republic*

Probiotic preparation on base of *Enterococcus faecium* with content of spore  $2.0 \cdot 10^{10}$  cfu.g<sup>-1</sup> and thrs application 6.6 ml for two weeks in feed and 3.7 ml into water at the feeders chicken ROSS 308 gained up dynamic weight at the age of 42 days (end of feeding) about +111.00 g in ♀ ( $P \leq 0.01$ ) and about +185.00 g at ♂ ( $P \leq 0.001$ ). At the same time there was an increase of slaughtered chicken body after handling up to +157.22 g in ♂ ( $P \leq 0.001$ ) and about 80.36g in ♀ ( $P \leq 0.05$ ) in experimental group. Abdominal fat fraction has increased in experimental group within both sexes up to 0.24% in ♂ and up to 0.48% by little chickens, but without statistic differences ( $P \geq 0.05$ ). Stomach fat volume probiotic preparation did not affect and a heart volume fat was slightly increased in experimental group ( $P \geq 0.05$ ). After the evaluation of total inward fat from JOT we can state slight increase within the probiotic group at both sexes (+0.18% ♂, +0.39% ♀). A slight increase of inward fat in the group after application of probiotic preparation on the bacteria base *Enterococcus faecium* does not have a negative impact to economy production of chicken meat, but on the other hand it has a possitive influence on achieve utility and therefore there might be an increasement for rentability of poultry meat production.

## **Photoinducible expression of chicken period gene in the pineal gland**

Herichová I.<sup>1</sup>, Monošíková J.<sup>1</sup>, Zeman M.<sup>1,2</sup>

<sup>1</sup>University of Komenský, Bratislava; <sup>2</sup>Slovak Academy of Sciences, Ivanka pri Dunaji, Slovak Republic

The pineal gland is a key component of the avian circadian system. Besides its capacity to generate oscillations, the avian pineal gland is able to synchronize to external light-dark cycles and generate an output signal – melatonin. The aim of our work was to study responsiveness of embryonic pineal gland to external light pulses. We measured expression of clock gene *per2* in 19-day old embryos by real time PCR after 3h lasting light pulse. We conclude that *per2* has capacity to mediate information about external light:dark cycle already on day 19 of development but this ability is limited to the phase of day when *per2* expression is highest.

## **Alteration of haematological and biochemical parameters in serological positive sows of *E. intestinalis***

Hisira, V.<sup>1</sup>, Kováč, G.<sup>1</sup>, Lešník, F.<sup>1,2</sup>, Novotný, J.<sup>1</sup>, Petrovová, E.<sup>1</sup>, Farkašová, Z.<sup>1</sup>, Link, R.<sup>1</sup>

<sup>1</sup>University of Veterinary Medicine, Košice, Slovak Republic; <sup>2</sup>Academy of Sciences of Czech Republic, Liběchov, Czech Republic

In our experiment we determined changes of haematological (erythrocytes, hemoglobin, hematocrit, MCV, leucocytes) and biochemical parameters (total proteins, albumin, creatinine, urea) in serological positive sows for *E. intestinalis*. These values we compared with physiological values. On the basis of acquired results we determined considerable divergence in two parameters of haematological profile and one parameter of biochemical profile.

## **The ultrastructure of m. gastrocnemius in Japanese quails under hypodynamy simulating microgravity in space**

Holovská, K., Kočíšová, J., Cigánková, V., Almášiová, V., Tomajková, E., Škrobánek, P.<sup>1</sup>, Zibrín, M.

*University of Veterinary Medicine, Košice; <sup>1</sup>SAS, Ivanka pri Dunaji, Slovak Republic*

Hypodynamy is one of the models simulating weightlessness in space. We studied the effect of long-term hypodynamy of Japanese quails on ultrastructure of skeletal muscle (m. gastrocnemius) since hatching up to 126 days. Three days after hatching the quails were suspended in the special shirts so their feet did not touch the floor. They could consume food and water ad libitum. Experimental and control animals were killed after 14, 28, 42, 56 and 126 days of hypodynamy. The most extensive morphological changes were found in the m. gastrocnemius after 14 days of hypodynamy. Internal structure of mitochondria was altered and they accumulated on the larger areas. After 28 days of hypodynamy we observed the occurrence of giant mitochondria. After 42 days of hypodynamy sarcoplasmic reticulum were dilated, mitochondria were vacuolised and after 126 days of hypodynamy vacuolised mitochondrial matrix and preserved sarcomeres were found.

## **Verification of homeopathics effect on selected physiological parameters in weaned piglets**

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The benefit of homeopathics is that no residues persist in organism in comparison to classic therapeutics. This fact positively acts on health creation not only in animals but also in humans. We applied homeopathics in post weaning piglets as prevention to diarrheic syndrome occurrence. Preparation PVB Diarrhéas is veterinary homeopathic specialty from Boiron Company that is used for diarrhea treatment and prevents complications like weight loosing, chronic enterocolitis and hepatitis. Experiment was realized in ŠPP in Zemlinska Teplica on 28 piglets. Each swine was applied 2 ml of

solution orally during the period of 3 days before weaning and 70 days after weaning.

### **Diagnosics of hereditary disease – feline polycystic kidney disease using DNA test in Slovakia**

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Feline polycystic kidney disease (PKD) is autosomal dominant disease, that causes the formation of fluid-filled renal cysts, often leading to renal failure. That disease is most diagnosed by ultrasound, but 98% accurate is after approximately 10 month of age. A genetic test for feline PKD will provide breeders with an efficient and accurate means to selectively breed their cats and remove PKD from the population. PKD is most common in the Persian breed and breeds that are related to Persians or have used them in their breeding programs. In our report, feline PKD1 gene was screened for transversion C>A, causing a stop codon (OPA) in exon 29 (Lyons et al., 2004), in Slovak different breeds of cats. This mutation has identified in the heterozygous state in 23 affected cats, including Persian and Exotic breeds. The causative mutation has not observed in SBI, SIB, BRI breeds.

### **Heavy metals and structure of testes and uterus of Apodemus flavicollis from area of nuclear power station**

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In testes and uterus of individuals *Apodemus flavicollis* coming from vicinity of nuclear power station Mochovce an AAS method used to discover relatively low average concentrations of Cu, Fe, Mn, Zn and Cd. Copper, zinc and cadmium were accumulated in higher level in testes when compared with uterus. The levels of iron and manganese were higher in uterus. Microscopically picture of testes showed normally formed structures without pathological changes, what was

also confirmed by quantitative morphometric analysis. Relative volume of epithelium of *Apodemus flavicollis* is 67.552%, lumen was 24.688% and stroma 7.760%. The tubular diameter was 142.5  $\mu\text{m}$ . An average number of sperm elements on the surface of the sample 10 000  $\mu\text{m}^2$  was 90.53. In the yellow-necked mouse the relative volume of the endometrium is 61.5045% and of the myometrium 38.4955%, the stroma forms the highest relative volume (83.515%). The relative volume of surface epithelium is 13.651% and glandular epithelium forms 2.834%. The results of analysis indicate that vicinity of nuclear power station Mochovce is not highly contaminated with heavy metals and it is not risky for health condition of small mammals.

### **Induction of oestrus in anoestrous ewes with combination Fluorogestone acetate and Equine chorion gonadotrophine**

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In our study we followed the effect of combination Fluorogeston acetate (FGA) and differ doses of Equine chorion gonadotrophine (eCG) for induction of oestrus in anoestrous ewes. Blood samples (n=10) were taken to determine concentrations of hormones (progesterone, P<sub>4</sub> and estradiol 17 $\beta$ , E<sub>2</sub>). Every of experimental ewes were subsequently induced to oestrus with 40 mg of FGA per head/13 days (intravaginal sponges). After 8 days, we taken blood sampling and next five ewes (group 1) were treated with 500 IU eCG per head and to other five ewes (group 2) with 1000 IU eCG per head. Seven days after application of eCG blood samples were taken again to determine concentrations of P<sub>4</sub> and E<sub>2</sub> using RIA methods. There was no significance changes in hormones concentrations determined in blood samples in group 1 before even after treatment. Concentration of P<sub>4</sub> was significantly decreased (P<0.05) and E<sub>2</sub> was significantly increased (P<0.001) in group 2. From our results follow, that the combination of FGA and eCG can stimulate secretion of gonadotrophin and ovulation in aneestrous ewes.

## **Histological findings in the adrenal glands from slaughtered cattle**

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Adrenal glands from healthy slaughtered cattle, 13 bulls, 10 heifers and 10 cows, were examined by means of histology and immunohistochemistry.. The samples were fixed in 10% buffered formalin, processed by common paraffin technique and sections were stained with haematoxylin and eosine. Presence of S100 protein, chromogranin A and synaptophysin was studied in duplicate sections by means of immunoperoxidase method. The following findings were recorded: Unequal thickness of connective capsule and nodular formations of zona glomerulosa, eosinophilic granules in the cells of zona glomerulosa, globoid arrangement of zona fasciculata, strips and nests of cortical tissue in the medulla, mutual interlace of superficial and deep zones of the medulla, growth of cortical or medullary cells into the wall of blood vessels in the medulla and focal infiltrates composed of lymphocytes or granulocytes. Cortical cells and noradrenergic cells in the medulla expressed positivity for S100 protein. Both adrenergic and noradrenergic cells in the medulla were positive for synaptophysin. Majority of the cells in the cortex and noradrenergic cells in the medulla displayed positivity for chromogranin.

## **Mineral Profile in Rabbits after an Experimental Nickel and Zinc Administration**

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In this study the effect of selected trace elements – nickel and zinc, on serum mineral profile of rabbit was studied. Adult females (Californian and New Zeland white breed, n=25) were fed ad libitum with different concentration of nickel and/or combination with zinc. In first group (P1; n=5) animals were fed with granular feed mixture with addition of

17.5 g NiCl<sub>2</sub> per 100 kg of mixture and second group (P2; n=5) with 35 g NiCl<sub>2</sub> per 100 kg of mixture. In group P3 (n=5) animals were fed with mixture containing 17.5 g NiCl<sub>2</sub> and 30 g ZnCl<sub>2</sub>, and in group P4 (n=5) 35 g NiCl<sub>2</sub> and 30 g ZnCl<sub>2</sub> per 100 kg of mixture. All results were compared with control group (n=5). After experimental period (90 days) concentration of serum mineral elements (sodium, potassium, chlorides, calcium, phosphorus, magnesium) was detected. The level of sodium was similar in all groups reaching 142.70 – 159.80 mmol.l<sup>-1</sup>. The highest potassium level was in group P3, but the differences were not significant (4.75 – 5.25 mmol.l<sup>-1</sup>). Average concentration of chlorides was 106.34 – 110.45 mmol.l<sup>-1</sup>. Phosphorus level was very similar in all groups (1.32 – 3.74 mmol.l<sup>-1</sup>) with the highest level in group P4. Calcium concentration was lower in control group (3.12 ± 0.36 mmol.l<sup>-1</sup>) in comparison with other groups where the values reached 3.23 – 3.49 mmol.l<sup>-1</sup>. During 90 days of experiment a significantly higher (P < 0.05) calcium concentration was detected in group P4 in comparison with control. The average level of magnesium was in control group 1.03 mmol.l<sup>-1</sup>, P1-1.15 mmol.l<sup>-1</sup>, P2-1.13 mmol.l<sup>-1</sup>, P3-1.06 mmol.l<sup>-1</sup> and in P4-1.17 mmol.l<sup>-1</sup>.

### **The role of some biological active substances in regulation of porcine sexual maturation**

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The aim of our experiments was to study the influence of level of progesterone (P4), insulin-like growth factor I (IGF-I), IGF-binding proteins-3 (IGFBP-3) in blood plasma and the release of these substances by porcine ovarian granulosa cells cultured in vitro on sexual maturation. Besides the role of IGF-I, oxytocin (OT), cAMP and intracellular messengers in the control of ovarian function and sexual maturation was studied in pigs. Gilts were divided in two groups according to the sexual maturation (sexually immature gilts and sexually mature gilts). We determined the basal secretions of substances by granulosa cells, isolated from immature and preovulatory ovaries, together with the effect of treatment by IGF-I (10 ng/ml), OT (100

ng/ml and dbcAMP (10 µg/ml) during culture. The following products were assayed in plasma, cell culture medium and cells using RIA, Western-immunoblotting and immunocytochemistry: P4, IGF-I, IGFBP-3, IGFBP-4, protein kinases A (PKA) and G (PKG), phosphotyrosine (PhT), ERK1,2 related MAP kinase, CREB1 and proliferation-associated (PCNA, CDC2/p34, cyclin B1), apoptotic (Bax) and antiapoptotic (Bcl-2) peptides. It was observed, that sexual maturation of gilts is associated with an increase in the production of P4, IGF-I, IGFBP-3 in both blood plasma. There were also increases in content of IGFBP-3 and the expression of PCNA, CDC2/p34, Bcl-2, Bax, PKA, CREB1, PhT and decreases in the presence of IGFBP-4, cyclin B1, PKG and ERK1,2 in cultured granulosa cells. In both groups of animals, dbcAMP increased the secretion of P4 and IGF-I and the expression of ERK1,2 and PKG and decreased the content of IGFBP-3, IGFBP-4 and the expression of PKA in cultured granulosa cells. In immature animals, dbcAMP stimulated PCNA, CDC2/p34 and inhibited cyclin B1, Bcl-2, Bax, CREB1 and PhT, whilst in mature animals effect was opposite. In both groups, IGF-I increased the content of P4, IGFBP-3 and the expression of PCNA but decreased the expression of CDC2/p34 and PhT. IGF-I induced the production of IGFBP-4 and the expression of Bax, PKA and CREB1 in immature gilts but had the opposite effect in mature animals. In both groups, OT increased the content of IGF-I, IGFBP-3, IGFBP-4 and the expression of PCNA but decreased the expression of Bcl-2, PKA, PhT and ERK1,2. In immature gilts, OT increased the expression of CDC2/p34, Bax, CREB1, PKG and decreased P4 secretion. It had the opposite effect in mature animals. These data demonstrate that in pigs IGF-I, OT, cAMP, IGFBP-3 and IGFBP-4 control the proliferation and apoptosis of ovarian cell, the secretion of ovarian substances and sexual maturation. They also indicate differences in contents of P4, IGF-I, IGFBP-3 and in the expression of IGFBP-4, PCNA, CDC2/p34, cyclin B1, Bax, Bcl-2, PKA, CREB1, PKG, PhT and ERK1,2 produced by granulosa cells during sexual maturation in gilts. These findings suggest the potential application of OT, IGF-I and cAMP preparation for regulation of porcine gonadal functions.

## **Dairy cow milk quality and metabolic disorders**

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The aim of our experiments was to study technological quality of milk in relation to parameters of internal milieu in dairy cows. We discovered that in milk of dairy cows with metabolically malfunctions was detected higher level of urea, lower level of calcium and fall of titrate acidity. From all studied biochemical parameters (urea, total lipids, total protein, calcium, phosphor) the highest negative effect of blood urea on determined parameters of technological quality of milk is reported. Significant dependence between parameters of blood and milk were registered in the group with metabolically malfunctions of ketosis between blood urea and milk urea, urea calcium and milk calcium, calcium and glucose. In the group with metabolically malfunctions of asidosis were detected significant correlation between total lipids in blood and level of fat in milk.

## **In vitro nickel toxicity on porcine granulosa cells**

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In this study effects of nickel (NiCl<sub>2</sub>) on production of sexual hormone (progesterone), ultrastructure and cell apoptosis were analyzed. Granulosa cells were aspirated using a 2 ml syringe and separated from the follicular fluid by centrifugation. The medium was replaced with new medium of the same composition containing, in addition, NiCl<sub>2</sub> (Sigma, St. Louis, MO) in required concentrations (62,5; 125; 250; 500 and 1000 μM.l<sup>-1</sup>). Control medium contained no NiCl<sub>2</sub>. Quantification of progesterone was performed after 48 h of culture directly from aliquots of the media from control and treated porcine granulosa cells by RIA. Quantification of apoptosis cells was performed using TUNEL assay. From each experiment 20 electron microscopy sections from three different replicates were examined. The highest production of progesterone was found in control group without NiCl<sub>2</sub>. After NiCl<sub>2</sub> administration, there was a decrease of progesterone production. The

lowest progesterone production was after addition of 1000  $\mu\text{M}$   $\text{NiCl}_2$ . Tunel assay detected higher occurrence of apoptosis after  $\text{NiCl}_2$  administration. An increased apoptosis in each of experimental groups apart from concentration of 250  $\mu\text{M}$   $\text{NiCl}_2$  with the lowest percentage of apoptosis cells (42%) was detected. The highest percentage of apoptotic cells was found after administration of 1000  $\mu\text{M}$   $\text{NiCl}_2$  (79%). Also the ultrastructure of granulosa cells was altered after  $\text{NiCl}_2$  administration. We observed higher frequency of euchromatin in nuclei, lipid droplets, microfilaments and vacuoles in cytoplasm after  $\text{NiCl}_2$  treatment. There was also relatively lower frequency of mitochondria and smooth endoplasmic reticulum. Our findings suggest a negative effect of  $\text{NiCl}_2$  on steroidogenesis as well as on the ultrastructure and death of granulosa cells.

### **Influence of melatonin on anxiety in hypertensive rats with up regulated renin-angiotensine system**

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The purpose of the study was to investigate effects of melatonin (MEL) on anxiety behaviour of hypertensive rats. Mature Sprague-Dawley control normotensive rats (N, n=20) and hypertensive rats with high activity of renin-angiotensin system (H, n=20) were used. Half of each group was treated with MEL in drinking water (40  $\mu\text{g}/\text{ml}$ ) for 3 weeks. The influence of MEL on anxiety was measured in the elevated plus-maze test (EPM). H rats spent more time in closed arms ( $P<0.05$ ) and showed low frequency of total arm entries ( $P<0.005$ ) than N rats in EPM. MEL treated N rats did not exhibit differences in behaviour observed in EPM. MEL treated H rats spent less time in closed arms ( $P<0.05$ ) and increased frequency of total arm entries ( $P<0.05$ ) in EPM than untreated H animals. Our results suggest that MEL decreased anxiety related behaviours in H rats that were induced by over-expressed renin-angiotensin-aldosteron system.

## **Expression of CD14 during apoptosis of polymorphonuclear leukocytes in resolution of inflammation**

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The goal of the study was to prove the effect of bacterial components (toxins of Gram-positive and Gram-negative bacteria) on the expression of CD14 during an induced inflammatory reaction of the heifers mammary gland. The experimental inflammatory reaction was induced by the toxins from Gram-positive and Gram-negative pathogens, muramyldipeptide (MDP) and lipopolysaccharide (LPS); phosphate buffered saline (PBS) was used as a control. The course of the inflammatory reaction was monitored in four time points – 24 hours and 48 hours (initiation of inflammatory reaction), 72 hours and 168 hours (resolution of inflammatory reaction). Flow cytometry was used to determine portion of apoptotic polymorphonuclear leukocytes (PMNs) and portion of CD14+ PMNs. Intramammary administration of used inductors (PBS, MDP, LPS) resulted in an inflammatory reaction. with different character in the initial phase and in resolution. The results of this study reveal that, during an initiation, apoptosis of PMNs occurs, which shows an increasing trend. On the other hand a decline in the portion of apoptosis of PMNs in resolution then indicates its termination. The difference in the portion of apoptotic PMNs was the greatest after PBS, whereas after MDP and LPS it was manifested with more gradual increase. The higher portion of CD14+ PMNs was observed 72 hours after induction with PBS. A statistically highly significantly lower portion was observed after induction with MDP ( $P < 0.01$ ), and a statistically lower portion after induction with LPS ( $P < 0.05$ ). Decrease in the portion of CD14+ PMNs followed. The results of this study indicate that just the bacterial toxins prolong the duration of an acute inflammation and have shown that expression of CD14 is controlled by the factors inducing inflammatory response as well as by the mechanisms of resolution.

## **The effect of selected miRNA constructs on the functions of human ovarian granulosa cells**

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The aim of our experiment was to investigate the effects of several miRNA constructs, recently discovered regulators of gene functions via RNA interference, on ovarian cell functions. Human ovarian granulosa cells were isolated, transfected with 40 different miRNA constructs and cultured for 2 days. Release of hormones (estradiol, testosterone and progesterone) and apoptosis (expression of BAX) was analyzed using radioimmunoassay and immunocytochemistry. It was observed that the majority of miRNAs decreased estradiol (mir-15a, mir-29a, mir-126) and testosterone (mir-25, mir-20, mir-26a) release. Some miRNAs were able either inhibit or stimulate progesterone release (inhibitors: mir-181a, mir-20, mir-29, stimulators: mir-182, mir-122, mir-125). Several miRNAs appear to be inhibitors (mir-17-3p, mir-20, mir-135) or promoters (mir-136, mir-29a and mir-125a) of ovarian cell apoptosis. The obtained results represent the first demonstration of involvement of miRNAs in control of ovarian cell functions.

## **Metabolic parameters and composition of sow's milk after administration of probiotic on bases of genus Bacillus**

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There were 2 x 16 hybrid sows included into the trial. Control and BioPlus 2B groups were balanced according to the sow parity number. The trial lasted from 2 weeks before farrowing until weaning at 4 weeks after farrowing. Control group was fed with standard feed for lactating sows, experimental one was fed with control feed (same as that of the control group) with 400 g BioPlus 2B/t feed which equals 1.28x10<sup>6</sup> CFU/g feed. Probiotic preparation BioPlus 2B consisted of equal

proportion probiotic bacteria *Bacillus licheniformis* and *Bacillus subtilis*, i.e.  $1.6 \times 10^9$ /g of preparation. Milk-samples of all sows were collected on the 3rd and 14th day of lactation. Milk-samples were analyzed for cholesterol, total lipids, protein, lactose and total solids. Blood samples for determination of parameters of protein and lipid profile was taken on day 1 and 15 after parturition. The experimental group had higher content of lipids and cholesterol in milk compared with control group on day 3 and also on day 14 of lactation. The concentration of cholesterol and total proteins had slightly decreasing tendency in both groups, e.g. cholesterol decreased from 1.54%, 1.42% respectively, to 1.2% or 0.91%. On the other hand, concentration of lactose increased on day 14 of lactation in both groups. No significant differences between groups were observed in total solids and lactose concentration in sow's milk. Level of cholesterol in blood of experimental group increased. As a result, significant higher cholesterol level was found in experimental group compared to control group on day 15 ( $P=0.038$ ). Similar to cholesterol, total lipids in blood of experimental group was significantly higher in comparison to control group on day 15 ( $P=0.007$ ). Results of our experiment indicate that the probiotics based on representatives of the genus *Bacillus* are able to affect the nutrient composition, e.g. total lipids and cholesterol, of sow's milk and consequently improve performance and average daily gains of suckling piglets.

### **Concentration of trace elements in insemination doses of foxes and spermatozoa quality**

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Development in industry and agriculture produce an infiltration of elements in the food chain. This also promotes the distribution of essential elements in the animal body and changes their interactions. The purpose of this study was to determine cadmium, copper, lead, nickel, iron and zinc concentration in fox insemination dose and to find relation between these contaminants and the occurrence of pathological spermatozoa. The semen samples were analyzed by atomic absorption

spectrophotometry (AAS). For analysis of pathological spermatozoa samples fixed with Hancock's solution and stained with Giemsa were prepared. The concentrations of copper, zinc and iron in insemination doses of foxes were found  $2.16 \pm 0.53 \text{ mg.kg}^{-1}$ ,  $13.09 \pm 5.22 \text{ mg.kg}^{-1}$ , and  $33.16 \pm 24.36 \text{ mg.kg}^{-1}$ , respectively, on wet weight basis. Concentration of cadmium was low ( $0.07 \pm 0.05 \text{ mg.kg}^{-1}$ ). The levels of lead and nickel in the insemination doses of foxes were  $0.08 \pm 0.06 \text{ mg.kg}^{-1}$  and  $0.35 \pm 0.24 \text{ mg.kg}^{-1}$  respectively. The total percentage of pathological spermatozoa was  $7.76 \pm 1.33\%$  with predominance of knob twisted flagellum. In relation to trace elements the analysis showed significances ( $P < 0.001$ ) between iron level and total number of pathological spermatozoa. These results also indicate, that concentration of trace element in insemination dose of foxes they have direct effect to the sperm quality.

### **Reproduction parameters in chronic intoxication with heavy metals in rats**

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The aim of the present investigation was to evaluate the effects of chronic intoxication with low doses of lead, mercury and cadmium dissolved in drinking water (200-times above maximal permissible dosage) on reproductive potency of 80 Wistar rats (40 males; 40 females) and the physical health of their progeny. The animals were divided into 4 groups - control (C) and 3 groups intoxicated by metals (Pb, 100  $\mu\text{mol/l}$ ; Hg, 1  $\mu\text{mol/l}$ , Cd, 20  $\mu\text{mol/l}$ , respectively). Females gave births from 13<sup>th</sup> to 78<sup>th</sup> week of experiment. Parameters of reprotoxicity such as number of litters, total number of newborns (assigned in the birth day), number of newborns per litter and number of weanlings (raised youngs that reached 28th day of life) were measured in 13-week intervals. The data revealed that the number of litters and number of newborns in rats exposed to either Pb or Hg were significantly higher compared either to C or Cd group ( $P < 0.05$ ) and increased in the following order: Hg > Pb > Cd. Thus, while the highest numbers of litters and total count of offsprings was observed in Hg group ( $P < 0.05$ ), in Cd group they barely differed from control. From 13<sup>th</sup> to 39<sup>th</sup> week of exposition the number of newborns per litter was

higher in all intoxicated groups compared to control ( $P < 0.05$ ). The burst of fertility ceased on 52<sup>nd</sup> week and later the number of newborns per litter decreased in intoxicated animals dramatically. In contrast to above, the numbers of weanlings in intoxicated groups evolved in entirely opposite way. The numbers obtained from intoxicated groups were always lower compared to control ( $p < 0.05 - 0.001$ ) and the survival rate decreased in the order:  $Cd > Pb > Hg$  ( $Cd$  vs.  $Pb$   $p < 0,05$ ,  $Pb$  vs.  $Hg$   $p < 0.001$ ). The exposition to mercury, thus, induced the highest reproduction rate on account of lowest survival rate of offsprings. Similar co-relations applied to other metals, too. The results suggest, that increase in reproduction rate early after intoxication onset may disclose certain reactive adaptation mechanisms. Nevertheless, number of weanlings appears to be most practical marker as to the outcome of progeny in reprotoxicity tests.

### **The effect of bioflavonoids on glycaemia levels in alloxan diabetic rats**

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The aim of the present study was to evaluate the possible protective effects of quercetin and chrysin in experimental alloxan-induced diabetes in rats ( $n=60$ ). Alloxan was injected at a single dose of 60 mg/kg (to the tail vein) for diabetes induction. Flavonoids quercetin (single dose 50 mg/kg; orally) and chrysin (single dose 50 mg/kg; orally) were administered daily for 3 days prior and 7 days after alloxan injection. Alloxan induced a significant increase of glycaemia ( $p < 0.001$ ) in comparison with control animals. Both quercetin and chrysin prevented serum glucose elevation, although, protective effect of chrysin was weaker ( $p < 0.001$  vs.  $p < 0.05$ ). On the other hand, glycosuria was increased in all groups of animals receiving alloxan. We suggest that protective effect of used flavonoids in experimental diabetes mellitus may be related to their antioxidative/chelatory properties. Increased glycosuria indicated that inhibition of renal glucose reabsorption may also play role in hypoglycemic effect of both flavonoids.

## **Effect of teat placement and milk flow pattern on milk flow parameters**

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Milk flow is influenced by anatomy of udder, teat (size, shape, placement teat) and neuro – humoral reaction ewes on milking. Mainly traits of teat and cistern are considered very good parameters of milkability ewes by machine milking. The trial was performed with 70 ewes. We evaluated milk flow, cisternal depth and teat placement. Cisternal depth and teat placement were evaluated by a linear evaluation system (cisternal depth /HC/: 1 point – shallow, 9 points – deep; teat placement /PC/: 1 point – vertical, 9 points - horizontal). From 165 measured milk flow were 27% unimodal (1V), 48% bimodal (2V), 22% plateau I (PI) and 3% plateau II (PII). Ewes with more horizontally positioned teats had higher the volume of milk yield  $0.233 \pm 0.041$  l ( $\leq 3$  points),  $0.343 \pm 0.031$  l (4 points),  $0.394 \pm 0.028$  l (5 points),  $0.411 \pm 0.023$  l ( $\geq 6$  points); ( $P < 0.0004$ ) and machine milk yield  $0.180 \pm 0.038$  l ( $\leq 3$  points),  $0.279 \pm 0.028$  l (4 points),  $0.296 \pm 0.026$  l (5 points),  $0.303 \pm 0.022$  l ( $\geq 6$  points); ( $P < 0.0183$ ). The traits of milkability were  $63 \pm 2$  s,  $15 \pm 1$  s,  $0.946 \pm 0.046$  l/min,  $0.217 \pm 0.012$  l by the time of machine milking, latency, maximal milk flow and milk yield of the first emission at bimodality. There were observed positive and significant correlations ( $P < 0.0001$ ) between these traits (maximal flow, yield in 30s, yield in 60 s, yield after the first peak of milk flow from machine milking, except latency) and milk yield, machine milk yield. Positive and significant correlations were between yield (milk yield, machine milk yield) and PC, HC.

**Two stage induction and synchronisation of oestrus in flock of sheep determinate by number and health condition of rams.**

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This work was focused on oestrus induction and synchronisation of Tsigai sheep that occurred from physiology anoestrus period in spring. Implementation of rational biotechnical reproduction control program to be properly „tailored“ for the individual flock of ewes proves to be an assumption how to obtain high fertility, higher yields of slaughter milk lambs production for Christmas markets. Oestrus induction and synchronisation by means of the combination of gestagen- and eCG-based preparation consisted in (1) application of vaginal sponge impregnated with gestagen, (2) i.m. injection of eCG based preparation at the time of gestagen discontinuing, and (3) observation and making records on fertilization. This biotechnical control of oestrus was carried out during two stages in the experimental flock sheep which was determinate by limited number rams in the breeding period.

**Physiological monitoring of processes recruitment, selection and dominance of tertiary follicles in ovaries of sheep**

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Presented paper is focused on physiological monitoring of the tertiary antral follicles during growth and development in processes: (1) recruitment, (2) selection, (3) dominance, (4) ovulation and luteinisation (corpus luteum – developing and growing) based on simultaneous use of laparotomy with digital photo documentary evidence, ultrasound investigation, histological evaluation and determination of the oestradiol-17 $\beta$  and progesterone in the blood serum and the follicular fluid of the largest follicles from the ovaries of the Improved Wallachian ewes. It can be concluded from performed observations that the results suggest on possibility of monitoring of ewes ovarian

folliculogenesis and its phases in vivo conditions during experiments and investigations.

### **Influence of hyperthermia to the biochemical parameters of blood in rabbit and selected feeder indices**

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Thermal stress is the main constraint in farm animal production in warm season. It starts to manifest by environmental temperature above 32 °C. As a result the parameters of growth and reproduction utility decrease due to alterations of biological functions. The biochemical parameters of blood and feeding parameters were monitored. In our study, we used flesh- lines of rabbits. The blood of rabbits was taken from auricular marginal vein every morning. Physiological parameters from blood serum were defined by automatical biochemical analyser Microlab 300. We analysed the following parameters: glucose (energy profile), level of total protein, cholesterol ( lipid profile) and calcium (mineral profile). All of these observed parameters of experimental young rabbits were lower as compared to the control group (from – 10.04%). Total growth of live weight (g/ks) of experimental young rabbits was found to decrease by about 235.2 g as compared with control group. Quantity of total protein in experimental group was from 50,60,04±3,78 to 60.93±4.39 g.l<sup>-1</sup>. It decreased as compared with control group (49,02±4,62–60,13,63±3,51 g.l<sup>-1</sup>). Quantity of glucose was found to be increase almost in all experimental groups (5.15±0.50 – 6.24±0.67 mmol.l<sup>-1</sup>) as compared with control group (5.05±0.52 – 5.56±1.14 mmol.l<sup>-1</sup>). Level of protein profile and level of calcium profile also decreased. Level of cholesterol was increased (except for the first intake) in experimental group (3.02±0.1.02-3.34±1.17 mmol.l<sup>-1</sup>) as compared with control group (1.74±0.27-2.25±3.34 mmol.l<sup>-1</sup>). In our study we found that biochemical parameters of blood were affected by higher temperature of feeding environment.

## **Differences in femoral bone structure of yellow-necked mouse (*Apodemus flavicollis*) and wood mouse (*Apodemus sylvaticus*)**

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Femoral bone structure of yellow-necked mouse (*Apodemus flavicollis*) and wood mouse (*Apodemus sylvaticus*) was investigated in this study. Altogether 40 bones were analysed. We compared bone length, bone weight and histological structure of the femora between examined animals. Our results indicate the measured values for bone length were higher in yellow-necked mouse ( $P < 0.01$ ). On the other hand, wood mouse disposed higher values of bone weight ( $P < 0.01$ ). Statistically significant differences for bone length and bone weight between females and males of both species were identified. Histological observation of thin sections through a shaft of femora of both species revealed an outer and inner essentially non-vascular lamellar layer around a poorly developed reticular layer, containing unorganized vascular canals. We did not identify differences in qualitative histological characteristics of examined bones between *Apodemus flavicollis* and *Apodemus sylvaticus*.

## **Hepatodystrofy – steatosis**

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In this study occurrence of hepatal steatosis is described, where optical empty vacuoles in hepatocytes were observed. In developing steatosis in the beginning small adipose droplets in hepatocytes cytoplasm later bleding to big droplets with increasing size were found. Finally a large adipose droplet fill the cell and replace cytoplasm to the marginal zone. Steatosis affected at central, intermedium and peripheral zone of lobe, perspectivevely all lobe. The finding of pathological changes of liver is also supported by metabolic profile where alteration in energy and hepatal profile were detected. Results of metabolic profile correlate with pathohistological finding and represent the disease development.

### **Protective effect of zinc against cadmium activity in poultry**

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In the 2 experiments on Japanese quails and turkeys was investigate markedly important depressive effect of cadmium on the level of cellular immunity after long-term exposition, however after short-time exposition, it was not manifested. Feeding of higher Zn amounts together with Cd assigned protective effect. Statistically important ( $p < 0.05$ ) protected effect of Zn against immunodepressive effects of Cd was found at cellular immunity. Accumulation of Cd was 36 multiple lower in muscle in comparison to kidney and 26 multiple lower in comparison to liver. Per os zinc application statistically important ( $p < 0.05$ ) decreased Cd accumulation in kidneys and livers of birds.

### **Quo vadis of animal experiments in university teaching and research?**

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There are few areas of animal use that arise so many emotions and questions as that of their use in education. The physical presence of an animal (whether alive or dead) is a dramatic event experience for most students, and their effects it has will depend heavily on their previous experience with that species, their moral values and the perceived necessity of the practical. Most of the current literature on this subject is highly emotive, based on relative little data and provide few conclusions. This paper attempts to clarify the issues raised and present an overview of the alternatives available with their strengths and weakness. Finally, it offers guidelines for humane education that take into consideration both the practical issues and the feelings of all those involved.

## **Using of paracetamol in therapy of respiratory diseases of pigs**

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The work presented was aimed at evaluation of therapeutic using of paracetamol and its effects on haematological profile, as well as body weight and concentration of total immunoglobulins in blood serum of pigs with respiratory diseases symptoms. Fifteen piglets were divided to 3 groups (1<sup>st</sup> experimental group – treatment with paracetamol; 2<sup>nd</sup> experimental group – paracetamol + inj. marbofloxacin; and control group). A four days supplementation of paracetamol resulted in a positive effect on concentration of leukocytes and body weight of pigs (in the 1<sup>st</sup> and the 2<sup>nd</sup> experimental group).

## **Hyperthermia effects on preimplantation rabbit embryos in vitro and the role of proteins Hsp70**

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The aim of our study was to test the influence of short exposure (6 h) of preimplantation rabbit embryos to elevated temperatures (41.5°C and 42.5°C) in vitro on their developmental capacity and evaluate a role of Hsp70 in response of rabbit preimplantation embryos to hyperthermia. Fertilized rabbit eggs isolated from oviducts of slaughtered females 19 hpc were cultured in k-DMEM medium + 10% FCS at standard temperature (37.5°C) until 72 hpc. Afterwards, the embryos which reached morula stage were divided into two groups - hyperthermic (HT, 41.5°C or 42.5°C) and control (C, 37.5°C). In a half of embryos from both groups an Hsp70 was blocked by the adding anti-Hsp70 to the medium. The embryos were cultured during 6 h. Then the embryos from HT group were transferred to 37.5°C and post-incubated overnight (16-20h) along with control group. Following incubation the embryos were evaluated for developmental stages and processed for apoptosis (TUNEL), actin cytoskeleton and presence of

heat-shock-specific proteins Hsp70. Embryo samples were analyzed using Leica fluorescent microscope. It was observed that hyperthermic conditions at 41.5°C did not alter advancement of embryos to higher preimplantation stages (expanded and hatching/hatched blastocysts). There were no damages in actin filament structure compared to control embryos. Western-blotting revealed the presence of heat-stress-induced 72 kDa fraction of Hsp70 proteins in lysates of embryos exposed to 41.5 °C. Following elevation of temperature to 42.5 °C the embryo development was dramatically compromised. The embryos were arrested at early blastocyst stage and did not advance in development. The structure of actin filaments in blastomeres was damaged and such blastomeres often contained apoptotic nuclei. In this group Western-blotting did not reveal a presence of heat-stress-induced fraction of Hsp70. Blocking of Hsp70 before developmental block resulted in no embryos (0%) at blastocyst stage. From Hsp70-blocked embryos after developmental block, 27.6% (C+anti-Hsp) and 22.6% (HT+anti-Hsp) of blastocyst were formed, versus 71.4% (C) and 69.7% (HT) in non-Hsp70-blocked embryos. These results demonstrate a threshold of thermotolerance of preimplantation rabbit embryos to short hyperthermic exposure in vitro. The embryos before genome activation (GA) are more sensitive to HT. The resistance of embryos before GA to HT is related to Hsp70, whilst after GA, Hsp70 is not probably a major factor of thermotolerance and the existence of additional protective mechanism is hypothesized.

### **Chemoprotective effect of Diclofenac in mammary carcinogenesis in female Sprague-Dawley rats**

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The chemopreventive effect of non-steroidal anti-inflammatory drugs in mammary carcinogenesis was frequently reported. In this work we evaluated the tumosuppressive effect of non-selective cyclooxygenase (COX) inhibitor diclofenac in N-methyl-N-nitrosourea-induced (NMU) mammary carcinogenesis in female Sprague-Dawley rats. Diclofenac was administered in drinking water in the concentration of 0.01 mg/ml (0.001%). In the last (22nd) week of experiment we recorded incidence

decrease by 11.54%. Tumor frequency per group decreased by 39.45% a tumor frequency per animal decreased by 31.58% when compared to control group. Latency period (time span from first carcinogen administration till the first palpable tumor development) was lengthened by 16%. Diclofenac as a preventive substance (non-selective COX inhibitor with predominantly COX-1 inhibition) altered basic NMU-induced mammary carcinogenesis parameters in female rats.

### **Leucopenia of the broiler rabbits under the influence of hyperthermy**

Parkányi, V., Ondruška, L., Rafay, J., Chrástínová, L., Pivko, J., Massányi, P.<sup>1</sup>, Lukáč, N.<sup>1</sup>, Capcarová, M.<sup>1</sup>

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Exposure of the young and adult rabbit's males and females to hyperthermic conditions ( $36\pm 3^\circ\text{C}$ ) negatively affects on their physiological processes and interferes with their internal homeostasis. The changes are demonstrated on all observed biological levels: fodder intake and conversion, growth intensity, haematological characteristics and viability. There is a significant leucopenia ( $7.380 \times 10^9/\text{L}$ ), together with a significant lymphopenia ( $3.700 \times 10^9/\text{L}$ ) of young rabbits ( $p < 0.05^*$ ), typical accompanying effect of the stress reaction to hyperthermy. The hyperthermic collapse is attended by increased mortality (young rabbits: 25–44%, adult rabbits: 20–50%).

### **Catecholamine levels in the hypothalamo–pituitary structures after hormonal stimulation**

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The effect of hormonal stimulation on catecholamine levels in the eminentia mediana, area preoptica, corpus mamillare and pituitary gland of ewes in the estric period by the radioenzymatic method. The estrus of ewes was synchronized with Agelin sponges (Agelin, Spofa) containing 20 mg chlorsuperlutine. After complete synchronization we

induced superovulation in the experimental groups by means of 2000 IU serum gonadotropin (PMSG). Catecholamine were determined radioenzymatically using the Catechola test. The results indicate that hormonal serum gonadotropin stimulation decreases significantly ( $p < 0.05$ ) the levels of dopamine, norepinephrine and epinephrine in the eminentia mediana of sheeps hypothalamus. An increase of norepinephrine levels ( $p < 0.001$ ) following 2000 IU PMSG in the corpus mamillare was pronounced. Its administration is associated with increase dopamine and epinephrine levels in the pituitary gland. We suspect that given changes in catecholamine levels can be associated in relationship to steroid alteration after administration of gonadotropic hormone PMSG.

### **The analysis of beef cattle internal environment**

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The aim of the study was to compare some biochemical parameters of blood plasma of different beef cattle category (bulls, heifers and steers) during the fattening. In each group, there have been 11 beef cattle crossbreeds observed during the period from day 26 to 618 of age. Animals live weight measurement and blood samples collecting were provided during this period in day 26, 310, 403, 448, 479, 512, 554 and 618. Blood plasma samples were analyzed for total protein, glucose, urea, calcium and phosphorus concentration. The values of blood plasma metabolic and mineral profile parameters in all monitored groups during experimental period ranged in intervals stated for healthy animals. Significantly highest body weight gain during experimental period was found in the group of bulls. There were found significant differences in blood plasma total protein concentration between the groups. Sporadic differences in other objected internal environment parameters between the groups were noted.

## **Effect of sodium selenite supplementation into the diet for sheep on selenium and antioxidant status**

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In experiment on sheep, the effect of supplementation of sodium selenite on superoxide dismutase (SOD) in erythrocytes, glutathione peroxidase (GPx) in blood, tissues and fractions of rumen fluid, malondialdehyde (MDA) and Se concentration in plasma, blood and tissues was investigated. Ten animals were randomly divided into two groups and were fed experimental diets for 2 months. Control group of sheep received BD providing a daily intake of Se 42.35 µg only. The diet for second group consisted of BD enriched with Se 0.3 mg.kg<sup>-1</sup> in the form of Na<sub>2</sub>SeO<sub>3</sub> and provided total daily intake 270.4 µg of Se per head. Increases in Se concentrations in whole blood and tissues as well as increases in the activities of blood and tissues GPx were highly significant in group of sheep fed diet supplemented with selenite. Interestingly, the concentration of Se in muscle was significantly increased in this group of animals too. Activity of SOD in erythrocytes as well as the contents of MDA in tissues was significantly reduced in animals fed diet supplemented with Se. The cumulation of Se in rumen microflora resulted in its significant increased in the bacterial and protozoal fractions of rumen fluid. The results suggest on the important role of rumen microflora in the creation of selenomethionine from selenite which is the only form of selenium able to build significant body deposits. The benefit of Se supplementation on the maintainance of antioxidant and selenium status of sheep was demonstrated.

## **Effects of gravidity on number of leukocytes and T cells subsets in selenium supplemented ewes**

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The aim of the work was assessed influence of reproduction cycle at the dynamics of leukocytes and T cells subsets in selenium supplemented ewes. The experiment was conducted on fifteen ewes of the Sumava sheep breed at 18 months of age. The ewes were in the stage of non-pregnancy, pregnancy and lactation during the experiment. Blood for analysis were collected in monthly intervals. After parturition blood samples were taken from lambing ewes on day 30 and 60. Number of leukocytes in blood smear was detected by microscopically analysis by the norm no. 84 3206, and the CD4<sup>+</sup> and CD8<sup>+</sup> T cells subsets in blood were detected by flow cytometry. The number of leukocytes was in physiological norm without statistically significant differences during the experiment. In the dynamics of the T cells subsets was founded statistically significant difference between stadium of gravidity and stadium after lambing ( $P < 0,001$ ).

## **The effect of trehalosis addition on fertilization capacity of frozen ram semen**

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In this study the effect of various trehalosis addition concentration (0, 25, 50, 75 and 100%) in place of glucosis to yolk-citrate diluent on fertilization capacity of frozen and re-frozen ram semen was analyzed. Re-frozen insemination doses were analyzed using CASA method, where sperms with progressive motility, non-linear progressive motility, non-progressive logal motile and non-motile sperms were recorded. Results of our experiment proved that for frozen ram semen is optimal combination of 50% glucosis and 50% of trehalosis where a high significant difference ( $P < 0.001$ ) in total motility as well as in progressive motility (64.67% resp. 45.01%) was detected. Results of this

study also indicate that the freezing successfulness is dependent on addition of 4% glycerol to diluent after an equilibration period tightly before freezing.

### **The role of metabolic profile test in relation to health and production**

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Good health status of animal is important condition for achievement and keeping of high utility. The aim of our experiments was to analyze some metabolic parameters of dairy cattle blood in agricultural enterprises Žiar, Mariková and Naháč. It was observed that metabolic parameters of mineral profile (Na, K) analyzed respond to physiological interval in all farms. At the same time we determined that concentration of glucose in blood serum was in the physiological interval. The higher concentration of cholesterol in dairy cattle blood was observed in agricultural enterprises Mariková a Naháč but in the middle lactation.

### **Zinc cummulation in tissue and Japanese quail eggs**

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The effect long-term addition of zinc on quality of Japanese quail eggs and tissues was investigated. Japanese quails (n=48) were divided into 2 groups. Each group consisted of 24 birds. Group 1 was the control group. In the experimental group G3, Zn was administered daily in form of water solution in dose 12 mg of Zn for one quail. The levels of Zn in tissues and eggs were evaluated with method AAS.

### **Mercury induced alterations in rat testes in vivo**

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In this study effects of mercury administration on the testicular structure of adult rats were evaluated. Wistar rats received mercury (as

HgCl<sub>2</sub>) in single intraperitoneal dose 20 mg HgCl<sub>2</sub> (group A), 10 mg HgCl<sub>2</sub> (group B) and 5 mg HgCl<sub>2</sub> (group C) per kilogram of body weight and were killed after 48 hours following mercury administration. After the preparation of histological samples the results were compared with control group (K). Qualitative analysis showed that the shape of seminiferous tubules was typically oval, without degenerative alterations. On the other hand, these observations revealed dilatation of blood vessels in interstitium, undulation of basal membrane and occurrence of empty spaces in germinal epithelium. Decreased relative volume of germinal epithelium, increased relative volume of interstitium and increased occurrence of apoptosis suggests damaged interstitium and revealed occurrence of edemas. The relative volume of seminiferous tubules showed higher luminization. Also other evaluated criteria demonstrated significant differences between control group and experimental groups. This study reports a negative effect of mercury on the structure and function of testis. The changes of nutritional characteristics of pork meat during the long period of freezing storage

### **The changes of nutritional characteristics of pork meat during the long period of freezing storage**

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Freezing is one of the most cautious ways of meat preservation. It enables long-term storage without application of chemical additives and significant side-effect. However, criteria of rozen meat have not been unambiguously determined. The changes in the nutritional characteristics of pork meat (m. longissimus dorsi) of normal quality, and that of altered quality (PSE: pale, soft, exudative) were observed in dependence on the length of freezing storage. The aim was to deepen and to make objective the knowledge on the effect of freezing process on the parameters of quality of pork meat. During freezing storage, the differences in the contents of total proteins and aminoacids between the PSE and control meat were eliminated, the values of valine, lysine, leucine, custine, histidine and arginine significantly decreased, probably in the form of free aminoacids due to the elevated losses of meat juice after defreezing.

## **The analysis of products of hydrolytic and oxidative degradation of adipose tissue**

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The changes in the basic lipid constants (peroxidation number, number of fat acidity and thiobarbituric acid) of pork meat (m. longissimus dorsi) of normal quality, and that of altered quality (PSE: pale, soft, exudative) were observed in dependence on the length of freezing storage. The aim was to deepen and to make objective the knowledge on the effect of freezing process on the parameters of quality of pork fat. Statistically significant changes in the basic lipid constants in thermally untreated frozen pork fat occur in general after month 6 of freezing storage in both groups.

## **Milk and dairy products consumption in Slovak population**

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The aim of the study was to evaluate milk and dairy products consumption in adult population in Slovak Republic – in the case of 483 random selected adults at the age from 25 to 95 years (52.80% females and 47.20% men), with average age  $47.60 \pm 15.01$  years. Used research method was nutritional questionnaire. Body mass index was assessed according to criteria of WHO (2004). In the sample occurred overweight in 56.31% of respondents (45.88% females and 67.98% men) and obesity in 16.98% of adults (13.73% females and 20.61% men). Android risk of metabolic complications of obesity was determined according to waist-to-hip ratio values in 28.63% of females and 89.47% of men. The most preferred consumed milk was semi-bold milk (56.71%) and low-fat milk (26.29%). Milk consumption refused 9.03% of females and 8.77% of men, low-fat milk did not consume more men than females (20.30% versus 30.77%), acidified milk refused 28.14% of females a 25.00% of men, yoghurts with higher fat content refused 52.69% of females and 53.66% of men.

## **Healthy Eating Index in adult women**

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The aim of the study was to evaluate the nutrition in Slovak adult women and to analyse the actual nutrition according to Healthy Eating Index. 24-hours dietary records (n = 161) were evaluated by using software Alimenta version 4.3e. 41.61% of protocols contained five food groups (cereals, vegetables, fruit, milk, meat). The ground of the nutrition was cereals and/or their products in the case of 98.89% protocols, 89.44% of anamnesis contained vegetables and/or its products, 88.82% of protocols contained milk and/or dairy products, and 82.23% of records contained meat, poultry and/or fish. Fruit or fruit products represented the component part of 68.32% of retrospective protocols. Receipt of cholesterol (maximal 300 mg a day) exceeded 30.43% of nutritional records, daily fat receipt (maximal 30% from daily energy receipt) was higher in the case of 67.08% protocols, receipt of saturated fat was in 70.37% of dietary records (was is too much) higher as is recommended (10 % of total energy content).

## **Morphological changes in the uterus of sheep after application of cloprostenol and equine chorion gonadotropine**

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Qualitative and quantitative histological changes in the uterus Slovak merino sheep were investigated following the synchronization and hormonal treatment. The investigations were carried out on 40 sheep in the period of physiological anoestrus. Oestrus induction in anoestrus sheep was achieved by intravaginal instillation of fluorogestonacetate (40mg/sponge) oestrus was detected 2 – 5 days after removal of sponges. During induced oestrous cycle in sheep on 7 day were administered of Cloprostenol (synthetic PGF<sub>2α</sub>) 250 ng/head, as first injection on day 1 and as second injection on 11 days was used in sheep that did not responded on first treatment. Next day after first

application of Cloprostenol was administrated of the equine chorione gonadotropine (eCG) hormone in dose of 750, 1000 and 1500 IU. The animals were killed on average 72 h following the Cloprostenol injection. The uterus samples were processed by common histological methods for examination under a light microscope and under an electron microscope. A scanning electron microscope Stereoscan Cambridge 2A and Joel were used to examine the samples. Our investigations showed that the administration of PGF<sub>2</sub>α resulted in a less significant decrease in the mass of uterus ( $p < 0.05$ ) than that observed after the application of eCG ( $p < 0.001$ ). An increase in the uterus cervix mass, observed following the administration of PGF<sub>2</sub>α was of low significance ( $p < 0.1$ ) however that recorded after the administration of eCG (750 and 1500 IU) was more significant ( $p < 0.01$ ) and the most significant increase was elicited by administration of 100 IU eCG ( $p < 0.001$ ). The number of glands in the uterus cervixes increased significantly ( $p < 0.05$ ) only after the highest administered dose of eCG (1500 IU), however the epithelium height increased significantly ( $p < 0.001$ ) after all doses of the hormones administered (i.e. also after PGF<sub>2</sub>α). The height of the surface epithelium of cervix increased on the level of significance  $p < 0.001$  following the administration of all hormonal preparations tested. Higher statistical significance was recorded for changes in the cervical epithelium. Cervical epithelial cilia of experimental sheep were many times elongated in comparison with those in the control animals. Secondary and tertiary projections were also observed. SEM examinations showed that the cervical epithelial surface was covered with dense microvillus and cilia and secretion blebs could be observed in many places. The administration of oestrus inducing preparations elicits responses in the uterus of anoestrus sheep similar to those observed during the natural oestrus.

### **The influence of microclimatic parameters of stabling environment on milk quality of cows in free stabling system**

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The experiment was realized in a brick stable with stocker floor and boxes with plastic litter made from separated slurry. The average

number of the animals was 509 Holstein cows with average production 7741.65 liters of milk per year, during the experiment. The ventilation was provided by an overhead inlet shaft and by lateral windows. Temperature and relative humidity of the stabling environment were measured in hour intervals. Through the experiment were founded MSCC and microorganisms tendencies to grow up, and milk protein (casein) and fat to decline in DM after growing temperature.

### **The evaluation of micronucleus frequency in the bone marrow of mice treated with morphine and protease of *Aspergillus oryzae***

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In this study we have used 4 groups of mice with 4 animals in each, using 1 negative control -K- (obtained 1ml of 0.9% NaCl sol. (PS) i.p. and 20 µl of the PS s.c. into tail), 1 positive control - KA+ (obtained 30mg of morphini HCl (M) .kg in 0.1% sol. i.p. and 20 µl of PS s.c. into tail) and 2 experimental groups of mice. The first of them - B-group (obtained except of M as in group KA+ also *A. oryzae* protease (PAO) diluted in relation 1:20 in 20 µl of PS s.c. into tail & also C-group of mice which obtained except of M as in group KA+ also PAO diluted in relatio1:10 in 20 µl of PS s.c. into tail). The effects of both agents in mice were observed via following the dynamics of the body temperature, genotoxicity & cytotoxicity criteria of studies. The experiments demonstrated that used M induced already alone significant decrease of body temperature ( $P < 0.05$ ) as well as in groups with the PAO, however body temperature decrease in KA+ was not significant ( $P > 0.05$ ) when we compare the data in relation with values before administration of agent. However in cases when were taken the comparisons in relation with the K- data the  $P < 0.05$  decreases of body temperature were observed not only in B- and C-groups but also in KA+group of mice. Significant differences in micronucleated polychromatic and normochromatic erythrocytes was observed ( $P < 0.001$ ); resp. ( $P < 0.001$ ) in experimental group of mice which obtained 30 mg/kg b. w. of morphine and PAO (1:10) into tail against

negative control. The ratio of polychromatic to normochromatic erythrocytes of female mice of both exposed groups was not significantly changed in comparison to negative control. Supported by Vega grant No. 1-2408-05.

### **Does increased maternal testosterone level have an effect on the second-to-fourth-digit ratio (2D:4D) in rats?**

Talarovičová, A., Kršková, L., Blažeková, J.

*University of Komenský, Bratislava, Slovak Republic*

The purpose of the study was to investigate effects of increased maternal testosterone level on the second-to-fourth-digit ratio (2D:4D) in rats. Digit length of the 2<sup>nd</sup> and 4<sup>th</sup> of both forepaws in control (C) and testosterone treated (T) groups of male and female rats (C: 8 males, 8 females; T: 8 males, 8 females) was measured at 90 days of age. Animals of the T group were prenatally exposed to testosterone (a single intramuscular injection of 2.5 mg testosterone isobutyrate) on gestation day 14. Our results showed that males and females of groups prenatally treated with testosterone had the 2D:4D ratio lower than C ( $P < 0.001$ ) on both left and right forepaw. The 4<sup>th</sup> digit was longer in T group than in C group in both genders ( $P < 0.001$ ). Our results suggest that higher level of maternal testosterone during intrauterine development influence digit ratio in laboratory rats. Since a similar relationship is known also in humans, this observation may be useful for noninvasive evaluation of androgen status in rats under experimental conditions.

### **Possible interaction of somatic cell count and quarter milk flow patterns**

Tančin, V., Ipema, A.H.<sup>1</sup>, Mačuhová, L., Uhrinčat', M., Hogewerf, P.<sup>1</sup>

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Milk flow parameters at udder and quarter levels were studied in relation to somatic cell count (SCC) and other risk factors for mastitis (duration of decline and overmilking phase). Thirty-eight Holstein cows

in their first to sixth lactations were investigated during 10 mo of lactation. Monthly milk samples were collected for SCC during morning milking. The SCC differentially affected whole udder milk flow and quarter level milk flow. At both the udder and quarter levels milk yield was reduced in groups with high SCC. Quarters with high SCC (over  $500 \times 10^3$  cells/mL) had lower peak flow rate and longer overmilking phases when compared with quarters with low SCC (less than  $200 \times 10^3$  cells/mL). The quarters with a longer duration of the decline phase (over 80 s) had greater SCC and peak flow rate but had lower milk yield compared with quarters with a shorter duration of the decline phase (less than 27 s). Duration of the overmilking phase influenced all measured parameters except SCC. We conclude that for good udder health the duration of the decline phase at the quarter level should be considered for milking parameters and udder preparation before milking.

### **Acute phase proteins in relation to selected parameters of internal status in dairy cows**

Tóthová, Cs., Nagy, O., Konvičná, J., Petrovič, V., Pisarčíková, J., Kováč, G.

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The aim of this study was to evaluate the concentrations of selected acute phase proteins – haptoglobin (Hp) and serum amyloid A (SAA) – in blood serum of dairy cows in relation to different phases of reproduction cycle, as well as to some variables of protein metabolism (total proteins, albumin, urea, creatinin, total immunoglobulins). The analysis were performed in dairy cows of a low-land black spotted breed divided into 9 groups according to the reproduction period – from 4 weeks before parturition to 10 weeks after parturition. In case of Hp and SAA we found significant differences in average values of their cocentrations in several groups during the monitored period. The Hp and SAA concentrations in cows during early postparturient period were significantly higher compared with later postparturient period. We found significant differences of average values throughout the monitored time in total proteins ( $P < 0.001$ ), urea ( $P < 0.001$ ), and total immunoglobulins ( $P < 0.05$ ). The concentration of albumin, like one of representant of negative acute phase proteins, decreased progressively

in postparturient period until 4 weeks after parturition. In assessment of correlation relations we found significant correlations of dynamics between Hp and SAA ( $r=0.916$ ;  $P<0.001$ ), total proteins and urea ( $r=0.668$ ;  $P<0.05$ ), total proteins and total immunoglobulins ( $r=0.826$ ;  $P<0.01$ ), and between total immunoglobulins and urea ( $r=0.899$ ;  $P<0.001$ ). Our results suggests, that in time around parturition there are significant changes in concentrations of acute phase proteins, as well as in complete protein metabolism of dairy cows.

### **Viability of preimplantation rabbit transgenic embryos**

Turanova, Z.<sup>1,2</sup>, Makarevich, A.V.<sup>1</sup>, Bauer, M.<sup>1</sup>, Chrenek, P.<sup>1,2</sup>

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The objective of this study was to compare quality of transgenic (TR) and non-transgenic rabbit embryos in relation to preimplantation developmental rate, a number of blastomeres in ICM and percentage of apoptotic index. The TR embryos were produced by microinjection of the EGFP and hFVIII genes into both pronuclei of fertilized rabbit eggs. The developmental rate and allocation of EGFP positive cells was examined at blastocyst (96h PC) by fluorescent microscope or by PCR as in case of hFVIII gene using. The transgenic rabbit embryos with hFVIII gene were also produced by mating of transgenic rabbits and flushing at 1-cell stage. Percentage of transgenic embryos reached blastocyst stage (90 and 88%) did not differ significantly from those in non-transgenic embryos (98% resp.). Based on fluorescence analysis the integration rate of EGFP gene in blastocyst stage embryos was 20%. The efficiency of hFVIII transgene integration as determined by PCR was 17%. No differences were observed in the total cell number, ICM area cell number and apoptotic nuclei between the groups. The higher number of total cell (142) and ICM area cells (36) were found in non-transgenic rabbit embryos. The index of apoptotic nuclei (2.8%) was detected in non-transgenic rabbit embryos. Our preliminary results indicate that transgenic rabbit embryos (with EGFP or hFVIII gene construct) are of comparable quality with non-transgenic ones in concern of blastocyst rate, total and ICM area cell number and apoptotic index.

## **Ultrasonographic and histological study of hormonal oestrus induction effect in anestrus ewes**

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*University of Veterinary Medicine, Košice, Slovak Republic*

Ultrasonic imaging of ovarian structures is rapid and highly accurate method for veterinary applications in sheep reproduction. In the present study, ovaries were USG analysed with 5.0 MHz linear and 7.5 MHz convex transducer. Follicular data were analysed quantitatively and qualitatively. The ovaries were picked up at the end of laparotomy and fixed in 10 % formalin. Sections of ovarian tissue were stained with haematoxylin and eosin and Azan staining. Ovarian slides were microscopically studied and quantitatively and qualitatively analysed by LUCIA-G ver. 4.6. Number of follicles < 3 mm monitored by USG was significant lower ( $P < 0.01$ ) after CIDR-G treatment. Image analysis of ovary sections determined not significant increase in total number of follicles and follicles < 3 mm opposite to that monitored by USG. Half of total number of antral follicles visible on the ovary surface is prepared for recruitment. The rate of atresia is not significantly higher after CIDR-G treatment and follicles in selection undergone atresia in 100 %. Increase in the number of follicles < 3 mm monitored by USG is due to impossibility of USG to notice image of follicles beginning to create the cavity and with very small cavities. We observed better effect on the follicle surviving after used FGA with 1000 IU eCG treatment.

## **The effect of water salinity on the embryonic and larval development in the common frog *Rana temporaria* L.**

Zamachowski, W., Stawarz, R., Formicki, G.

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Research was carried out on the embryos and larvae of the common frog *Rana temporaria* L. The effect of different concentrations of NaCl on the development of approx. 2,200 embryos and approx. 2,000 larvae was studied. It was found that the 5‰ solution of NaCl was a lethal concentration for both the embryos and larvae. In the 1‰ solution of NaCl the development of eggs was similar to that in pure water; the same concerned the mortality of embryos. It was found that the

vulnerability of larvae to salinity depended on their developmental stage. Young larvae with external gills were most vulnerable to salinity, whereas older larvae were less vulnerable. The mortality of embryos and larvae increased in line with the growth of NaCl concentration in the solution. The duration of metamorphosis became prolonged as well. The present studies have shown that the common frog *Rana temporaria* is a typical freshwater amphibian with a very small tolerance for salinity.

### **The circadian organisation and disturbances of circadian rhythms in the cardiovascular system**

Zeman, M.<sup>1</sup>, Bada, V.<sup>2</sup>, Dulková, K.<sup>2</sup>, Liška, D.<sup>2</sup>, Kováčiková, Z.<sup>1</sup>, Košťál, L.<sup>1,3</sup>, Herichová, I.<sup>1</sup>

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The paper deals with disturbances of circadian rhythms in blood pressure and heart rate in hypertensive patients. Increased frequency of circadian rhythms disturbances in cardiovascular system in developed countries is well known but their ethiology is not understood. We hypothesize that a changed life style with shift of activity to late nights, light contamination of environment and shift work may represent risk factors that can contribute to higher incidence of cardiovascular diseases in developed countries. According to our present results conditions of continuous ambulatory blood pressure monitoring substantially influence the final share of different rhythm abnormalities, e.g non-dippers, reversed dippers or extreme dippers. Frequency of all three rhythms disturbances is increased when measurement is performed in hospital in comparison with home conditions. The findings underlay a role of stress and activated sympathetic system in development of rhythm abnormalities in cardiovascular system.

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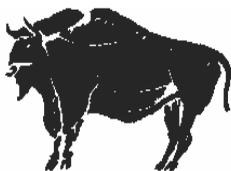
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