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Želáme všetkým účastníkom vedeckej konferencie tvorivé prostredie pre prezentáciu svojich príspevkov, veľa podnetných myšlienok a príjemne strávený čas v spoločnosti kolegov.

Vyslovujeme poďakovanie členom vedeckého a organizačného výboru za ich významný podiel a spoluprácu pri organizovaní tejto konferencie a všetkým účastníkom za ich aktívnu účasť.

prof. Ing. Jaroslav Kováčik, PhD.
za organizačný výbor

INJURY TO THE SMALL INTESTINE OF RABBIT AFTER INTRAMUSCULAR APPLICATION OF T - 2 TOXIN

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ABSTRACT

T – 2 toxin is a toxic member of trichotecenes (group A) generated by numerous *Fusarium* species (mainly *F. sporotrichoides*). They may contaminate feed and unfavourably influence human and animal health. The numerous toxicological studies had been carried out after peroral administration, but there are other important paths of intake such the inhalation or percutaneous route. As the intestine plays a crucial role in the metabolism of T – 2 toxin, in this study we focused on evaluation of structure of duodenum and jejunum of rabbits after single intramuscular application of T – 2 toxin. Adult male rabbits of meat line M91 at age 150 days and body weight 4 ± 0.5 kg were divided into control (C, n=5) and experimental (E, n=5) groups. Experimental rabbits were administered by a single injection of T-2 toxin (Romer Labs Division Holding GmbH, Tulln, Austria) at dose 0.08 mg/kg, 72 hours before they were euthanased. Tissue samples were processed by a common histological technique, stained with haematoxylin & eosin and documented by LM Zeiss Axio Lab A1 with camera Axio Cam ERc5. Both examined sections of the small intestine revealed serious diffuse histopathological changes of the mucous membrane. Intestinal villi and crypts were damaged and almost totally devoid of epithelial covering. The lumina contained quantities of sloughed damaged cells and numerous lymphocytes. The loose connective tissue of lamina propria contained high amount of inflammatory cells. Next constituents of the intestinal wall such the submucosa, muscular coat or serous membrane seemed to be unaffected.

Keywords: T – 2 toxin, rabbits, small intestine, structure

COMPARISON OF GLUTATHIONE PEROXIDASE ACTIVITY AND REDUCED GLUTATHIONE CONCENTRATION IN FETAL CHICKEN BRAIN STRUCTURES ONE DAY BEFORE HATCH

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ABSTRACT

Neural system is efficiently protected by enzymatic and non-enzymatic antioxidants against ROS. Among the non-enzymatic antioxidants special role is attributed to the reduced glutathione. This tripeptide cooperates with enzymatic antioxidants to neutralise free radicals and protects thiol groups of the active enzyme sites. Freshly laid zero-day-old fertilised eggs from the same commercial broiler breed flock of Ross 308 line They were incubated under standard conditions. The samples of brain structures: cerebellum, cerebrum, medulla and optic lobe from chicks were collected and subjected to biochemical analysis. GSH measurements will be performed in deproteinized supernatant thanks to the reaction of thiol groups of GSH with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) (Ellman, 1959). The measurements of glutathione peroxidase (GPx) activity are based on the conversion of oxidised glutathione (GSSG) to reduced glutathione (GSH) with a concomitant oxidation of the reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Lück, 1962). GPx activity was the highest in cerebellum comparison to remaining structure. The highest content of GSH was observed in optic lobe. The results demonstrate the differences in GPx activity and GSH concentration in the investigated brain structure.

SUPEROXIDE DISMUTASE ACTIVITY AFTER TAURINE INJECTION IN MICE BRAIN TREATED WITH ETHANOL

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ABSTRACT

Oxidative stress can be defined as an imbalance between cellular oxidants and antioxidants. It is well - documented that alcohol consumption is involved in tissue injury, as a consequence of enormous oxidative stress, whereas taurine is an amino acid recognized as potential antioxidant which is capable to scavenging free radical. The use of antioxidant enzymes (e.g. superoxide dismutase) which metabolizing highly reactive compounds as a markers of alcoholism is supported by many studies. The goal of present study was to investigate taurine influence on SOD activity in mice brain treated with ethanol. In each series (1 day, 14 days and 56 days) 20 adult males were used and divided into 4 groups: control (tap water), ethanol (treated with 15% ethanol added to tap water), taurine (injected with 20 mg/kg of taurine) and ethanol-taurine (15% ethanol + 20 mg/kg taurine). We detected that taurine enhanced SOD activity in mice brain treated with ethanol in all time periods.

Keywords: antioxidant, ethanol, oxidative stress, superoxide dismutase, taurine

THE IMPACT OF GREEN TEA AND BLACK TEA ON RABBIT SPERMATOZOA MOTILITY *IN VITRO*

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ABSTRACT

Tea is one of the most popular drinks in the world. Many studies inform that consumption of green tea has beneficial effect on human health. Reactive oxygen species (ROS) are involved in many physiological functions of mammalian spermatozoa, however under pathological conditions may lead to oxidative stress generation. Tea contains flavonoids, which have strong antioxidant properties therefore, may protect cells and tissues against free oxygen radicals. Tea may have preventive activity against chronic disease including some forms of cancer. This study presents the effect of green tea and black tea on the motility of rabbit spermatozoa *in vitro*. In our investigation ejaculates from male New Zealand White rabbit bucks (n=5) were used. Three different concentrations of both teas solution (4 g/250 mL, 2 g/250 mL and 1 g/250 mL in 0.9% NaCl) were analyzed using CASA methodology. The measurement was performed *in vitro* after 1 hour of spermatozoa incubation at 37°C. Our results suggest that green tea, as well as, black tea concentrations have significant impact on the spermatozoa motility. The highest decrease of spermatozoa motility was detected at concentration of 4 g/250 mL in the case of green as well as black tea.

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Keywords: tea; *in vitro*; spermatozoa; CASA

THE INFLUENCE OF PROPOLIS AS SUPPLEMENT DIET ON OXIDATIVE STABILITY OF CHICKEN MEAT

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ABSTRACT

The aim of this work was to evaluate the effect of different propolis extract added to feed mixtures on the oxidative stability of breast and thigh muscles of chicken broilers Hubbard JV during refrigerated storage (6 months, -18 °C). Ninety pieces of one day-old chicks were included in the experiment, which were divided into 3 groups (control, E1 and E2). Chicken were fed by *ad libitum* system until the age of 42 days. Propolis extract was added into feed mixture for experimental groups in an amount 600 mg.kg⁻¹ (E1) and 800 mg.kg⁻¹ (E2). During whole period of refrigerated storage were higher values of malondialdehyde (MDA) determined in control group compare to experimental groups. The higher average MDA value determined in breast muscles was in samples of control group (0.157 mg.kg⁻¹) compared to experimental groups E1 (0.127 mg.kg⁻¹) and E2 (0.113 mg.kg⁻¹) after 6-month of storage. Significantly higher values of MDA were determined in control group compare with both experimental groups from fourth month to the end of storage. Trend of thigh muscle oxidation stability of chicken hybrid combination Hubbard JV was during 6 months of refrigerated storage similar than in breast muscle. The higher average MDA value determined in thigh muscles was in samples of control group (0.170 mg.kg⁻¹) compared to experimental group E1 (0.139 mg.kg⁻¹) and E2 (0.126 mg.kg⁻¹) after 6-month of refrigerated storage. Significantly higher values of MDA were determined in control group when compared to both experimental groups from fourth month to the end of storage. Higher amount of MDA in thigh muscle in comparison to breast muscle was observed due to higher amount of fat occurred in thigh muscle.

Keywords: oxidative stability, chicken meat, propolis

Acknowledgments: This work was financially supported by the Ministry of Education, Science, Research and Sports of the Slovak Republic project VEGA 1/0129/13.

MOŽNOSTI EKOLOGICKÉHO ZEMĚDĚLSTVÍ SE ZAMĚŘENÍM NA PRODUKCI VAJEC

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ABSTRAKT

Bylo provedeno dotazníkové šetření, které vypovídá o informovanosti veřejnosti o ekologickém zemědělství, biopotravinách a jejich kvalitě. Sledovány byly údaje zahrnující faktory týkající se nákupu vajec, dále byla hodnocena znalost rozdílů mezi konvenčním a ekologickým chovem nosnic. Pro účely výzkumu bylo osloveno 150 respondentů majících možnost chovat nosnice doma. Byla zvolena nejpoužívanější a nejjednodušší metoda kvantitativních výzkumů, dotazníkové šetření. Z výsledku šetření vyplývá, že stále více spotřebitelů ustupuje od drobného chovu nosnic a vejce nakupuje. Pro 42,1 % respondentů je rozhodujícím faktorem při nákupu výrobce. Rozdíl mezi klecovým a ekologickým chovem nosnic vnímá 48,2 % dotázaných, rozdílné způsoby chovu nosnic pak vidí 56,0 % respondentů. Způsob chovu nosnic se odráží i v ceně vajec. Vejce z ekologických chovů jsou dražší oproti konvenčním. Vzhledem k tomu, že většina spotřebitelů považuje za prioritní ukazatel poptávky cenu, pak právě cena většinu spotřebitelů odradí od nákupu z ekologických chovů. Pouhých 39,0 % respondentů je ochotno si za vejce v biokvalitě připlatit.

ANALYSIS OF PORCINE PITUITARY GLAND TRANSCRIPTOME ON DAYS 10-11 OF THE ESTROUS CYCLE AND PREGNANCY

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ABSTRACT

Pigs are domestic animals frequently used as a model organism for the study of different physiological processes, including the regulation of HPG axis. The control of gonadotropin-releasing hormone (GnRH) and gonadotropin secretion appeared to be incomplete, since the new substances, affecting GnRH- LH system - such as gonadotropin-inhibitory hormone and kisspeptins - had been discovered. Previous studies proved usefulness of transcriptomic experiments for evaluation of genes expression associated with changes in intrauterine environment of pregnant and cyclic pigs. The transcriptomic profile of porcine pituitary during the estrous cycle and early pregnancy is unknown. Therefore, the aim of the study was to compare transcriptomes of porcine pituitaries harvested on Days 10-11 of the estrous cycle (n=4) and pregnancy (n=4). Porcine (V2) Gene Expression Microarray, 4x44K (Agilent Technologies) and bioinformatic analyses using: GeneSpring (Agilent Technologies), PANTHER (Protein Analysis Through Evolutionary Relationships) and DAVID (Database for Annotation, Visualization and Integrated Discovery) were performed. Analysis of microarray data allowed to indicate 319 differentially expressed genes (fold-change ≥ 1.2 , $p < 0.05$) in pituitary of pregnant vs. cyclic gilts. From these genes, 236 were up-regulated and 183 down-regulated. The most significantly enriched signaling pathways included: PPAR, actin cytoskeleton, Toll-like receptors and purine metabolism. Further analysis revealed the greatest numbers of genes with changed expression in the following ontologies: metabolic processes, cell communication, protein transport, immune response and response to stimulus.

In conclusion, presented microarray study and preliminary data analysis made it possible to identify biological pathways that undergo the most significant changes in pituitary of pregnant compared to cyclic pigs (on Days 10-11). It is necessary to undertake further investigation of specific genes and molecular pathways engaged in the local modulation of gonadotropin secretion at the pituitary level in cyclic and pregnant pigs.

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PLASMA OREXINS CONCENTRATION DURING DIFFERENT PERIODS OF ACTIVITY IN THE EUROPEAN BEAVER (CASTOR FIBER L.)

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ABSTRACT

In this study, we report for the first time, the concentration of orexins, as well as sex-, seasonal and age-related changes in plasma orexins (A and B) level in the European beaver (*Castor fiber L.*) – the largest free-living rodent of Eurasia. Beavers are characterized by seasonal patterns of reproduction (long day breeders). The mating of animals takes place in late Winter and juveniles are born in late Spring and early Summer (May and June). The peak activity of a beavers family is in the Spring, during raising of offspring and in the Autumn, when individuals collect food for the Winter, build and repair burrows and lodges. During the Winter, beavers do not hibernate. The phenomenon of seasonal breeding may be related to seasonal changes in the secretion of orexins (A and B) – the hormones control feeding behavior, wake cycle and arousal, energy homeostasis, stress response and even reproductive functions. Therefore, the aim of our experiment, conducted on a population of free-living male and female beavers, was to find gender, seasonal and age-dependent changes in plasma orexin levels.

Blood samples were collected under general anesthesia in April – pregnancy in females (8 males and 5 pregnant females), July (postbreeding) – the end of lactation and offspring rising (4 males and 6 females) and November (prebreeding) – preparing for Winter (6 males and 5 females). In July (2 males and 3 females) and in November (3 males and 2 females), the blood samples were also collected from young beavers (the first year of life). The blood for the determination of the plasma hormones concentration was collected from the carotid artery. The level of orexins was measured by enzyme-linked immunosorbent assay (ELISA). Analyses were performed using Statistica software (StatSoft Inc., USA). Data (ng/mL; mean±SEM) were analyzed by two-way ANOVA followed by the Duncan post hoc test. The level of significance was set at $P < 0.05$ for all analyses.

Seasonal, but not sex-dependent changes in plasma OXA levels were observed. In males, the highest OXA concentrations were noted in July ($n=4$, 27.74 ± 5.127 pg/mL) and in females – in November ($n=4$, 24.49 ± 1.38 pg/mL). Neither sex nor season-related variations in plasma OXB levels were observed. Additionally, we did not notice changes in plasma orexin A and B concentrations between the old and the young beavers.

Our data suggests that only the levels of orexin A altered dependently on season. Moreover, those changes might be connected with seasonal secretion of glucocorticoids.

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EFFECT OF DIETARY ZINC SOURCES ON MINERAL DEPOSITION AND SUPEROXIDE DISMUTASE ACTIVITY IN TISSUES OF RABBITS

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ABSTRACT

This study was conducted to investigate the effect of feed supplementation with different zinc sources on deposition of Zn, Cu and Mn in liver, kidney and muscle tissue (musculus longissimus dorsi) of rabbits. Simultaneously the activities of total superoxide dismutase (SOD) and specific Cu/Zn SOD in liver and kidney were measured. Ninety six 42-day-old broiler rabbits (both sexes) were allocated to 4 dietary treatments with 6 replicates, each containing 4 animals per replicate. For the subsequent 6 weeks, the rabbits were fed the identical basal diet (BD) supplemented with equivalent dose of Zn (100 mg/kg) from various sources. Group 1 (control) was given the unsupplemented BD. Diets for group 2, 3 and 4 consisted of same BD supplemented with Zn in the form of Zn sulphate, Zn chelate of glycine hydrate (Zn-Gly) and Zn chelate of protein hydrolysate (Zn-Pro), respectively. Intake of the diet supplemented with inorganic Zn sulphate and organic Zn-Pro significantly increased Zn deposition in liver compared to control animals given unsupplemented BD. No significant difference in Zn levels was observed in kidney cortex and muscle tissue. Concentration of Zn in plasma was significantly elevated only in Zn sulphate group. Feeding the diet enriched with Zn-Pro resulted in significantly higher copper deposition in liver than in control and Zn sulphate groups. Copper concentrations in plasma, kidney and muscle tissue were not influenced by dietary treatments. No differences in tissues and plasma concentration of manganese were found due to source of Zn supplemented. The intake of feed supplemented with zinc did not result in any change of SOD or Cu/Zn SOD activity in analysed tissues. The presented results indicate that dietary organic zinc has similar effect on zinc deposition and Cu,Zn-containing superoxide dismutase activity in tissues as traditionally used inorganic zinc source. In conclusion, our results suggest on bioequivalence of zinc chelates and Zn sulphate as efficacious source of zinc in nutrition of rabbits.

Keywords: rabbit, zinc chelate, superoxide dismutase

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IMPACT OF LOAD ON WHITE BLOOD CELL COUNTS IN HORSES

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ABSTRACT

White blood cells are part of the immune system. They play an important role in protecting the body against infection. Therefore, in infection and in blood cell count determine their number increased. Increased levels however, occur not only during infection, but also caused by excessive load, for example when working in extreme conditions or in heavy load.

In the experiment was included 6 horses (3 operating horse, 3 sport horses), bred at the Experimental center of livestock on DAH. We studied the impact of load on a conveyer of 30 minutes on selected haematological parameters during the trial (C - control), the first day after load after 5 and 30 minutes (1a5, 1a30) and seventh day after exercise also at 5 and 30 minutes (7a5, 7a30). We collected blood from the jugular vein at specified intervals. In blood we determined the total number of white blood cells (WBC), lymphocyte count (LYM), the mean size of cells including monocytes and eosinophils (MID) and the number of granulocytes (GRA) immediately after the collection with an automated hematology analyzer Abacus Junior Vet. From the results, we calculated the basic statistics, and the differences were tested by t-test and ANOVA in SAS program.

WBC Operating horses had an average level of leukocytes at rest (C) $7.33 \times 10^9/l \pm 1.48$, (1a5) $5.77 \times 10^9/l \pm 1.50$, (1a30) $6.63 \times 10^9/l \pm 1.01$, (7a5) $5.49 \times 10^9/l \pm 1.57$, (7a30) $6.74 \times 10^9/l \pm 1.95$. The sport horses (C) $5.43 \times 10^9/l \pm 1.50$, (1a5) $4.7 \times 10^9/l \pm 0.53$, (1a30) $8.28 \times 10^9/l \pm 0.78$, (7a5) $4.59 \times 10^9/l \pm 0.39$, (7a30) $7.78 \times 10^9/l \pm 1.04$.

LYM For operating horses were recorded mean levels at rest (C) $3.29 \times 10^9/l \pm 0.82$, (1a5) $1.46 \times 10^9/l \pm 0.51$, (1a30) $1.93 \times 10^9/l \pm 0.54$ (7a5), $1.73 \times 10^9/l \pm 0.49$, (7a30) $2.03 \times 10^9/l \pm 1.58$. For sport horses are ranged at a level (C) $2.03 \times 10^9/l \pm 0.11$, (1a5) $2.04 \times 10^9/l \pm 0.63$, (1a30) $3.28 \times 10^9/l \pm 0.54$, (7a5) $1.45 \times 10^9/l \pm 0.12$, (7a30) $2.99 \times 10^9/l \pm 0.84$.

MID Levels ranged in operating horses (C) $0.32 \times 10^9/l \pm 0.21$, (1a5) $0.22 \times 10^9/l \pm 0.25$, (1a30) $0.35 \times 10^9/l \pm 0.06$, (7a5) $0.17 \times 10^9/l \pm 0.14$, (7a30) $0.27 \times 10^9/l \pm 0.09$. At sport horses (C) $0.10 \times 10^9/l \pm 0.13$, (1a5) $0.11 \times 10^9/l \pm 0.12$, (1a30) $0.18 \times 10^9/l \pm 0.12$, (7a5) $0.18 \times 10^9/l \pm 0.12$, (7a30) $0.59 \times 10^9/l \pm 0.25$.

GRA Operating horses had levels of granulocytes in the control (C) $3.72 \times 10^9/l \pm 0.98$, (1a5) $4.09 \times 10^9/l \pm 1.55$, (1a30) $4.63 \times 10^9/l \pm 0.60$, (7a5) $3.59 \times 10^9/l \pm 1.00$, (7a30) $4.44 \times 10^9/l \pm 0.46$. Sports horses (C) $3.30 \times 10^9/l \pm 1.44$, (1a5) $2.58 \times 10^9/l \pm 0.74$, (1a30) $4.83 \times 10^9/l \pm 0.26$, (7a5) $2.95 \times 10^9/l \pm 0.18$, (7a30) $4.21 \times 10^9/l \pm 0.18$.

Neither among the groups nor between operational and sport horses have not seen significant differences. The highest levels of the monitored parameters, we found 30 minutes after exercise.

Keywords: white blood cells count, horses

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THE INFLUENCE OF DAGO ON ESTRADIOL SECRETION BY PORCINE ENDOMETRIUM DURING THE ESTROUS CYCLE AND EARLY PREGNANCY – PRELIMINARY STUDY

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ABSTRACT

Introduction

Endogenous opioid peptides (EOP) are divided into three families: endorphins, enkephalins, dynorphins. EOP derive from three main opioid precursors: proopiomelanocortin (POMC), proenkephalin (PENK) and prodynorphin (PDYN). Peptides originating from these precursors preferentially act through one type of opioid receptors – i.e. mu, delta or kappa, respectively. It was found that EOP affect the functioning of reproductive axis at all levels. Previous studies have shown the effect of opioids on steroidogenesis in ovary and adrenal cortex. Therefore the aim of this study was to determine the influence of DAGO (mu receptor agonist) on estradiol (E2) secretion by porcine endometrium during the estrous cycle (n=4, days 2-3, 10-11, 12-13, 15-16 and 18-20) and early pregnancy (n=4, days 10-11 and 15-16).

Material and Methods

Uteri were collected from mature cross-bred pigs (Large White x Polish Landrace, weight 90-110 kg) during the estrous cycle and early pregnancy. Uterine horns were placed in ice-cold phosphate buffered saline (PBS) with 100 IU/ml of penicillin and 100 µg/ml of streptomycin and transported to the laboratory for endometrial tissue isolation. Endometrium was sliced into 100-110 mg parts and placed in culture vials containing 2 ml of Medium 199 with the addition of 0.1% BSA fraction V and 20 µg of gentamycin. Endometrial slices were preincubated (2 h at 37°C under an atmosphere of 95% O₂ and 5% CO₂) and subsequently incubated for 6 and 24 h without or with addition of DAGO (10⁻⁹, 10⁻⁸, 10⁻⁷ M). After the incubation, media were collected and frozen (-20°C) until the radioimmunoassay (RIA) of estradiol concentration.

Results

The study has shown stimulatory effect of DAGO on estradiol secretion during both – the estrous cycle and pregnancy. During the estrous cycle, DAGO stimulated the secretion of E2 on days 10-11, 12-13 (10⁻⁸ M) and 15-16 (10⁻⁷ M) after 6 h incubation, as well as on days 2-3 (10⁻⁸ M), 15-16 (10⁻⁷ M) and 15-16 (10⁻⁸ M) after 24 h incubation. During pregnancy, it increased E2 secretion on days 10-11 (10⁻⁸ and 10⁻⁷ M) and 15-16 (10⁻⁸ M) only after 24 h incubation.

Conclusion

It has been shown that DAGO may affect the secretion of estradiol in the endometrium of gilts during the estrous cycle and early pregnancy. This indicates that POMC system is potentially engaged in the regulation of intrauterine environment in pigs.

CADMIUM INDUCED CHANGES IN METTALLTHIONEIN AND SOME APPOPTOSIS FACTORS mRNA EXPRESSION IN KIDNEY OF DEVELOPING CHICKEN EMBRYOS

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ABSTRACT

Cadmium (Cd) is a heavy metal whose ions are easily absorbed and accumulated in the tissues of both plants and animals. In animals exposed to cadmium compounds, particularly high concentrations of cadmium ions have been found in kidneys, heart and liver, and lower concentrations in pancreas and brain. Moreover, cadmium is known to cause teratogenesis in a wide variety of animals. Chick embryo exposed to Cd(II) ions demonstrated alterations in multiple organs and is useful model for study embryotoxicity aspects. Metallothioneins (MT) are low molecular weight proteins, binding a number of trace metals including zinc, cadmium, mercury, platinum and silver, and also protect cells and tissues against heavy metal toxicity. A number of studies have shown that increased MT expression is closely associated with tumor grade and proliferative activity in solid tumors, and that these proteins may be potential negative regulators of apoptosis. The aim of this study was analysis of mRNA expression of metallothionein, caspase-6 and Bcl-2 protein in kidney tissue of chicken embryos exposed in ovo to cadmium. Hatching eggs were injected on day 4th of incubation with cadmium at the dose of 0 and 7,8 µg/egg (80 eggs/group). The samples of kidney tissues were collected from embryos at E14, E18 and at hatching day- D1. Total RNA was isolated (TRI Reagent; Sigma-Aldrich) and after reaction of reverse transcription (High-Capacity cDNA Reverse Transcription Kit; Life Technologies), analysis of mRNA expression of metallothionein and apoptosis regulated factors: caspase-6 (responsible for apoptosis execution) and anti-apoptotic Bcl-2 protein, were analysed by using Real Time PCR method (SYBR Green, Life Technologies). In control group the metallothionein gene expression was highest in E14 and next significantly decreased during E18 and D1. However Cd induced significant decrease of this gene expression at E14 following increase at E18. Parallel results were previously detected in semi quantitative PCR analysis of metallothionein mRNA expression (not published data). Similarly, expression of caspase-6 and Bcl-2 anti-apoptotic protein mRNA in Cd-exposed kidney tissue decreased at E14, and next increased in E18 ($p > 0.05$) as well as at D1 ($p < 0.05$) in comparison to control group. In concluding, significant decrease at E14 and induction of metallothionein mRNA expression observed at E18 can be evidence for crucial role of this protein in cadmium detoxification and suggest, that mechanism protecting the body from heavy metals needs time for activation. These observation is supported by the changes in mRNA expression of determinants/inhibitors of apoptosis (caspase-6/ Bcl-2 protein). Similar direction of changes in mRNA expression of caspase-6 and Bcl-2 protein does not allow to draw conclusions on the effect of cadmium on apoptosis. We can only speculate that cadmium affects this process in a time-dependent manner.

EFFECT OF CURCUMIN ON ANTIOXIDANTS ACTIVITY IN SELECTED ORGANS OF MICE

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ABSTRACT

Curcumin, the principal curcuminoid found in rhizomes of *Curcuma longa*, is an effective scavenger of reactive oxygen species and reactive nitrogen species in the *in vitro* test. However, it is not clear whether curcumin acts directly as an antioxidant *in vivo*. Therefore, the purpose of the study was to investigate the effect of intraperitoneal injection of curcumin at the doses: 50 mg/kg b.w., 100 mg/kg b.w. and 200mg/kg b.w in inflammation caused by zymosan A administration (100 mg/kg b.w.). Twenty five adult male Swiss mice were divided into five groups: control group, zymosan A group and three groups being the combination of zymosan A and curcumin in different doses. In the liver, pancreas and kidneys of the mice the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were determined. After the injection of zymosan A, the activities of analyzed enzymes in the pancreas and kidneys of mice were significantly lower in relation to control. Curcumin injection before the administration of zymosan A significantly inhibited the decrease in the activities of the SOD, CAT and GPx compared with the zymosan A group. The results of our research, clearly indicate that curcumin is an anti-inflammatory agent.

Keywords: curcumin, zymosan, superoxide dismutase, catalase, glutathione peroxidase

EFFECTS OF ECHINACEA PURPUREA ON THE BLOOD CELLS

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ABSTRACT

Echinacea purpurea, one of the most important medical herbs, has been used to treat inflammation. It contains a variety of medically important substances that play a role in its therapeutic effects which include alkylamides, caffeic acid derivatives, glycoproteins, polysaccharides, polyacetylenes, phenolic compounds, cinnamic acids, essential oils and flavonoids. Several phenolic compounds have been reported to be inhibitors of chemical carcinogenesis and mutagenesis. The aim of this study was to investigate the effect of aqueous extract of the herb Echinacea and alcohol extract of the root in an animal model of inflammation induced by zymosan. Male mice (Swiss) were used in the experiment. The animals were divided into six groups, one control and five experiments. The control group - healthy mice treated with saline, and I experimental group - mice after intraperitoneal injection of zymosan, the second experimental group - mice after oral administration of the aqueous extract of Echinacea, the third experimental group - mice after oral administration of the alcoholic extract Echinacea root, the fourth experimental group - mice after a single intraperitoneal injection of zymosan and after oral administration of aqueous extract of the herb Echinacea, V experimental group - mice after a single intraperitoneal injection of zymosan and after oral administration of an alcoholic extract of the root of Echinacea. The experiment lasted four weeks, on day 29 of the experiment the animals were decapitated. The peripheral blood of all animals tested was determined the number of neutrophils, eosinophils, basophils, lymphocytes and monocytes. The study shows that in all treatment groups showed alterations in the total number of neutrophils, eosinophils, lymphocytes and monocytes compared to the control group. The largest statistically significant at $p < 0.01$ increase in the number of neutrophils, eosinophils, lymphocytes and monocytes were observed after administration of alcohol extract of Echinacea root, root alcohol extract of Echinacea along with zymosan and aqueous extract of the herb Echinacea with zymosan which confirms its immunostimulatory properties. Echinacea purpurea has many beneficial features, especially activation of immune system by increasing the number of circulating white blood cells.

THE EVALUATION OF STEROID HORMONE PRODUCTION AFFECTED BY EXPERIMENTAL APPLICATION OF *TERMINALIA BELLIRICA*

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ABSTRACT

Terminalia bellirica Roxb. (family Combretaceae) is a perennial deciduous tree of the tropics and found in South Asia, including India. Its fruits are one of the ingredients of TRIPHALA, an ayurvedic herbal formulation consisting equal parts of three medicinal plants namely *T. chebula*, *T. bellirica* and *Emblica officinalis*, which is credited with diverse beneficial properties like anti-stressor, anti-oxidant and immunostimulant properties. *T. bellirica* fruits are known to be astringent, acrid, digestive, antihelminthic, narcotic, ophthalmic, antipyretic and rejuvenating. The objective of this *in vitro* study was to examine the impact of fruit extract of *T. bellirica* on the release of steroid hormones by porcine ovarian granulosa cells. Granulosa cells were isolated from porcine ovaries and subsequently cultured without (control) or with fruit extract at three doses (10; 100 and 1000 µg/mL) for 24 h. The release of steroid hormones (progesterone, 17-β-estradiol) was assayed by ELISA. After application of the fruit extract to cultured granulosa cells, no significant ($p \geq 0.05$) change was observed in the release of steroid hormone progesterone as compared to control. Similarly, the release of 17-β-estradiol by ovarian granulosa cells was not affected, too, after application of fruit extract at any of the doses used. Our results indicate the importance of evaluating the bioactivity of herbal medicinal plants used in ayurvedic formulations, such as *T. bellirica* for their potential effect on mammalian model systems. In conclusion, the release of steroid hormones progesterone and 17-β-estradiol by porcine ovarian granulosa cells was not affected by experimental administration of *T. bellirica* fruit extract.

Keywords: *Terminalia bellirica* Roxb., porcine ovarian granulosa cells, steroid hormones.

Acknowledgments: This work was financially supported by the European Community under the Project no. 26220220180: Building Research Centre „AgroBioTech“, Ministry of Education, Science, Research and Sports of the Slovak Republic project VEGA 1/0022/13 and Slovak Research and Development Agency of the Slovak Republic APVV-0304-12.

EFFECT OF DIMETHYL SULFOXIDE ON LIVER IN THE CHICK EMBRYO

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ABSTRACT

Dimethyl sulfoxide (DMSO) is one of the most commonly used solvents for water-insoluble substances. DMSO is a hydroxyl radical scavenger, excellent cryoprotectant and topical analgesic. However, its widespread use has been restricted due to its toxic effects, which vary depending on the cells in question and concentration. In the present study, we observed morphological changes in the chick embryo on 9ED exposed to 50µl/egg of DMSO for 5 days. Samples of livers from 9-day chick embryos were fixed in 3 % glutaraldehyde and postfixed in 1 % osmium tetroxide. After dehydration in acetone tissues were transferred to propylene oxide and embedded in Durcupan ACM (Fluka). Semi-thin sections for light microscopy were cut using an ultramicrotome LKB Nova. The semi-thin sections were stained with toluidine blue and examined under a Jenamed light microscope. In the control group, we observed normal morphology of liver. Hepatocytes were either columnar or pyramidal shapes with round euchromatic nucleus and evident nucleolus. Hepatocytes were circularly arranged around the bile canaliculi forming the tubules. The tubules were surrounded by liver sinusoids. In some hepatocytes a few lipid droplets was observed. In the experimental group dilatation of liver sinusoids and hyperemia were observed. The shape and arrangement of hepatocytes were not changed markedly. Hepatocytes contained larger lipid droplets than in control group, but still in small amount. In some areas of liver, the bile canaliculi were markedly dilated. Our observations demonstrated that DMSO may adversely affect the liver of chick embryo, however further studies are needed to discover and identified more about the effect of DMSO on organism.

Keywords: DMSO, chick embryo, liver, structure

LEPTIN RECEPTOR (OB-R) EXPRESSION IN THE OVARY AND UTERUS OF THE EUROPEAN BEAVER (CASTOR FIBER)

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ABSTRACT

Leptin is 16 kDa hormone, which is synthesized and secreted mostly by adipose tissue. Through its receptor (Ob-R) leptin regulates food intake and energy balance. Moreover mRNAs and proteins of leptin and Ob-R were also identified in several reproductive tissues including: the ovary, oviduct, endometrium and placental trophoblast. It has been demonstrated that leptin plays an integral role in the regulation of reproductive functions including: stereogenesis, oocyte maturation, early embryonic development and implantation. It is known that the appetite, body weight and body fat mass in the European beaver (*Castor fiber*) increase in autumn and winter, when its reproductive system is prepared for mating. It has been reported that leptin is a critical link between the adipose tissue and the reproductive system, therefore we suspect that fat stores in winter improve the reproductive success of female beavers. The aim of our study was to determine the expression of leptin receptor protein in the beaver ovary and uterus (myometrium and endometrium). Tissues were collected from mature beaver females collected in different stages of their reproductive activity: in April – before beginning of breeding (n=5), July – the end of lactation and offspring rising (n=7), November – sexual silence (n=6). To detect the expression of OB-R protein, immunohistochemical technique was used. Tissue slices were incubated with rabbit primary anti-Ob-R antibodies and anti-rabbit IgG conjugated with horseradish peroxidase enzyme (HRP) were used as the secondary antibodies. 3,3'-Diaminobenzidine (DAB) solution was used to immunodetection and hematoxylin for nuclei staining was applied. Total 6 images per tissue were taken and DAB immunoreactive area was measured. Obtained data were analysed by one-way ANOVA followed by the Duncan post-hoc test.

Our study showed that leptin receptor protein was expressed in all tested tissues during different stages of the beaver reproductive activity. In ovaries, the Ob-R protein expression level was higher in April than in July or November. In endometrium, the highest level of Ob-R protein expression was noted in July, then lower in April and the lowest in November. In turn, in myometrium the expression of leptin receptor protein was at the same level in April and July and significantly higher than in November. Our findings suggest that leptin may be involved in the regulation of reproductive processes in beaver females.

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NEXT-GENERATION SEQUENCING (NGS) AND BIOINFORMATIC TOOLS FOR ESTABLISHING THE FULL SEQUENCE OF PORCINE ARYL HYDROCARBON RECEPTOR NUCLEAR TRANSLOCATOR (ARNT) GENE

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ABSTRACT

The aryl hydrocarbon receptor nuclear translocator (ARNT) is a protein containing basic helix-loop-helix domain and two Per-Arnt-Sim (bHLH PAS) domains. In the nucleus it binds to the ligand-AhR complex and promotes the activation of genes which have dioxin responsive element (DRE) in their regulatory regions, including cytochrome P450 genes and estrogen receptors. This study was performed to determine the complete nucleotide sequence of porcine ARNT gene. Total RNA was isolated from porcine livers. The integrity of RNA (RIN) was evaluated using 2100 Bioanalyzer. The complete sequence of ARNT gene was obtained by Next-Generation Sequencing (NGS). The sequencing was performed by OpenExome company using Illumina HiSeq2500 sequencer. We determined the complete mRNA sequence of the porcine ARNT gene and established the presence of two variants of ARNT. Variant 1 had a total length of 3857 bp, contained 21 exons and encoded a polypeptide of 776 amino acids. Variant 2 had a total length of 3902 bp, contained 22 exons and encoded a polypeptide of 791 amino acids. In addition, *in silico* analysis revealed that in the pig, ARNT gene is located on 4. chromosome. The full-length mRNA sequence of the porcine ARNT gene was demonstrated for the first time in the current study. The results of the study extend our knowledge concerning ARNT-dependent processes.

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DETERMINE THE POTENTIAL EFFECT OF 4-NONYLPHENOL ON cAMP STIMULATED TESTOSTERONE PRODUCTION OF MICE LEYDIG CELLS

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ABSTRACT

During the past decades, a large body of information concerning the effects of endocrine disrupting compounds (EDCs) on humans and animals has been accumulated. EDCs are of natural or synthetic origin and certain groups are known to disrupt the action of androgens and impair the male reproductive system. A lot of different classes of EDCs exist, such as pesticides, dioxins and other synthetic compounds. Nonylphenol (NP) is a xenobiotic compound classified as an endocrine disruptor capable of interfering with the hormonal system of numerous organisms. This substance is primary degradation product of nonylphenol polyethoxylate (NPE), frequently incorporated into insecticides, detergents, synthetic products and industrial surfactants. Nonylphenol is able to directly or indirectly affect the key enzymes in steroidogenesis, leading to increased or decreased hormone level. The principal testicular structure, which is responsible for androgen biosynthesis are the Leydig cells. These have a spherical shape and are located between the seminiferous tubules. It has been shown that the development of Leydig cells follows a specific pattern: mesenchymal, progenitor, newly formed adult Leydig cells, immature adult Leydig cells and mature adult Leydig cells. Mitochondria and endoplasmic reticulum have irreplaceable position within Leydig cells. The exact mechanism of action of endocrine disruptors is not completely understood however it may be associated with enzymatic damage and alterations in the content or function of cAMP (cyclic adenosine monophosphate). The potential impact of 4-nonylphenol (4-NP) on androgen production by interstitial Leydig cells during 44h in vitro cultivation was observed in the present study. The interstitial (Leydig) cells were obtained from NMRI mice testis and cultivated with addition of 0.04; 0.2; 1.0; 2.5 and 5.0 µg/mL of 4-nonylphenol, and compared to the control. Cell suspensions in all wells were cultured in the presence of cAMP. Concentration of testosterone in the media were determined using enzyme linked immunosorbent assay (ELISA). The testosterone production decreased at 0.04 – 5.0 µg/mL of 4-NP, however this decrease was non-significant. Results of our study indicate a decreased testosterone production in cAMP stimulated mice Leydig cells at concentrations of 0.04; 0.2; 1.0; 2.5 and 5.0 µg/mL 4-NP after a 44h in vitro cultivation. A considerably more detailed and systematic research in endocrine toxicology is however required for a better understanding of risks associated with endocrine disruption in humans and wildlife.

Keywords: mice, 4-nonylphenol, Leydig cells, testosterone

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EFFECT OF HERB FEEDING ON AMINO ACID LEVEL OF CHICKEN BLOOD SERUM

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ABSTRACT

The objective of the experiment was to determine if some herb supplements could have an effect on the essential amino acid level in blood serum of broiler chickens. There were used the rosemary leaves (RL), the yarrow blooms (YB), the plantain leaves (PL), the oregano talks (OT) or the red grape pomace (GP) in the amount of 1.5 % of a feed ration from 10 to 35 day of age as herb supplements. The experiment was carried out on the 192 female chickens Ross 308. The chickens were kept in the double-floor cage technology. All of them were fed by the same complete feed mixture BR1 (Broiler No. 1) for the first 10 days. After 10 days of age, the birds were randomly allocated to six 18 cages in both tiers corresponding to six dietary treatments with three replicates of each treatment. Each group comprised 3 cages containing 10-11 broilers each. The dietary treatments included a control diet and the diets containing 1.5% herb supplements dry powder with the other components remaining the same as in the control diet. The level of the essential amino acids was determined in samples obtained from 35 days old animals by high performance liquid chromatography with UV/VIS detection and post column labelling. The fodder supplement of the RL, the YB, the PL, the OT and the GP had no significant ($P>0.05$) effect on the level of the threonine, the leucine and the arginine in the blood serum of the broiler chickens. The highest level of the histidine in the blood serum was measured in the YB's fodder supplement. The lowest level of the amino acid was in the PL's fodder supplement. The difference found was significant ($P<0.05$).

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THE EFFECT OF YUCCA SHIDIGERA ON BASIC OVARIAN FUNCTIONS IN PORCINE OVARIAN GRANULOSA CELLS

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ABSTRACT

Yucca shidigera is a widely used medicinal plant for its antimicrobial, anti-inflammatory, antiviral, antioxidant and anti-cancer activity. These effects were studied on various animals, but its action on basic ovarian functions remains unknown. The aim of this study was to find the role of extract from *Yucca shidigera* in control of basic ovarian cell functions. For this purpose, we have examined the effect of yucca extract at the doses 0, 1, 10, 100 µg/ml on proliferation, apoptosis and steroidogenesis by cultured porcine ovarian granulosa cells. Accumulation of cyclin B1 (marker of proliferation) and caspase 3 (marker of apoptosis) were analysed by immunocytochemistry. EIA was used for evaluation of the steroid hormones (progesterone and testosterone) release.

It was observed, that addition of yucca extract inhibited the percentage of proliferative (cyclin B1-positive) cells at the all used doses (1, 10 and 100 µg/ml). The percentage of apoptotic (caspase 3-positive) cells was stimulated increased at 1 and 100 µg/ml but not 10 µg/ml. Yucca treatment inhibited progesterone release (at 10 and 100 µg/ml but not at 1 µg/ml) and not affected testosterone release (at 1, 10 and 100 µg/ml) by porcine granulosa cells. These results suggest a direct effect of yucca on proliferation, apoptosis and steroidogenesis in porcine ovarian cells. Taken together, these data showed, that extract of *Yucca shidigera* can negatively affect reproductive (ovarian) functions – suppress ovarian cell proliferation, promote their apoptosis and alter release of steroid hormones.

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THE CHANGES OF THE ACTIVITY OF LIVER ENZYMES FOLLOWING APPLICATION OF QUERCETIN IN THE RABBITS BLOOD

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ABSTRACT

The aim of present study was to determinate the effect of application of different doses of quercetin on the activity of liver enzymes (AST and ALT) in the rabbits blood. Adult male rabbits (n = 20) were divided into four groups (n=5 in each group), control group (C) and three experimental groups (E1, E2 and E3). The experimental groups were given quercetin by injection (intramuscular) 10 µg.kg⁻¹ in group E1, 100 µg.kg⁻¹ in group E2 and 1000 µg.kg⁻¹ in group E3 for 60 days three times a week. Blood was collected on 30th and 60th day of the experiment. Blood samples were analyzed by automatic clinic analyzer Microlab 300. When evaluating the measurement of ALT on 30th day of the experiment in group E1, we measured the lowest value of 0.47 ± 0.18 µkat.l⁻¹, whereas in the group E2 we measured the highest value of 0.77 ± 0.05 µkat.l⁻¹. Between groups E1 and E2 we observed a statistically significant difference (P<0.05). Other parameters were not influenced (P>0.05) after quercetin application. However, further investigations are needed to prove the final answer concerning the health promoting effects of quercetin.

Keywords: rabbits, quercetin, AST, ALT

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THE EXPRESSION OF ANDROGEN RECEPTOR IN THE PITUITARY AND ADRENAL GLANDS OF MALE TURKEYS (*MELEAGRIS GALLOPAVO*) DURING GROWTH AND DEVELOPMENT

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ABSTRACT

Introduction: Androgens take part in the regulation of puberty and support growth and development. They promote the pituitary development and the regulation of pituitary hormone secretion. Androgens are secreted primarily by the testes, but these hormones are also produced by the adrenal glands. These steroids play their biological role by binding to a specific androgen receptor (AR). The aim of this study was to evaluate the expression of AR mRNA and protein in the pituitary and adrenal glands of male turkeys at the age of 4, 8, 12, 16, 20, 24 and 28 weeks.

Materials and methods: Pituitaries and adrenal glands were collected from Big 6 male turkeys (*Meleagris gallopavo*) during growth and development (n=6 in each age group). The AR protein expression was analysed by Western Blot and the AR mRNA level was detected by real-time PCR method. The AR protein and mRNA expression in the pituitary and adrenal glands were analyzed by one-way ANOVA followed by a post-hoc Least Significant Difference (LSD) test. The results were expressed as means of groups \pm SEM. Differences were considered statistically different at the level of $p < 0.05$.

Results and conclusions: Pituitary levels of AR mRNA were the lowest at 4 and 8 weeks, higher at 12 and 16 weeks and the highest at 20, 24 and 28 weeks of age ($p < 0.05$). Pituitary concentrations of AR protein were the lowest at 4, 8, 12, 16 and 20 weeks, higher in weeks 24 and 28 ($p < 0.05$). AR mRNA levels in the adrenal glands were higher at 12 weeks of age and the highest at 8 weeks of age, relative to successive growth stages ($p < 0.05$). AR protein concentrations in the adrenal glands were higher in weeks 4, 8 and 12, relative to weeks 20, 24 and 28 ($p < 0.05$). Our study revealed a difference between the AR expression in pituitary and adrenal glands during growth of male turkeys. During the maturation of male turkeys, the concentrations of androgens in plasma increase (Kiezun et al., 2015), but the mechanism of androgen receptor regulation seems to be different in the pituitary and adrenal glands. An increase in the AR expression in the pituitary may be responsible for the maintenance of high androgen concentrations in the plasma and testes. A drop in the adrenal expression of AR could point to the presence of a compensatory mechanism, that enables turkeys to avoid the potentially detrimental effects of high androgen concentrations. Our results will contribute to better understanding of the ARs role in the pituitary and adrenal glands during the growth and development of birds.

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ADIPONECTIN AFFECTS REPRODUCTIVE FUNCTIONS VIA GABBR2, GRB14, PTGER4 AND NPR2 GENES REGULATION IN THE PITUITARY OF CYCLIC GILTS

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ABSTRACT

Introduction: Female reproductive success is closely associated with nutritional status and energy balance. In this context, adiponectin appears to be one of the key hormones connecting reproductive system functions and metabolism regulation. This 30 kDa adipokine acts via two distinct widely expressed receptors (AdipoR1 and AdipoR2), which suggests its pleiotropic effect. The expression of both, adiponectin and its receptors, has been indicated at all levels of the porcine hypothalamo-pituitary-gonadal axis (HPG). For more, the in vitro effect of this hormone on LH and FSH secretion by anterior pituitary (AP) cells has been proved. Nevertheless, the mechanisms of adiponectin action in the anterior pituitary remains unclear. The aim of this study was to identify the most potent genes connected to the female reproductive functions in the AP cells of cyclic gilts, which expression is regulated by adiponectin.

Material and methods: Anterior pituitaries were harvested from gilts on days 10 to 12 of the oestrous cycle. AP cells were cultured in vitro for 24 h (water-saturated atmosphere of 5% CO₂ and 95% air) with the addition of 10 µg/ml of adiponectin or without treatments (control). Total RNA isolated from cells after culture was assigned for microarray analysis and real-time PCR validation of microarray results. DAVID, KEGG and BioCarta databases, as well as IPA tool were used to select group of genes connected to the female reproductive functions, which expression was affected by adiponectin. Real-time validation was conducted to confirm the obtained data. Statistical analyses were performed using one-way ANOVA followed by LSD post hoc test.

Results and conclusions: Microarray analysis allowed us to select group of genes including GABBR2, GRB14, PTGER4 and NPR2, regulated by adiponectin, which are found to be a potent regulators of female reproductive functions at the pituitary level of HPG axis. GABBR2, also called gamma-aminobutyric acid (GABA) B receptor type 2 gene, which encodes G-protein coupled GABA receptor subunit, was up-regulated by adiponectin. The stimulatory effect of GABA on LH secretion was recently described. This suggests that adiponectin may affect LH secretion via enhancement of GABA action in AP cells. GRB14 gene encodes growth factor receptor-bound protein 14, which interacts with insulin receptor to inhibit insulin intracellular signalling. Down-regulating effect of adiponectin on GRB14 expression indicated in this study, may boost stimulatory action of insulin on LH secretion. Moreover, observed in this experiment up-regulating effect of adiponectin on PTGER4 gene, encoding prostaglandin E₂ (PGE₂) receptor type 4, may be alternative way of influencing gonadotropins secretion, since PGE₂ is known to stimulate LH and FSH secretion. Also

NPR2 gene, encoding receptor type B for natriuretic peptide, was up-regulated by adiponectin. NPR2 mediate natriuretic peptide (BNP) signalling via cGMP activation, which plays an important role in the regulation of gonadotropins release. It is suggested that cGMP activates protein kinase G that causes exocytosis of gonadotropins secretory granules by the AP cells. In conclusion, the obtained data seem to confirm our earlier hypothesis on the important role of adiponectin in the regulation of female reproductive functions at the pituitary level of the HPG axis. Moreover, the adipokine affects pituitary functions GABA, insulin, PGE2 or natriuretic peptide.

Acknowledgments: This research was supported by National Science Centre of Poland (project no: 2011/01/B/NZ4/01596)

EFFECTS OF HIGHER INTAKE OF TWO MANGANESE SPECIES ON SHEEP RUMEN MICROBIAL POPULATION AND ACTIVITIES

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ABSTRACT

We studied the effects of higher intake of manganese on sheep rumen microbial ecosystem after three month treatment. We compared the total and generic count of rumen ciliates, bacteria, and enzymatic activities (α -amylase, carboxymethyl-cellulase, xylanase) in sheep fed a diet with basal Mn content (34 mg/kg dry matter; control) and sheep fed daily with either inorganic Mn supplement (183 mg /kg dry matter) or organic Mn (184 mg/kg dry matter). Sheep were fed with basal diet of ground barley and hay (300 + 800 g/kg DM per animal and day) in two doses. The rumens of experimental sheep were harbored with ciliates of the genus Entodinium, Epidinium, Diplodinium, Eudiplodinium, Dasytricha and Isotricha. The rumens of two sheep of group fed with organic Mn supplement were not harbored with ciliates. We have not observed significant differences in the total and generic number of ciliates, as well as total number of bacteria between groups. Alpha-amylase catalytic activities were significantly lower in sheep supplemented with inorganic Mn. CM-cellulase catalytic activities were lower in both Mn-supplemented groups. Xylanase catalytic activities were not influenced. Alpha-amylase specific catalytic activities were decreased in group with inorganic Mn. CM-cellulase specific catalytic activities were increased in both Mn-supplemented groups. Xylanase specific catalytic activities were not influenced. Very low protein concentrations in rumen samples of defaunated animals resulted in different values of enzymatic activities in comparison to faunated animals. Rumen bacteria dominated in cellulolytic and amylolytic activities in contrast to ciliates which dominated in xylanolytic activities. We can conclude that higher intake of inorganic and organic manganese influenced the specific groups of bacteria with probable impact on rumen cellulose and starch digestibility.

Keywords: rumen, sheep, ciliates, bacteria, α -amylase, carboxymethyl-cellulase, xylanase

Acknowledgement: This research was supported by the Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences (VEGA 2/0009/14).

PROTEOME ANALYSIS OF PORCINE EMBRYOS FROM THE PERIOD OF EARLY PREGNANCY – PRELIMINARY STUDIES

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ABSTRACT

Porcine blastocysts undergo extensive morphological changes between days 10 and 16 of gestation. Pregnancy recognition in pigs occurs between days 12 and 13 when blastocysts undergo rapid elongation. The porcine embryos remain free-floating in the uterine lumen until days 13-14 of gestation when they appose to attach to the uterine luminal epithelium. The aim of the current study was to compare the proteomic profiles of porcine embryos harvested from the time of maternal recognition of pregnancy (days 12-13) with proteomic profiles of the embryos from the time of peri-implantation period (days 15-16). Total proteins were extracted from embryos (n = 4, days 12-13 and n = 4, days 15-16) directly into a rehydration buffer. Tissue extracts were used for two dimensional gel electrophoresis (2DE). Then, MALDI TOF mass spectrometry was performed. To identify the proteins ProFound search engine was used. We found 5 and 107 proteins which were unique for embryos harvested during days 12-13 and 15-16 of pregnancy, respectively and we identified 24 proteins. Because of limited databases within 5 determined unique spots from days 12-13 it was not possible to identify any proteins from this period of pregnancy. Among the identified proteins afadin, centromere protein J, human BRCA1 homolog were unique for embryos from days 15-16. We suppose that obtained results may enable to identify protein markers uniquely expressed in embryos during important days of early pregnancy and to define protein factors characteristic for them. However, further research is needed to confirm/establish the importance of identified proteins for the support of early pregnancy.

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ASSESSMENT OF 17-B-ESTRADIOL LEVEL IN BLOOD PLASMA OF RABBITS AFTER SHORT-TERM AMYGDALIN THERAPY

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ABSTRACT

Cyanogenic glycoside as an amygdalin was detected in apricot kernels, bitter almonds and peach, plum, pear and apple seeds. Previous studies related to amygdalin, have primarily focused on its purification, antitumor mechanism, determination in plants, as well as on its toxicity caused by the release of cyanide. Amygdalin itself is non-toxic, but its HCN production decomposed by some enzymes is toxic substance. Previous studies describe the effects of natural compound amygdalin on female and male reproductive systems focused on process of steroidogenesis, spermatozoa motility and morphological abnormalities of spermatozoa. Previous studies examined the effects of natural compound amygdalin on female reproductive system concentrated on secretion activity of porcine ovarian granulosa cells *in vitro*. Objective of this *in vivo* study was to evaluate the potential effect of amygdalin (as a bioactive component of apricot seeds) on the plasma levels of 17- β -estradiol, using rabbits as a model organism. Adult rabbit males (n=20, age: 150 days) were divided into to 5 groups: control without amygdalin addition, 2 experimental groups received intramuscular injection of amygdalin (at dose 0.6 and 3.0 mg/kg b.w.) during 2 weeks period and other 2 experimental groups were fed by apricot seeds (at dose 60 and 300 mg/kg b.w.) mixed with feed during the same period. After this period the blood was collected and plasma levels of the steroid hormone was assayed by ELISA. Our results showed no significant ($P \geq 0.05$) differences in 17- β -estradiol plasma level after short-term period of amygdalin application in comparison to untreated control group. In conclusion, amygdalin, like a reportedly anticancer agent, did not affect the plasma steroid hormone level of 17- β -estradiol after short-term exposure.

Keywords: amygdalin, 17- β -estradiol, rabbit.

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EFFECT OF A-TRICHOHECENES ON STEROID SECRETION BY PORCINE OVARIAN GRANULOSA CELLS AFTER 12 HOURS EXPOSURE *IN VITRO*

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ABSTRACT

T-2 and HT-2 toxins are type A trichothecene mycotoxins produced by *Fusarium (F.) poae*, *F. sporotrichioides*, *F. kyushuense* and *F. langsethiae*. HT-2 toxin is furthermore described as the main metabolite after T-2 toxin application. T-2 toxin has been found to contaminate foods, animal foods and agricultural products. Surveys for T-2 toxin and HT-2 toxin revealed their presence in grains such as wheat, maize, oats, barley, rice, beans, and soya beans as well as in some cereal-based products. The aim of the *in vitro* study was examine the effect of A-trichothecenes on secretion activity of porcine ovarian granulosa cells (GCs) *in vitro*. No significant changes in progesterone release by porcine ovarian GCs after 12 hours long exposure of T-2 toxin at various doses 0.01, 0.1, 1, 10 and 100 ng/ml were observed. On the other hand, significant ($P < 0.05$) stimulation of progesterone secretion by GCs after addition 100 ng.ml⁻¹ (but not at 0.01, 0.1, 1, 10 ng/ml) of HT-2 toxin was detected. 17- β -estradiol release by ovarian GCs was not significantly affected by T-2 toxin at the doses 0.01, 0.1, 1, 10, 100 ng/ml. Similarly, no significant ($P > 0.05$) changes in 17- β -estradiol release by HT-2 addition at the doses 0.01, 0.1, 1, 10, 100 ng/ml were found. Results in this study showed that trichothecenes as T-2 toxin and HT-2 toxin have ability to modulate ovarian steroidogenesis depending on used dose. Currently, occurrence of mycotoxins in food and feed is a worldwide problem and therefore study of these toxins could be beneficial for better understanding of mechanism of their toxic effects in organism.

Keywords: T-2 toxin, HT-2 toxin, ovaries, steroidogenesis.

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**POTENTIAL EFFECTS OF GREEN TEA EXTRACT ON APOPTOTIC AND
PROLIFERATIVE MARKERS OF PORCINE OVARIAN GRANULOSA CELLS
IN VITRO**

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ABSTRACT

One of the most popular beverages worldwide is a green tea. Green tea and its bioactive components have been extensively studied for their beneficial effects, including cancer chemoprevention, anti-cancer properties and such as promising agent to prevent obesity and diabetes. In the present study, we examined the potential effects of green tea extract at the selected doses (0,1; 1; 10; 100 and 200 µg/mL) for 24 hours on basic regulators of cellular proliferation (PCNA, cyclin-B1) and apoptosis (caspase-3, p53) in porcine ovarian granulosa cells *in vitro*. The detection of proliferative and apoptotic markers was assessed by immunocytochemistry. No significant ($P \geq 0.05$) differences in PCNA, as well as cyclin-B1 in ovarian granulosa cells were noted after administration of green tea extract, compared to control without addition. However, addition of green tea extract to cultured ovarian cells increased the percentage of apoptotic cells (caspase-3 positive) only at the highest used dose (200 µg/mL). Similarly, the percentage of cells containing other apoptotic marker p53 was statistically ($P \leq 0.05$) increased after green tea extract addition at the dose 200 µg/mL, but not at lower doses. Data obtained from our *in vitro* study indicate that green tea did not affect the markers of cell proliferation in porcine ovarian granulosa cells. On the other hand, apoptosis, as a programmed cell death, can be induced in ovarian cells by bioactive components of green tea at dose-dependent manner.

Keywords: green tea, ovaries, proliferation, apoptosis.

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THE EFFECT OF HYPERTHERMIA ON THE OCCURRENCE OF APOPTOTIC POSITIVE CELLS IN THE TESTICULAR TISSUE

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ABSTRACT

High temperature - hyperthermia, is considered to be the most stressful factor influencing various biological systems. Organs of urogenital system belong to the most dynamic ones in the animals' organism and they are highly sensitive to different environmental factors. The aim of this work was to study the effect of high ambient temperature (34, 36 and 38 ° C for 28 days) on the occurrence of apoptosis in testes of rabbits (in vivo). Methodology: For in vivo experiments broiler rabbits were used. Samples from the organs of the animals were histologically analysed and eventual apoptotic changes were evaluated on the testicular tissue using TUNEL analysis. Results: The pathological-anatomical autopsy of parenchyma of the organs showed no signs of pathological damage, but the histological preparations of individuals exposed to the temperature of 38°C led to significant damage of the basic testicular structures. The negative effect of hyperthermia was also evident by statistically significant ($P < 0.001$) presence of apoptotic positive cells, number of which was increasing with increasing temperature. We confirmed the increase apoptotically positive cells (16.80 ± 6.51) at $800\ 000\ \mu\text{m}^2$ area already at 34 ° C of testicular tissue of rabbits and their number has gradually increased with the increasing temperature of 36 ° C and 38 ° C (33.90 ± 10.1 and 61.80 ± 22.2). Conclusion: The study found a statistically significant negative effect of high ambient temperature (34°C, 36°C, 38°C applied for 28 days) on seminiferous tissue which may significantly alter the spermatogenesis and thus ultimately the ability of male fertility.

Keywords: hyperthermia, testes, apoptosis, rabbits

Acknowledgments: The work was supported by research project VEGA 1/0857/14 and APVV-0304-12.

THE PREVALENCE OF CARDIOVASCULAR RISK FACTORS IN MIDDLE-AGED ADULTS HOSPITALIZED IN CARDIOCENTRE NITRA

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ABSTRACT

Cardiovascular diseases (CVD), including coronary heart disease (CHD) and stroke, currently represent the major causes of mortality and morbidity all over the world. The aim of this work was evaluating the prevalence of risk factors for cardiovascular disease in middle-aged adults hospitalized in Cardiocentre Nitra. Methodology: We evaluated the prevalence of modifiable risk factors for cardiovascular disease (CVD) and the relationship between dietary habits and lipid profile in a group of 231 randomly selected middle aged patients hospitalized in the Cardiocentre Nitra, of which 71 were women (30.74%) and 160 men (69.26%). Respondents were 45-59 years old, where the average age of women was 55.33 and the average age of men was 53.87 years. The following parameters were evaluated: total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides and blood glucose, because these parameters are considered to be one of the major risk factors for cardiovascular diseases. We used the diagnostic criteria of the metabolic syndrome (MS) according to ATP III criteria for the evaluation of the risk factors, as the MS brings together components that are associated with the risk of CVD, in particular, with the risk of coronary heart disease. Results: The most frequent risk determinants among the respondents were overweight and obesity, when BMI ≥ 25 was recorded in 71.83% of women and 81.25% of men. Up to 52.11% of women and 45.63% of men in the study group had blood pressure higher than $\geq 130/85$ mmHg. The cholesterol level higher than 5.2 mmol/L was recorded in 26.76 % of women and 18.76% of men. The triglycerides ≥ 1.7 mmol/L were recorded in 14.08% of women and 25.00% of men and fasting blood glucose levels ≥ 5.6 mmol/L were recorded in 43.66% of women and 53.13% of men. There was no statistically significant difference ($P > 0.05$) in the occurrence of elevated levels of cholesterol, triglycerides, blood pressure and blood glucose between both genders. The combination of several factors, high cholesterol, hypertension and smoking markedly enhances the risk of cardiovascular accidents. Simultaneous occurrence of two of risk factors such as BMI ≥ 25 with impaired fasting glucose levels ≥ 5.6 mmol/L was present in 28.17% of women and 26.88% of men. Up to 16.90% of women and 16.25% of men had three risk factors present (BMI ≥ 25 , fasting blood glucose ≥ 5.6 mmol/L, blood pressure $\geq 130/85$ mmHg). When assessing the dietary habits we have seen frequent consumption of meat and meat products, insufficient consumption of fish and milk. Fruits and vegetables are indeed a part of daily diet of almost all patients, but in serving one or two pieces a day, which is compared with insufficient dietary recommendations. Conclusion: Through the research carried out, we recorded high incidence of risk factors, in particular overweight and obesity, high blood pressure and high prevalence of hypercholesterolemia and hypertriglyceridemia.

Eighty percent of cardiovascular accidents could probably be avoided by lifestyle adjustment (weight control, smoking abstinence, physical activity, and a healthy diet), together with proper management of clinical and biological risk factors.

Keywords: cardiovascular disease, risk factor, dietary habits, lipid profile, body mass index

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SEASONAL VARIATIONS OF HEPATIC ENZYMES AND BILIRUBIN IN BLOOD SERUM OF SHEEP (*OVIS ARIES*)

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ABSTRACT

Sheep are among the oldest domesticated livestock. The great importance of keeping them is mainly in their performance (meat, wool, milk, leather). The objective of this work was to examine the possible effect of seasonality on selected levels of liver enzymes (AST, ALT) and bilirubin. Hepatic and enzyme profiles are mainly used in the diagnosis of functional disorders of the liver associated with myopathy, or bone and cardiac muscle diseases. Specificity of monitored enzymes is following: AST - heart, liver, muscles; ALT - muscle, liver. The serum bilirubin is influenced by functional changes of hepatic parenchyma, bile ducts and blood. The increase is typical for some bacterial, viral, parasitic diseases and intoxication.

The blood samples were collected from 10 sheep (Merino) in two intervals (spring and winter). The blood serum was separated using centrifugation at 3000 rpm for 20 minutes. Parameters were measured by semi-automated analyzer RX Monza (Randox Laboratories Ltd., United Kingdom) using commercial kits DiaSys (Diagnostic Systems GmbH, Germany). Statistical analysis was performed with statistical software SAS. To compare results, the analysis of variance t-test was used.

The average annual value of AST in the group of sheep was $2.695 \mu\text{kat.l}^{-1}$, level of ALT was $0.32 \mu\text{kat.l}^{-1}$ and bilirubin $9.71 \mu\text{mol.l}^{-1}$. Significant differences between the groups based on seasonality were observed, AST at the highest level of significance ($p < 0.001$) and ALT at the intermediate level of significance ($p < 0.01$). Differences between groups in bilirubin were not statistically significant. In conclusion, all the monitored parameters were increased in group 1 (spring) when compared to group 2 (winter), which could be caused by the change of diet (environmental contaminants).

Keywords: sheep, merino, AST, ALT, bilirubin

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INTERNAL ENVIRONMENT CHANGES RELATED TO SOME CIVILISATION DISEASES

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ABSTRACT

Several scientific studies in recent years confirm the importance of evaluation the level of selected parameters of the internal environment as important markers of metabolic manifestation in relation to lifestyle diseases such as cardiovascular and oncological diseases. The change in the acid-base balance, related to the acidity of the body due to excessive intake of acid-forming substances in food, is well discussed problem. Some authors in their work argue, that the blood which has its strictly defined pH range, has got excellent compensation mechanisms in order to keep it within these borders. Regulation of blood pH is so effective that even exogenous factors including nutritional factors can't influence it. On the other hand, some authors remind the fact that, while the pH of the blood remains relatively constant, in order to compensate possible changes in acid-base balance in the blood, ionic composition dis-balance occurs often in various tissues of the body with a consequent negative effect on the physiology of the cells. According to Brönsted theory, acid is a substance which is a proton donor and a base is the acceptor. In connection with the evaluation of the biological effect of the metabolic process would appear Lewis theory to be more consistent - the acid is a substance capable of binding pairs of electrons and other particles on the contrary the principle of the free electron pair donor. In this context, there is considerable similarity between the redox reactions of the organism, Lewis acids and bases conception and the effect of antioxidants in the elimination of oxidative stress. This way, it is often explained by different metabolic effects of some so-called acidifying substances presented in food which are interesting in terms of their impact in the pathogenesis of civilization diseases.

Keywords: acid-base balance, acid, base, internal environment, civilization diseases

THE CONTENT OF REDUCED GLUTATHIONE IN THE SEMEN OF THE M91 BREED RABBITS NEW ZEALAND AFTER ADMINISTRATION OF ACRYLAMIDE

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ABSTRACT

Acrylamide was found in food, mainly heat-processed potato products, bread, cereals and coffee. Extensive studies revealed that this compound may cause neurotoxic, genotoxic and cytotoxic effects and has been classified as probable human carcinogen by IARC. Apart from human diet, another source of acrylamide is smoking. Acrylamide was found to react with glutathione giving rise to an *S*-conjugate of acrylamide. Acrylamide toxicity has been extensively studied in recent years including toxicokinetic studies and cellular effects of treatment with acrylamide in rodent and human cell lines. In the cells acrylamide is metabolized either enzymatically or non-enzymatically by conjugation with glutathione (GSH). The possible mechanisms of acrylamide toxicity were also investigated; one of them may be the enhancement of cellular oxidative stress by inducing the reactive oxygen species generation and disturbing redox balance by depleting the cellular pool of glutathione. Oxidative stress may affect cell function and contribute in triggering cell death. To of research material (semen rabbit) was added eight different concentrations of acrylamide. As a result of of the experiment it was observed statistically significant decrease in the concentration of reduced glutathione observed in two trials. The highest concentration of acrylamide did not differ much from the control group.

Key words: acrylamide, sperm, rabbit, reduced glutathione

EFFECT OF RESORCINOL ADMINISTRATION ON MOTILITY PARAMETERS AND MORPHOLOGY OF RABBIT SPERMATOZOA IN VIVO

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ABSTRACT

Reproduction is one of the first areas where biotechnological procedures and methods were used. In present study we have analysed the effect of resorcinol per os on the sperm motility parameters and sperm morphology of rabbit in vivo in different periods of the experiment (at the beginning, after 10 applications, after 20 applications). The experimental group we served resorcinol, which we prepared by dissolving 1250 mg resorcinol in 5 ml of tap water. Solution we applied using a syringe the mouth at a dosage of 50 mg per 1 kg body weight of the rabbit. Experimental group were compared with the control group, which wasn't give resorcinol. Motility parameters – the motility and progressive motility of sperm we evaluated using CASA analysis immediately after arrival at the laboratory.

Assessment of the motility parameters of sperm, we determined that the highest motility (92.52%) and progressive motility (83.17%) were recorded in the experimental group given the resorcinol 20 days. These differences were statistically significant. Achieved values compared to the control group were characterized by significant upward trend in all sampling periods ejaculate. Based on our results, we can say that the effect of resorcinol on the parameters of motility has a positive effect, which causes an increase in sperm motility in rabbits, thereby increasing their fertilizing capability.

Assessment of the morphological analysis, we found that the percentage occurrence of morphologically changed spermatozoa in the experimental group at the beginning, after 10 applications and after 20 applications was a higher than in control group. From the total number of morphologically abnormal spermatozoa in the experimental group represented in all the periods of collection ejaculate the highest percentage representation broken flagellum, while in the control group it was loop twisted flagellum. Despite of higher amount of morphologically abnormal spermatozoa in the experimental group compared with the control group had resorcinol no significant effect on incidence of morphologically changed spermatozoa of rabbits.

Keywords: spermatozoa, resorcinol, ejaculate, motility, CASA, morphology.

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THE EXPRESSION OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTORS (PPARS) IN THE PORCINE MYOMETRIUM

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ABSTRACT

In this study we demonstrate the presence of three isoforms of peroxisome proliferator activated receptor (PPAR) mRNA in the porcine myometrium. We also evaluate a pattern of genes expression in the tissue of gilts on days 10-12 and 14-16 of the estrous cycle or pregnancy. PPARs represent a family of nuclear receptors. They are transcription factors that are mostly involved in the regulation of processes related to fatty acid and glucose metabolism. Their expression has been also demonstrated in the ovary (granulosa and luteal cells) and the uterus of rodents, dogs, cows and pigs. So far, it was reported that PPARs regulate steroidogenesis in the corpus luteum and endometrium of the pig.

Material and Methods

The study was performed on crossbred pigs (100 kg, 7 month-old) from a commercial farm. The animals were divided into the experimental groups (n=5) of cyclic (days 10-12 and 14-16) and pregnant (days 10-12 and 14-16). During slaughter tissues were dissected and transported to the laboratory on ice in sterile PBS. In the laboratory the myometrium was separated from the endometrium, washed with PBS and snap frozen at -80°C for total RNA isolation and real-time RT-PCR quantification.

Results

We noted mRNA presence of all PPAR isoforms (α , β , γ) in the porcine myometrium. The mRNA level of α and β isoforms was significantly ($p < 0.05$) higher on days 14-16 of the estrous cycle (late-luteal phase) than on days 10-12 (mid-luteal phase). There were no changes in the expression of γ isoform during the estrous cycle. During pregnancy, only PPAR γ mRNA level was higher ($p < 0.05$) on days 14-16 (time of implantation) than on days 10-12 (maternal recognition of pregnancy). The remaining PPAR isoforms did not fluctuate during the analysed stages of the estrous cycle and pregnancy.

Conclusion

We found that all isoforms of PPAR are expressed in the porcine myometrium. The changes in their expression profile suggest that the receptors can be involved in the regulation of reproductive processes during the estrous cycle (α and β isoforms) and periimplantation (γ isoform). To date, the role of PPARs activation in the regulation of physiological processes in porcine myometrium has not been investigated and further analyses are required.

INTERACTION OF MTOR PATHWAY AND HIPPOCAMPAL ENDOGENOUS OPIOID SYSTEM UNDER HYPERGLYCEMIA

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ABSTRACT

Metabolic changes associated with impaired glucose conditions nowadays are defined as diseases of civilization. Hyperglycaemia is listed as one of the major etiopathogenic factors for neurodegenerative disorders such as Alzheimer's (AD), Parkinson's (PD) and Huntington's disease (HD). Main subcortical structure in the central nervous system infected by neurodegenerative process is hippocampus which is involved in learning, neuronal plasticity and memory formation. In diabetic individuals early symptoms of dementia are associated with elevated blood glucose level, and also in AD named "type III diabetes" glycemic abnormalities might be factors leading to the development of neurodegeneration. mTOR pathway, which takes part in maintaining neuronal homeostasis is susceptible on numerous signals from neural systems, including the endogenous opioid peptides. The level of endogenous opioids, particularly Met-enkephalin, is closely correlated with glucose metabolism. Thus the aim of the study was to investigate the interaction between mTOR pathway and opioid system in hippocampus of hyperglycemic rats.

The experiment was performed on 40 male Wistar rats weighing about 250-280 g. The animals were housed in cages of 3-4 animals, under standard conditions, with free access to water and commercial feed. Animals were divided into four groups: I - control (normoglycemia), II - hyperglycemia, III - normoglycemia + rapamycin (mTOR inhibitor), IV - hyperglycemia, IV - hyperglycaemia + rapamycin. Hyperglycemia was induced using a single intraperitoneal injection of streptozotocin (STZ 55mg / kg). Rapamycin was administered in three intraperitoneal injections (1 mg / kg /day).

Streptozotocin-induced hyperglycemia caused IL-6 increase in hippocampus, and rapamycin treatment resulted in decrease of cytokine level what confirmed its anti-inflammatory properties. In hyperglycemic rats 5-fold increase in Met-enkephalin concentration was noted. GFAP and Raptor mRNA expression was raised while Rictor mRNA expression decreased. In normoglycemia rapamycin administration stimulated Met-enkephalin synthesis while in hyperglycemia opioid concentration was diminished. Unexpectedly, rapamycin did not affect the mRNA expression of mTOR subunits - Raptor and Rictor in both conditions, however reduction of mRNA GFAP expression was observed.

mTOR pathway inhibition effect on opioid system in hippocampus was demonstrated under normoglycemia and impaired glucose conditions. Hyperglycemia might cause stimulation stress-dependent signaling pathways which strongly affect mTOR activity.

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EFFECT OF ASCORBIC ACID INJECTED IN OVO ON THYROID AND ADRENAL HORMONES OF CHICKEN EXPOSED ON HYPERTHERMIA DURING HATCHING

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ABSTRACT

Chick embryos during hatching are exposed to stress caused by effort-related with the leaving the eggs and microclimatic conditions prevailing in hatcher. Seems that the in ovo injection of antioxidative substances, such as ascorbic acid, can attenuate its effects.

The aim of study was to examine the influence of the ascorbic acid the in ovo injected on concentration of the hormones of adrenal cortex and the medulla in blood plasma of chicks exposed to heat stress during hatching.

The 480 hatching eggs of broiler line F15 were supplemented in ovo by ascorbic acid at a dose of 0 or 2,5 mg/egg in 18th day of incubation. Embryos/chicks were incubated in t=37,2°C and RH=60% (control group) or t=38,5°C and RH=65% (experimental group).

There was found that the maximum plasma concentration of corticosterone was observed in newly hatched chicks but the catecholamines before the start of hatching process. Heat stress disturb the secretion these hormone. However in heat stress conditions the ascorbic acid in ovo administrated may regulate the concentration of adrenaline and noradrenaline in the initial stage of the hatching. Moreover, thyroid hormones level in embryos`/chicks` blood was depended on stage of hatch. Ascorbic acid seemed to have the regulating properties TH concentrations in chicken embryos exposed to hyperthermia during hatching .

Ascorbic acid injected in ovo can regulate the concentration of adrenal and thyroid hormones in blood plasma of chicks exposed on hypertermia during hatching.

THE EFFECT OF THE SHEEP MILKING GROUP ORDER BY ENTERING INTO MILKING PARLOUR ON THEIR MILKABILITY

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ABSTRACT

The aim of the present investigation was to evaluate the effect of the order of the milking group in which the ewes enter the milking parlour on their milkability. The ewes were milked in one-platform milking parlour with 24 stall. During six evening milkings, ewes were rated according to milking group order in which they were milked. In all, the sheep were milked in 15 milking groups. Sheep entering the parlour in first milking group achieved 15 points and sheep of each next milking group one point less, i.e. last group achieved 1 point. Only the ewes with highest and lowest average number of points were included in the analysis. Ewes, which had the highest average number of points were arrangement to first group (FG, N=19) and with the lowest average points to last group (LG, N=29). The recording of milkability parameters was performed by the equipment for graduated electronic recording of milk level in jar at one second intervals. After each milking of sheep, the individual milk samples were collected from the jar for analysis of composition and somatic cells count. Milk yield, machine milk yield (for both $P<0.05$), maximal milk flow rate ($P<0.001$) and latency time ($P<0.01$), were higher in FG than SG. The occurrence of milk flow with ejection reflex was higher in sheep of FG (69%) than in LG sheep (28%). The differences in milk composition between FG and LG were found out only in solids-not-fat ($P<0.05$).

Thus, it seems that ewes which enter the milking in early milking groups have better parameters of milkability than those milked in later groups. Therefore the reasons affected the order entering of ewes into parlour should be evaluated more in detail.

Keywords: Sheep, milking, order of the milking group, milkability

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STEROIDOGENIC ACTIVITY OF OVIDUCT IN GILT DURING THE ESTROUS CYCLE

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ABSTRACT

Steroid hormones act as a potent regulators of cycling changes within the female reproductive tract. We hypothesize that oviducts of gilts from various days of the estrous cycle may be a source of estradiol-17 β (E2), androstendione (A4) and progesterone (P4). Oviductal production of steroids may supplement the amount of steroid hormones produced by uterus and in this manner may enhance an action of those of ovarian and uterine origin in order to optimize tubal milieu.

Steroid synthesis requires activity of specific enzymes: 3 β -hydroxysteroid dehydrogenase/ Δ -5-4 isomerase enzyme (3 β HSD) and aromatase P450 (P450arom) - product of CYP19 gene. 3 β HSD converts pregnenolone into P4 or androstendione (A4). P450arom converts A4 and testosterone (T) into E2. Thus, in this study, the expression of 3 β HSD and CYP19 mRNAs in ampulla and isthmus of oviducts harvested on Days 2-3 (n=5), 10-11 (n=5), 12-13 (n=5), 15-16 (n=5) and 18-20 (n=5) of gilts during the estrous cycle were determined. Moreover, in oviductal fluid concentrations of E2, P4 and A4 were studied.

The expression of 3 β HSD mRNA in ampulla was significantly lower on Days 10-11 and 15-16 of the estrous cycle in comparison to Days 2-3, 12-13 and 18-20. Expression of 3 β HSD in isthmus was significantly lower on Days 12-13 and 15-16, increased on Days 10-11 and 2-3 and was the highest on Days 18-20 of the estrous cycle. The expression of CYP19 in ampulla and isthmus was low on Days 2-3, 10-11 and 12-13 of the estrous cycle, then increased on Days 15-16 and was the highest on Days 18-20 in ampulla and decreased in isthmus at the end of the estrous cycle (P<0.05). Concentration of E2 in oviductal fluid was low during studied days of the estrous cycle with exception of Days 12-13, when this concentration significantly increased. Concentration of P4 was high during Days 2-3 and it decreased on other Days of the estrous cycle (P<0.05). Concentration of A4 was significantly higher on Days 2-3 and 18-20 vs. Days 10-11, 12-13 and 15-16 of the estrous cycle. In conclusion 3 β HSD and CYP19 mRNAs are present in ampulla and isthmus of gilts' oviducts during the estrous cycle. Moreover, oviducts have a potential for production of E2, P4 and A4. The level of 3 β HSD and CYP19 gene expression and the production of E2, A4 and P4 and as well, are differentiated within days of the estrous cycle. Results demonstrate that steroid hormones, produced by oviducts, may potentially affect reproductive functions of female during the estrous cycle.

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THE EFFECT OF MISTLETOE (*VISCUM ALBUM*) EXTRACT ON PORCINE OVARIAN FUNCTIONS

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ABSTRACT

Viscum album (mistletoe) is a plant parasite, which has been used in traditional and alternative medicine for many years. The positive biological effect of mistletoe has become a key factor of anticancer, antimycobacterial, antiviral, secretory activity induction and immunomodulatory processes. The aim of our study was to examine the effect of mistletoe extract at different doses (0,1; 1; 10; 100 and 1000 µg/mL) for 24 hours on cellular regulators of proliferation and apoptosis in porcine ovarian granulosa cells *in vitro*. The detection of proliferative markers (PCNA and cyclin-B1) and apoptotic marker (caspase-3) was analysed by immunocytochemistry. Administration of mistletoe extract to cultured ovarian granulosa cells did not cause significant ($P \geq 0.05$) changes in presence of proliferative peptide PCNA, as well as in presence of cyclin-B1 in ovarian granulosa cells. Similarly, presence of apoptotic marker caspase-3 in porcine granulosa cells was not significantly ($P \geq 0.05$) affected by experimental application of mistletoe. Results from this *in vitro* study showed that mistletoe did not affect the presence of substantial cellular regulators of proliferation and apoptosis in cultured granulosa cells and thus the modulatory activity of mistletoe was not confirmed.

Keywords: ovarian granulosa cells, intracellular regulators, proliferation, apoptosis,

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THE EFFECT OF FRESH YOLK ON SHORT TURKEY SEMEN STORAGE

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ABSTRACT

Optimization of the management of turkey breeder males includes the need for efficient methods of semen storage. Since the late 1930's, egg yolk was considered an essential additive to maintain viability of cooled frozen sperm. Among 9000 species of birds throughout the world, domestic chickens (*Gallus gallus domesticus*) egg yolk is a common component as a cryoprotectant agent for spermatozoa storage in different animals. There have also been numerous reports that egg yolk from avian species such as the turkey, quail, duck or chicken has different combinations of fatty acids, phospholipids and cholesterol, which could result in different cryopreservation effects on the spermatozoa. Semen of the domestic turkey usually cannot be stored for longer than 6 h without a loss in fertilizing capacity, even when oxygenated and stored with the appropriate diluents at a reduced temperature, generally 2 – 5 °C. The present study was undertaken to investigate the influence of turkey egg yolk on spermatozoa motility parameters during *in vitro* cultivation at 41°C. The experiment was carried out in cooperation with the poultry farm Branko a.s. located at Cabaj, Slovakia. Semen was obtained by penel massaging of the turkeys of the line Big 6 (BUT – British United Turkeys Ltd., Chester, United Kingdom). Semen samples were a mixture of several groups of identical individual turkeys. Row semen was diluted in a ratio of 1 part of semen and 100 parts of physiological solution (Sodium chloride 0.9% Braun, B. Braun Melsungen AG, Melsungen, Germany) – control sample – KMT. At the same ratio the semen was diluted with five different turkey egg yolk solutions: 5% - MT5, 10% - MT10, 15% - MT15, 20% - MT20 and 30% - MT30. Samples were cultured at 41 °C and recorded at 7 time periods: 0, 1, 2, 3, 4, 5 and 6 hours. The experiment was realized in 6 replicates. Each of thus prepared samples were evaluated using a Computer Assisted Semen Analyzer (CASA) system – Sperm Vision (Minitub, Tiefenbach, Germany) equipped with a microscope (Olympus BX 51, Japan) to assess the spermatozoa motility. As a result motility (MOT/%), progressive motility (PRO/%) and beat cross frequency (BCF/Hz) were evaluated. In all total spermatozoa motility (MOT) experimental samples and in all times significantly higher values were recorded comparison to the control group ($p < 0.001$). The highest percentage of (MOT) 84.93 % was detected in the sample MT5 at the beginning of *in vitro* culture. Samples results for progressive motility provided better use in practice condition. In all experimental groups significantly higher values in comparison to the control were measured ($p < 0.001$) even after four hours of incubation. Results of beat cross frequency showed similar values as progressive motility.

These results confirm that turkey fresh yolk as a supplement in extender is useful in short liquid storage and 41°C.

Keywords: Turkey, Yolk, Spermatozoa, Motility, CASA

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EXPRESSION OF AQP1, 5 AND 9 IN THE PORCINE OVARIAN FOLLICLES DURING THE ESTROUS CYCLE

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ABSTRACT

Aquaporins (AQPs) are a family of membrane channel proteins which facilitate bulk water transport. To date, 11 isoforms of AQPs have been reported to be expressed in the female and male reproductive systems. Our previous study have shown that expression of AQP1 and AQP5 protein was the highest on Days 18-20 of the estrous cycle. AQP9 protein expression did not change during the estrous cycle. The purpose of this study was to determine changes in the expression of AQP1, 5 and 9 within the pig ovary during different stages of the cycle in pigs. Tissue samples were recovered from mature gilts at the early (Days 2-4), middle (Days 10-12), and late (Days 14-16) stage of the luteal phase and the late (Days 18-20) stage of the follicular phase of the estrous cycle. The expression of AQP 1, 5 and 9 was determined by Real Time PCR analysis. All data were analyzed by one-way ANOVA and least significant difference (LSD) post hoc test and were reported as the means \pm SEM from five independent observations. The results demonstrated that the highest expression of AQP5 and AQP9 was on Days 10-12 in granulosa cells. The expression of AQP1 was higher in middle and late stage of luteal phase and in follicular phase but significantly increase only on Days 18-20. In theca cells expression of aquaporins was varied in relation to the granulosa cells. Expression of AQP1 was higher on Days 14-16 and 18-20. During the follicular phase expression of AQP5 change significantly in theca cells. The expression of AQP9 did not change significantly between all days of the estrous cycle. In conclusions, studied AQPs are present in different cells populations of the follicles and may have an important role for the follicular development in pigs.

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DETERMINATION OF THYMOL IN BROILER PLASMA BY SOLID - PHASE MICROEXTRACTION - GAS CHROMATOGRAPHY – MASS SPECTROMETRY AFTER ADDITION THYME ESSENTIAL OIL TO DIET

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ABSTRACT

Compounds derived from plant feed additives can affect several physiological processes in the animal organism. It has been already shown that thyme essential oil (EO) can improve health, whereas substances specific for thyme EO can interact with animal and human cells in vitro. The monoterpen thymol often accounts for more than half of the essential thyme oil.

One-day old non-sexed broiler chickens were fed a basal diet supplemented with different concentrations of thyme EO in which thymol achieves around 48%. Forty-eight chickens were divided randomly to six groups with thyme EO in diet in amount 0.01 %, 0.02 %, 0.03 %, 0.04%, 0.05% and 0.1 %. Concentrations of thymol in 1 ml of blood plasma were analysed after cleaving thymol from thymol glucuronide and thymol sulphate during incubation using the enzyme β -glucuronidase. The amount of thymol was determined by solid - phase microextraction (SPME) prior to gas chromatography coupled to mass spectrometry.

Thymol originating from thyme EO was found in the blood plasma from all groups of broiler chickens indicating the efficient transport of thymol to plasma after feeding a diet with thyme EO. Significant differences in the concentrations of thymol in the first and fifth group were observed. Significant increasing of thymol concentration in groups of animals with 0.05 and 0.1% of EO in their diet in comparison with other groups were observed.

The distribution of thymol to target organs and tissues after absorption in digestion system is limited by concentration of thymol in plasma. Levels of thymol in plasma can be affected by absorption in digestive system. Fullness of gastrointestinal tract and efflux proteins located in the intestinal wall can influence the absorption of thymol. Desired effect of thymol in tissues is influenced by its amount in plasma.

Keywords: thyme essential oil, thymol, plasma

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INVESTIGATION OF THYME ESSENTIAL OIL COMPOUNDS IN TISSUES AND BLOOD PLASMA OF BROILERS

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ABSTRACT

It was shown that natural plant products used as feed additives in animals can improve health and performance in animals. Animal health can be influenced by plant substances during biochemical reactions at the cellular level of tissue. Compounds of plant derived feed additives are probably absorbed in cranial segments of gastrointestinal tract and transferred by blood to organs.

Considering that thyme essential oil (TEO) can influence health in broiler chickens, presence of its compounds was investigated in intestine, plasma and organs of broiler chickens after feeding diet enriched with 0.1% TEO by using solid - phase microextraction (SPME) followed by gas chromatography coupled to mass spectrometry.

Our results indicate specifically which compounds from TEO were absorbed in gastrointestinal tract and transferred by blood to tissues. Thymol and other compounds of TEO detected in our study can promote suggestion, that plant bioactive compounds from feed additives are absorbed in digestion system and transported to target tissues, where biological activity of observed substances can be performed.

Keywords: feed additives, thyme essential oil, bioactive compounds, broiler chickens

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HOMOLOGY MODELING OF LIGAND BINDING DOMAIN OF THE PORCINE ARYL HYDROCARBON RECEPTOR (AHR-LBD) AND IN SILICO ANALYSIS OF THE INTERACTIONS BETWEEN AHR AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)

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ABSTRACT

INTRODUCTION: The aryl hydrocarbon receptor (AhR) is a nuclear transcription factor belonging to the bHLH-PAS (basic helix-loop-helix/Per-Arnt-Sim) gene family involved in the regulation of a variety of developmental and physiological events. The AhR is a biological sensor for some environmental chemicals and has been primarily studied in the context of the toxicity of dioxin-like compounds, particularly 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most potent activator of the AhR. Acute effects by TCDD altered sexual behavior, caused endocrine disruption as well as endometriosis-like symptoms, teratogenesis, abortion and diminished fertility. In pigs, TCDD affected follicular and luteal steroidogenesis. **MATERIAL AND METHODS:** Three-dimensional (3D) structure of the porcine AhR-LBD was established using the homology modeling. Two crystal structures of the human HIF-2 α protein (PDB ID: 3F1P and 3H82) were chosen as templates. Next, AhR and templates sequence alignments were exported to MODELLER 9v12 program and 100 models were generated. Models with the lowest DOPE (Discrete Optimized Protein Energy) values were selected for further analysis (ProSAIL and PROCHECK software). TCDD was docked into the binding site of AhR-LBD using AutoDock Vina 1.1.2 program. The binding-box encompassed binding site of AhR-LBD. The parameters of grind-box (X,Y,Z) was set to 15Å, 15Å, 15Å. The docking analysis was performed with exhaustiveness =32. **RESULTS:** From one hundred models of the porcine AhR-LBD that were generated in silico, the AhR-LBD model with 84.6% residues matching the 'most favored' region of the Ramachandran plot was selected. The AhR-LBD was found to be formed by 103 amino acids (residues 285-388 of the AhR) arranged in five β -strands and three α -helices, forming a hydrophobic pocket capable of a specific binding to a ligand. The binding energy of TCDD bound to the ligand binding pocket of AhR was calculated as -8.8 kcal / mol. TCDD was stabilized within the ligand binding pocket through hydrophobic interactions with the following AhR-LBD residues: H289, F293, P295, L306, L313, F322, F349, L351 and S363. In addition, TCDD formed a single hydrogen bond with Q381. **CONCLUSION:** The current study provides a framework for examining the key events involved in the ligand-dependent activation of the AhR.

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HODNOCENÍ INTENZITY OSVĚTLENÍ NA INSEMINAČNÍ STANICI KANCŮ

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ABSTRAKT

Práce je zaměřena na hodnocení světelných podmínek v chovu kanců, které jsou jedním z klíčů ovlivňujících úspěšný zdravý statut zvířat. Cílem experimentu bylo zjistit vliv intenzity osvětlení na parametry kvality ejakulátu: motilita spermií (%), objem ejakulátu (ml), koncentrace spermií (tis.ml-1) a podíl spermií s patologickými změnami (%). Pokus probíhal po dobu jednoho roku od března 2012 do března 2013 na ISK v ČR. Statisticky největší vliv byl prokázán u plemene Landrace, dále pak plemene Duroc. Nižší intenzita (52 lx oproti 66 lx) způsobila u plemene Landrace nejvyšší objem (445 ml oproti 322 ml), ale tato hodnota se projevila na úkor vysokého procenta abnormalit (12,3 % oproti 5,6 %) a snížené koncentrace spermií (220 tis.ml-1 z 289 tis.ml-1). Naopak vysoká intenzita snížila patologické změny až skoro o 7 %. Za vysoké intenzity osvětlení vykazoval nejvyšší koncentraci spermií duroc (473 tis.ml-1 z 390 tis.ml-1) a abnormality byly sníženy o 0,3 %. Durocovi tedy vzrostla za vyšší intenzity osvětlení koncentrace skoro až o 90 tis.ml-1. Lze konstatovat, že vysoká intenzita osvětlení způsobí pokles množství objemu ejakulátu, ale naopak pozitivně zapůsobí na kvalitu ukazatelů koncentrace a procenta patologických změn spermií.

ANALYSIS OF HAEMATOLOGIC PROFILE OF MOUFLONS (OVIS MUSIMON)

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ABSTRACT

Mouflons are primarily native from Mediterranean region. Since 1730 the first mouflons were carried in to Europe from Sardinia and Corsica islands. On the sustenance are these animals less demanding than other wild living animals. Haematological parameters observation are using as an aid in clinical diagnostics. The haematological parameters are used for utilization on the metabolic status. The main goal of our work was to investigate haematological parameters of mouflons. The experimental study included a total of 10 mouflons, they were only 5 years age females. Most animals proved to be clinically healthy. In the blood, by normal laboratory procedure was setting the haematological profile of blood components (Er, Htk, MCV, Hb, Lc). The obtained samples were examined by the Centre of Excellence Laboratory of Animal Ecology and man at Prešov University in Prešov. The middle level of hemoglobin was observed and detected in interval 12,18 – 13,06 g.dl⁻¹ with the lowest middle level in spring and with the maximum middle level in autumn. The middle level of hematocrit (Hk) was observed in seasons which were detected in interval 0,371 – 0,384 l.l⁻¹ with the lowest middle level in spring and with the maximum middle level in summer season. The middle level of erythrocyte (Er) was observed in seasons and detected in interval 8,07 – 9,37 T.l⁻¹ and middle level of lymphocyte (Ly) observed in seasons which were detected in interval 62,92 – 66,18 % with the lowest middle level in winter and with maximum middle level in autumn.

Keywords: analysis, blood, ecology, mouflons

Acknowledgement: This work was supported by grant OPV-2012/1.2/05-SORO, ITMS 26 11 02 30 100.

ANALYSIS OF HAEMATOLOGIC PROFILE OF MARES IN SELECTED SEASON

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ABSTRACT

The objective of our work was to evaluate the selected haematological parameters of our experiment group of mares. The analysis of blood was performed in spring at the model group of animals. The experimental group included a total of 13 mares they were until one year age. Most animals proved to be clinically and physical healthy. Evaluation was carried out according to welfare. The middle level of erythrocyte (Er) was observed in season and detected in interval 7,48 – 12,1 (T/l) and middle level of leukocyte (Lc) were observed in interval 5,8 – 12,4 (G/l) and middle level of Packet cell volume (PCV) were observed in interval 0,36 – 1,9 (l/l). The middle level of neutrophil segments showed 11 – 70 (%) and neutrophil sticks 0 – 2 (%), lymphocyte was 30 – 83 (%), basophil showed 0 – 1 (%), monocyte 0 – 4,2 (%) and eosinophil 0 – 4,9 (%). The main reason of studying the haematological profile of blood at horses consists particularly comparing and summarizing the biological processes. Haematological examinations and evaluations were made following the last standard laboratory methods.

Keywords: blood, erythrocyte, leukocyte, mares, spring

Acknowledgement: This work was supported by grant OPV-2012/1.2/05-SORO, ITMS 26 11 02 30 100.

NEXT-GENERATION SEQUENCING (NGS) AND BIOINFORMATIC TOOLS FOR ESTABLISHING THE SEQUENCE OF PORCINE ARYL HYDROCARBON RECEPTOR (AHR) GENE

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ABSTRACT

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor best known for mediating xenobiotic-induced toxicity. The AhR is a phylogenetically highly conserved receptor which physiological as well as toxicological significance in the organism development was reported by many researches. The present study was performed to determine the complete nucleotide sequence of porcine AhR. For this purpose, two livers were collected from cyclic gilts. Total RNA was isolated from a liver using AllPrep DNA/RNA Mini Kit (Qiagen) and RNA integrity number (RIN) was evaluated via microfluidic electrophoresis using a 2100 Bioanalyzer (Agilent Technologies, USA). For each sample, RIN calculated with the use of Agilent 2100 Expert software, was >7.8. The sequence of the entire porcine transcriptome was obtained by Next-Generation Sequencing (NGS). Sequencing was performed by OpenExome using Illumina HiSeq2500 sequencer. In silico analysis of nucleotide sequence showed that the full-length mRNA of porcine AhR gene had two variants: 1/ variant I - 5070 bp (GenBank accession No. KM379096.1) and 2/ variant II -5072 bp (GenBank accession No. KM817031.1). The same sequence length of coding region – 2550 bp (from 112 to 2661 bp) – was estimated for the two variants. In addition, computer analysis revealed that AhR gene in the pig is located on 9. chromosome (9: 95,509,942-95,542,785). The percentage of identity of the coding part of the porcine AhR gene with homologous genes of other species was very high. The porcine AhR gene was predicted to encode a polypeptide of 849 amino acids. Comparative analysis of porcine AhR sequence variants revealed eleven single nucleotide substitutions within the coding region of the gene. All of these substitutions were missense mutations. Determination of the AhR full sequence in pigs is important not only for extending the knowledge concerning AhR, but also in order to properly design experiments involving gene silencing as a part of the studies on the AhR-dependent processes.

Acknowledgement: This study was supported by The Ministry of Science and Higher Education in Poland (2012/05/B/NZ9/03333, UWM No. 528.0206.0806.).

EFFECTS OF ORAL ACRYLAMIDE INTAKE ON BLOOD MORPHOLOGY IN MICE

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ABSTRACT

Acrylamide (AA) is a neurotoxic, mutagenic and carcinogenic substance. In the organism acrylamide forms adducts with hemoglobin which theoretically may influence on blood morphology. Taking above into consideration the study over AA influence on basic blood parameters may be interesting. The aim of my research was to analyze the blood morphology of male swiss mice treated with AA. One experimental group of mice received 20 mg/kg/day acrylamide and the dose applied to the second group of mice was 40 mg/kg/day. The blood morphology was estimated using the Auto Hematology Blood Analyzer from Mindray. Analysis of the results indicated that AA have significant impact on WBC, RBC and HGB concentration. Also, our results indicate significant AA influence on lymphocyte concentration in blood of mice. Long-term exposure to AA indicated decrease WBC, RBC, HGB and limphocytes concentration. We did not observe significant differences between the effects of different doses of acrylamide.

EFFECT OF INTRAMUSCULAR APPLICATION OF EPICATECHIN ON SELECTED PARAMETERS OF MINERAL PROFILE

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ABSTRACT

The goal of the experiment was to determinate the effect of epicatechin, the natural antioxidant on selected parameters of mineral profile (calcium, phosphorus, magnesium) in blood of rabbits. Adult female rabbits (n=25), maternal albinotic line e (crossbreed New Zealand White, Buskat Rabbit, French Silver) and paternal acromalictic line (crossbreed Nitra's Rabbit, Californian Rabbit, Big Light Silver) were used in experiment. Rabbits were divided into three experimental groups E1, E2, E3 (n=5) and control group C (n=5). Experimental groups E1, E2, E3 received epicatechin in injectable form intramuscularly (*Musculus biceps femoris*) 10 $\mu\text{g}\cdot\text{kg}^{-1}$ in E1, 100 $\mu\text{g}\cdot\text{kg}^{-1}$ in E2 and 1000 $\mu\text{g}\cdot\text{kg}^{-1}$ in E3 group, for 15 days three times a week. The blood serum was used for the analysis of following components: calcium, phosphorus, magnesium using by Ecoline kits analyzer RX monza (United Kingdom) were used according to manufacturer conditions. No significant differences ($p>0.05$) in content of calcium, phosphorus, magnesium were found among the groups. The doses of epicatechin used in this study had no influence on observed parameters.

Keywords: epicatechin, pautlin, calcium, magnesium, phosphorus

Acknowledgments: This work was financially supported by VEGA scientific grant 1/0760/15 and APVV-0304-12.

INVOLVEMENT OF TRANSCRIPTION FACTORS IN CONTROL OF OVARIAN CELL FUNCTIONS

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ABSTRACT

We are the first who studied the control and the role of transcription factors in the regulation of reproductive functions. Our presentation represent a short review of original data concerning external (temperature, calories restriction) and internal (hormones, protein kinases, RNA interference) in control of selected transcription factors (p53, NFkB, STAT, CREB), as well as their role in control of basic ovarian cell functions (proliferation, apoptosis, secretory activity, response to hormonal stimulators).

It was shown, that stress (high temperatures, food restriction), hormonal regulators of reproduction (gonadotropins, GH, oxytocin, some growth factors), pharmacological or genomic regulators of protein kinases (protein kinase A, MAP kinase, CDC2 and other kinases), si RNAs and miRNAs control the expression of these transcription factors within porcine, rabbit and human ovarian cells. Furthermore, the transfection-induced overexpression of these transcription factors altered the proliferation (markers of cell cycle PCNA, cyclin B1, MAP kinase, CDC2 kinase/p34), both nuclear (TdT) and cytoplasmic (bax, bcl-2, caspase 3, p53) apoptosis, release of steroid (progesterone, testosterone, estradiol) and peptide (oxytocin, IGF-I) hormones and prostaglandins (F and G) by cultured ovarian granulosa cells, as well as their response to stress and hormonal treatments.

These observation demonstrate (1) the presence of transcription factors in ovarian cells, (2) the hierarchy of upstream regulators of ovarian transcription factors (environmental factors – hormones-protein kinases – miRNA – transcription factors), (3) the involvement of transcription factors in control of basic ovarian cell functions (proliferation, apoptosis, secretory activity and response to hormones) and (4) that transcription factors are mediator of the upstream regulators action on basic ovarian cell functions.

IMPACT OF VARIOUS TAURINE CONCENTRATIONS ON TURKEY SPERMATOZOA MOTILITY DURING THE *IN VITRO* CULTURE AT 41°C

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ABSTRACT

The aim of the present study was to evaluate effect of various taurine concentrations on the turkey spermatozoa motility during the *in vitro* incubation at 41°C. Selected motility parameters were monitored at six time periods: 0, 1, 2, 3, 4 and 5 hours. Semen samples of adult male turkeys of the line Big 6 were diluted a ratio of 1 part of semen and 100 parts of physiological solution (control sample – C) and with five different concentrations of taurine: 10 mg.ml⁻¹ – sample AT; 7.5 mg.ml⁻¹ – sample BT; 5 mg.ml⁻¹ – sample CT; 2.5 mg.ml⁻¹ – sample DT and 1.25 mg.ml⁻¹ – sample ET (diluted in physiological solution). Measurements were evaluated by the CASA system (Computer Assisted Semen Analyser) with the program Sperm Vision®. At the beginning of incubation significantly ($p < 0.001$) lower values of spermatozoa motility were detected in the sample AT (43.35%) in comparison to the control (57.53%). Sample AT, with the highest concentration of taurine, showed significant lower motility at the time 1, 2, 3 and 5 h. At the final time of incubation (5 hours) significantly lower motility were observed in all experimental samples. Spermatozoa progressive motility followed the tendency of spermatozoa motility. Significantly lower data were observed in samples AT after 0, 1, and 2 hours and in the sample BT after 1 hours of incubation. Non-significant differences were found in all experimental samples in other time periods of *in vitro* incubation. It can be concluded from this study that addition of taurine (from 1.25 to 10 mg.ml⁻¹) to turkey spermatozoa have no positive effect on monitored spermatozoa motility parameters at 41°C.

Keywords: turkey, taurine, spermatozoa, motility, CASA

Acknowledgments: This study was supported by projects VEGA 1/0760/15; 1/0857/14, APVV-0304-12 and KEGA 006/SPU-4/2015. The research leading to these results has received funding from the European Community under project no 26220220180: Building Research Centre “AgroBioTech”.

MILK FLOW KINETIC AND PERFORMANCE IN DAIRY GOATS

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ABSTRACT

The aim of the work was to find out the milkability of the dairy goats and evaluate the effect of the stage of lactation, the order of entry into the milking parlor, breeds, bimodality, number of kids and lactation numbers. The research was done at the farm located in the Orava region supported by Kega 006SPU-4/2014 and CEGEZ No. 26220120042. The measurements were performed using Lactocorder at two different stages of lactation in June (3rd to 4th month) and July (4th to 5th month). Both measurements were realized on 129 ones. Goats, which were included in the measurements were of different breeds, and formed the basis of White Shorthaired and Alpine goat, less likely to be represented Thuringia goat and crosses of different breeds. Both measurements were carried out during the evening milking. The following parameters were evaluated: maximum milk flow in one minute, milk yield in the 1st, 2nd and 3rd minute, the average milk flow in the main phase, time of milking, the milk temperature, electrical conductivity, maximum milk flow, time to peak flow, time ascending and descending phases and time the plateau phase.

Goat milk yield from the 3rd to 4th month of lactation (June) was significantly higher (1.04 ± 0.048 kg) than in goats from 4th to 5th month of lactation (July) (0.870 ± 0.052 kg). The significant difference between the parameters was recorded at the milk temperature, where from 3rd to 4th month lactation the temperature was 37.17 ± 0.12 °C and in next month it was 34.97 ± 0.13 °C. When the impact of the order of entry into the milking parlor was evaluated the highest milk yield was recorded in the last entering group, 1.140 ± 0.058 kg, and lowest in the first entering group, 0.73 ± 0.063 kg. The middle group had milk yield (the group between of both above mentioned groups) 0.997 ± 0.054 kg. In animals with bimodal milk flow there was recorded significant effect on milk yield. Goats with the one peak type of flow had milk yield lower (0.740 ± 0.037 kg) as compared to a bimodal curve flow (1.170 ± 0.068 kg). The goats with one young kid as compared with twins during pre-weaning period did not differ in milk yield during both studied stages of lactation.

In conclusion there is first time detail described milkability of dairy goat in Slovakia primary production and obtained data could be used in the selection. Also results contribute to a better understanding of the factors affecting milkability of goats during machine milking which could be implemented into primary to improve breeding conditions.

Keywords: goat, milking, milkability

ASSESSMENT OF EFFECTS OF VEGETABLE AND ANIMAL FATS AFTER HEAT TREATMENT ON OVARIAN FUNCTIONS *IN VITRO*

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ABSTRACT

Fats supplies to many food a distinctive flavor, aroma, texture and form an important component of energy. The aim was to investigate the effect of vegetable and animal fats (palm oil, lard, mixture of vegetable fats) after heat on the secretion of steroid hormones (progesterone, 17 β -estradiol, testosterone) and markers of proliferation and apoptosis (PCNA, caspase-3) to ovarian granulosa cells of gilts *in vitro*. Ovarian granulosa cells were incubated with fats (100 μ l/ml) for 24 hours, while the control group did not contain the fats after heat. Progesterone, 17 β -estradiol and testosterone were determined by ELISA. Markers of proliferation and apoptosis were determined by immunocytochemistry. The release of progesterone in palm oil has been conclusively inhibited and in lard unchanged. Release of 17 β -estradiol was conclusively inhibited by effect of fat after heat. The release of testosterone was unchanged. We have also investigated the effect of burnt fat (vegetable fat, palm fat) for the presence of markers of proliferation and apoptosis of granulosa ovarian cells *in vitro*. We have notice a reduction in the presence of the marker PCNA in the case of a mixture of vegetable fats and the presence of a marker caspase-3 in the case of palm oil. Our *in vitro* results indicate that the fat after heat treatment may affect the secretory activity of the ovarian granulosa cells of pigs and affect reproductive function - steroidogenesis.

Keywords: 17 β -estradiol, progesterone, testosterone, fats, ovary cells, apoptosis, proliferation

Acknowledgments: This work was financially supported by the European Community under the Project no. 26220220180: Building Research Centre „AgroBioTech“, Ministry of Education, Science, Research and Sports of the Slovak Republic project VEGA 1/0022/13 and Slovak Research and Development Agency of the Slovak Republic APVV-0304-12.

COMPARISON OF HEMATOLOGICAL PROFILE OF DAIRY COWS AT DIFFERENT LACTATION STAGES

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ABSTRACT

Hematologic analysis is very helpful not only for diagnose hematologic disorders, but also systemic and organ diseases. These disorders occur during transition period very often, but can be also observed in other lactation periods. The aim of this work was to compare selected hematological parameters of dairy cows at the beginning (BL) and at the peak of lactation (PL). In total, 42 Holstein-Fresian cows (21 at the beginning of lactation and 21 at the peak of lactation) were chosen. Blood samples were collected from *vena caudalimediana* directly into centrifuge tubes coated with ethylenediaminetetraacetic acid (EDTA). Hematological parameters - total white blood cell count (WBC), lymphocytes count (LYM), platelet count (PLT), red blood cell count (RBC), haemoglobin (HGB) and haematocrit (HCT) were determined in the whole blood. Analyses were provided in Biochemical and hematological laboratory at the Department of Animal Physiology (SUA in Nitra). GraphPad Prism 5 was used to conduct statistical analysis, using Mann-Whitney U test. Results showed that values of WBC, LYM, RBC and HGB complied with reference ranges. HCT was decreased in BL group (20.64%) and was at the lower limit in PL (21.12%). There was significant difference ($P < 0.05$) between the total number of erythrocytes (RBC) in BL ($5,16 \times 10^6 \mu\text{L}^{-1}$) when compared to PL group ($5,56 \times 10^6 \mu\text{L}^{-1}$). Slightly decreased levels of RBC and HGB in BL group can be caused by an increase of plasma volume during pregnancy. A significant decrease ($P < 0.05$) of platelets was observed in PL group ($172.4 \times 10^3 \mu\text{L}^{-1}$) in comparison to BL group ($280.1 \times 10^3 \mu\text{L}^{-1}$). Thrombocytopenia is found in excessive consumption, decreased platelet production or intoxication related destruction. The white blood cell parameters (WBC, LYM) were slightly increased in BL group in comparison to PL group. These results could point to an organism exhaustion or hepatitis during peak milk yielding, but diagnosis or prognosis should not be based on hematologic results only. In cattle, changes in CBC (complete blood cell), especially in leukogram, should be accompanied by findings from the clinical examination.

Keywords: dairy cow, hematology, beginning of lactation, peak of lactation

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CURCUMIN ADMINISTRATION TO A SEMEN EXTENDER HAS MOTION-PROMOTING EFFECTS ON BOVINE SPERMATOZOA

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ABSTRACT

The aim of this study was to assess the effects of curcumin (CUR) supplementation to a semen extender on bovine spermatozoa motility characteristics at different temperature settings (20°C, 4°C and -20°C) during various time periods (4h, 24 and 48h) of a 48-hour in vitro culture. Semen samples were collected from 10 adult breeding bulls, and diluted in a commercial bovine semen extender containing 0.5% DMSO with or without 50 µmol/L CUR. Spermatozoa motion parameters (motility, progressive motility, distance average path and average path velocity) were determined that using the SpermVision™ CASA (Computer Aided Sperm Analysis) system. The analysis at 20°C revealed that CUR had no significant effects on the motion parameters during a short-term culture, however its presence was able to significantly ($P<0.001$) prevent the decrease of spermatozoa activity at 24h and 48h. The motility-promoting properties of CUR became evident early on with respect to the 4°C culture, with significant outcomes at time 4h ($P<0.05$) and extending throughout the remaining time periods ($P<0.001$; times 24h and 48h). Although CUR supplementation did not prevent cell death caused by freezing temperatures (-20°C), it was able to offer at least a partial protection against the loss of spermatozoa activity, especially at time 4h ($P<0.05$). These results suggest that curcumin exhibits stimulating and protective effects on the spermatozoa physiology and activity during a routine in vitro storage and processing of male gametes, and could be used as a suitable supplement for media used in veterinary andrology.

Keywords: curcumin, semen extender, CASA, spermatozoa, bulls

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LITTER SIZE EFFECT ON BEHAVIOUR OF MULTIPAROUS DAMS OF SYNTHETIC SLOVAK DAIRY SHEEP POPULATION AT THE TIME OF PARTURITION

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ABSTRACT

The aim of the study was to characterise physiological levels of behaviours related to lambing time and investigate the litter size effect on maternal behaviour of the ewe. Observation was carried out at the NPPC – Research Institute of Animal Production Nitra, experimental farm in Trenčianska Teplá. The ewes were moved to individual lambing pens (1.0 x 1.8 m) with hay bedding 4 days before the expected lambing date and allowed to lamb undisturbed, human assistance was given if birth failed to progress normally. All activities of animals were continuously video recorded by 7 cameras linked to a two 4-channel digital recorders. We focused to monitor activities during two hours before and two hours after the birth, ewe behaviours were analysed as means \pm SE in seconds for ten minute periods. As a starting point we used the time when the fetus was completely out of the ewe's uterus. Before parturition we evaluated locomotor activity and feeding behaviour of dams, after lambing we analysed the grooming behaviour of ewes and lamb activities - the first movement, first attempt to stand up, the first standing on four limbs and the first placement of the head underneath ewe in the udder region. Ten multiparous dams of synthetic Slovak Dairy Sheep population were evaluated without assistance during lambing, 5 ewes with single lamb (SL) and 5 ewes with twins (TL). Differences between dams divided by litter size were analysed using a mixed model (SAS 9.1). The average time of standing for ten minutes period in SL was 372.42 ± 27.94 s, however we observed no significant differences in relation to TL (304.83 ± 27.07). A similar situation - no significant differences SL vs. TL, was also at the time of lying (221.42 ± 27.85 vs. 275.38 ± 27.95), at the time when kneeling (6.18 ± 2.56 vs. 19.78 ± 6.62), intake of concentrate (39.53 ± 16.19 vs. 12.42 ± 7.05) and intake of roughage (47.83 ± 17.59 vs. 39.40 ± 12.62). Statistically significant differences were found in the grooming behaviour between groups SL vs. TL (342.92 ± 24.42 vs. 468.17 ± 17.47 s, $F = 11.20$, $P = 0.01$) and also among the particular time periods ($F = 8.81$, $P < 0.001$). We can confirm the hypothesis that ewes spend less time grooming the lamb during increasing subsequent time periods after the birth and TL spend more time grooming the lambs than SL.

Keywords: ewe, delivery, litter size, behaviour before lambing, after lambing

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DOES IN VITRO FERMENTATION METHOD PREDICT THE USE OF FUNGAL ENRICHED SUBSTRATES IN RUMINANT NUTRITION?

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ABSTRACT

A current word trend in animal nutrition has increased the demand for feeds containing health beneficial polyunsaturated fatty acids (PUFA) as omega-3 and omega-6 fatty acids (FA) that are not produced by the body and must be obtained through diet or supplementation. Gamma-linolenic acid (GLA; C18:3n-6) is an intermediate in the conversion of linoleic acid (C18:2) to arachidonic acid (C20:4) and these omega-6 FA are precursors of a variety of metabolites regulating biological functions. An effective way to enhance the concentration of GLA in ruminant derived food products could be achieved by feeding GLA enriched diets. Over the last five years we have chosen to examine the effect of prefermented cereal substrates enriched with GLA by oleaginous fungi on lipid metabolism and ruminal fermentation. For the purpose of feed evaluation, in vitro ruminal fermentation methods involving RUSITEC and batch culture system of incubation were used as they are ethically superior, faster, and less expensive than in vivo methods and still offering degree of animal-feed interaction. The aim of the present study was to assess ruminal fermentation parameters and lipid metabolism through a meta-analytical approach of integrating related studies from published papers which described effects of various fungal GLA enriched substrates in ruminant feed in vitro. Apart from fatty acids, other related rumen fermentation parameters were also included in the database, i.e. degradability of dry matter, organic matter, neutral detergent fibre, acid detergent fibre, as well as gas production, methane concentration, ammonia N concentration, short-chain fatty acid profiles and protozoal counts. The data obtained from a total of 16 studies were subsequently subjected to a statistical analysis based on mixed model methodology. Different studies were treated as random effects whereas different GLA enriched substrates were considered as fixed effects. Data clearly indicated that GLA enriched substrates increase the outputs of GLA from ruminal effluent by approximately 80%. The effectiveness of GLA sources in increasing of ruminal GLA outputs varied on used filamentous fungi in the order *Cunninghamella echinulata* > *Thamnidium elegans*, however efficiency depends also on cereal substrate type. This review presents the most recent perspective for GLA enriched cereal substrates application in area ruminant nutrition, however, in vivo studies are needed to support in vitro results.

Keywords: gamma-linolenic acid, prefermented cereal substrates, ruminal fermentation characteristics

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PITUITARY HORMONES AFFECT ESTROGENS RELEASE BY THE MYOMETRIUM IN PIGS (*SUS SCROFA DOMESTICA*)

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ABSTRACT

Uterus is an important source of steroid hormones during early pregnancy and the estrous cycle in the pig. Our previous study also showed that myometrium express CYP19 (which encodes P450 aromatase) and secretes estrone (E1) and estradiol - 17 β (E2) during luteolysis and early pregnancy. The hormonal regulators of estrogens production by porcine myometrium are not known. In this study we hypothesized that pituitary hormones i.e. luteinizing hormone (LH) and follicle stimulating hormone (FSH) may affect E1 and E2 secretion by porcine myometrium acting via myometrial FSH and LH receptors (FSHR and LHR, respectively). To determine myometrial ability to response to LH and FSH the expression of LHR and FSHR genes in the myometrium harvested during luteolysis (days 15-16, n=5) and early pregnancy period (days 15-16, n=5) was investigated using Real-Time PCR. To determine the response of the myometrium to gonadotropins the tissue slices (200 mg) were first pre-incubated (18h, 37°C, in an atmosphere of 95% O₂ and 5% CO₂) and then incubated (6h, at the same conditions) in the presence or absence of LH (10 ng/ml or 100 ng/ml) or FSH (10 ng/ml or 100 ng/ml). Concentrations of E1 and E2 in culture media were determined by radioimmunoassay. We found the expression of LHR and FSHR mRNAs in the myometrium harvested during luteolysis and early pregnancy. The myometrial expression of LHR and FSHR in cyclic and pregnant pigs did not differ ($p > 0.05$). During luteolysis myometrial secretion of E1 was increased after stimulation with LH (both doses) and FSH (100 ng/ml). Myometrial E2 secretion was increased ($p \leq 0.05$) in the presence of LH (both doses) and FSH (10 ng/ μ l). Neither LH nor FSH affected E1 and E2 release in pregnant pigs. In conclusions: 1) porcine myometrium express LHR and FSHR mRNAs during luteolysis and peri-implantation period; 2) LH and FSH specifically affect myometrial secretion of E1 and E2 during luteolysis; 3) pituitary hormones do not affect estrogens release by porcine myometrium during peri-implantation period. The effect of gonadotropins on myometrial E1 and E2 release depends on the reproductive status of the female.

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THE BENEFITS OF SUPPLEMENTARY FAT AND PLANT MIXTURE IN FEED RATIONS FOR RUMINANTS

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ABSTRACT

Recently, natural products, such as probiotics, prebiotics, organic acids or secondary plant compounds, have been tested to mitigate negative effects of feeding high-concentrate diet to ruminants and to enhance the efficiency of feed utilization. Little information is available on the potential of traditional medicinal herbs to modify rumen fermentation pattern. It is possible that the addition of dietary fats together with a medicinal plant mixture could influence the microbial ecosystem in the rumen toward the enhancing of essential polyunsaturated fatty acids (PUFA) in ruminant products and could also mitigate the negative effects of high-concentrate diets. Therefore the aim of this study was to determine the effects of a medicinal plant mixture (MP), sunflower oil (SO) and a combination of the medicinal plant mixture and sunflower oil (MPSO) on fermentation end products, the fatty acid composition of the rumen fluid and the ruminal microbial population of sheep fed a high-concentrate diet. At first, 24 h in vitro ruminal incubations were performed and following treatments were tested: control (meadow hay/ barley grain, 400/600, w/w), MP (10% replacement of meadow hay), SO (3.5% DM) and MPSO. The MP and MPSO treatments enhanced the in vitro dry matter digestibility ($P < 0.001$) and decreased methane production ($P = 0.021$); in addition MPSO increased the concentration of PUFA ($P < 0.001$). Based on the results from the in vitro study an in vivo experiment was performed. Four rumen-fistulated rams were randomly assigned to a 4×4 Latin square design and fed a basal diet consisting of 720 g DM/day meadow hay and 540 g DM/day barley grain. The basal diet was supplemented with no additive (control), a medicinal plant mixture (MP, 10% replacement of meadow hay; 72 g DM/day), sunflower oil (SO, 36 g/day) and the combination (MPSO). There was no effect of treatment on the rumen fermentation characteristics. DGGE analysis was used to analyze the changes in the rumen eubacterial community induced by supplementation with MP, SO or MPSO, respectively. However, supplementing the animal diet does not affect the rumen eubacterial community composition in a substrate-specific manner. The beneficial effects of MP and MPSO on PUFA concentration observed in vitro were not fully confirmed in vivo, nevertheless, the results point to the promising impact of using a medicinal plant mixture in high-concentrate diets of ruminants without adverse effects on fermentation characteristics and microbial population in sheep rumen.

Keywords: fatty acids, medicinal plants, sunflower oil, 16S-PCR-DGGE, rumen fermentation

Acknowledgement: This study was financially supported by grant VEGA 2/0009/14.

MOLECULAR IDENTIFICATION OF PARAMECIUM BURSARIA SYNGENS AND STUDIES ON GEOGRAPHIC DISTRIBUTION USING MITOCHONDRIAL CYTOCHROME C OXIDASE SUBUNIT I (COI) AS MARKER

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ABSTRACT

The *Paramecium bursaria* (EHRENBERG 1831, FOCKE 1836) is composed of five syngens, which are morphologically indistinguishable but sexually isolated. The aim of the present study was to confirm by molecular methods (analyses of COI mtDNA) the identification of *P. bursaria* syngens originating from different geographical locations. The phylograms constructed using both the neighbor-joining and maximum-likelihood methods based on a comparison of 34 sequences of *P. bursaria* strains and *P. multimicronucleatum*, *P. caudatum* and *P. calkinsi* strains used as outgroup have revealed strains grouping into five clusters, which refers to results obtained previously by mating reaction. Our analysis show the existence of 24 haplotypes for the COI gene sequence in the studied strains. The interspecies haplotype diversity value was $Hd=0.967$. We confirmed the genetic polymorphism between strains of *P. bursaria* and the occurrence of correlation between geographical distribution and the correspondent syngen.

NOVEL SEQUENCE OF PREGNANCY-ASSOCIATED GLYCOPROTEIN GENES (WITHIN EXON 6) IDENTIFIED IN THE EUROPEAN BEAVER GENOME

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ABSTRACT

The aspartic proteinases superfamily (AP; EC 3.4.23) includes proteins present in vertebrates and non-vertebrates, e.g. pepsin A, C, F (PepF), pregnancy-associated glycoprotein family (PAGs / PAG-Like), renin, cathepsin D, as well as several other fungal and retroviral enzymes. The PAG-Like family (PAG-L) is a large group of secretory chorionic products, that is believed to be involved in proper embryo-maternal interaction and placenta development (see Szafranska et al. 2006). So far, multiple cDNAs have been cloned in the Artiodactyla (i.e. pig, cattle, sheep and water buffalo), whereas single genes in the Perissodactyla (horse and zebra) and the Rodentia (mouse only – mPepF/PAG-L). In domestic species, AP genes encompass 9 exons and 8 introns. However in some wild species, genomic sequences and exonic-intronic structure of the PAG-L genes have not been identified yet. The aim of this study was to identify internal nucleotide sequences of the PAG-L gene(s) in the genome of the European beaver, the largest rodent in Europe.

Genomic DNA (gDNA) templates isolated from leucocytes of adult beavers (females, males) and fetuses (N=15) were used for PCR amplification of the PAG-L with primers (ozEx5Se and ozEx7As) that should amplify the region of exons 5 and 7 with intron E and F, according to AP genes structures (2835–3253 nt), parallel to porcine PAG gDNA or beaver cDNA (468 nt) as control templates. After electrophoretic separation and UV-visualization, dominant beaver gDNA amplicons were gel-out purified, precipitated, and then subjected to sequencing in both sense and antisense directions (3130 Genetic Analyzer, Applied Biosystems). Obtained gDNA sequences were compared to placental PAG-L cDNA sequences identified by NGS (Illumina).

Sequencing of amplicons (99–841 nt) permitted to identify nucleotide sequences of the beaver PAG-L gene that was named CfPAG-L, according to Latin nomenclature of this species (*Castor fiber*). The consensus CfPAG-L sequence of the entire exon 6 (125 nt) shared the highest homology (74–84%), to the pepsinogen family known in other species (GenBank).

This is the first report concerning a pioneer nucleotide sequence identification of the CfPAG-L/PepF gene fragment (in the region of exon 6). Our results provide a novel information about the European beaver genome. Further complex studies are required to discover the entire structure of the CfPAG-L gene.

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EFFECT OF CHRONIC APPLICATION OF EPICATECHIN ON HAEMATOLOGICAL PARAMETERS OF RABBITS

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ABSTRACT

In this study haematological parameters (total white blood cell count, lymphocytes count, medium size cell count, granulocytes count, red blood cell count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, medium size cell percentage, platelet count, platelet percentage, mean platelet volume and platelet distribution width) in blood of rabbits after chronic administration of epicatechin were analysed using haematology analyzer Abacus junior VET (Diatron®, Vienna, Austria). Animals (adult female rabbits, body weight 4 ± 0.5 kg) were divided into four groups: control group (C) and experimental groups (E1 - E3). Experimental groups received epicatechin in injectable form at $10 \mu\text{g.kg}^{-1}$ in E1, $100 \mu\text{g.kg}^{-1}$ in E2 and $1000 \mu\text{g.kg}^{-1}$ in E3 for five weeks three times a week. Blood was collected from vena auricularis to EDTA-treated tubes for four times. The first collection was done at the beginning of the experiment before experimental intervention. Second and third blood collection during the experiment after two and four weeks was realized. The last time was blood collected at the end of the experiment after five weeks exposure of epicatechin. The results from first blood collection showed that all animals were healthy. Significant decrease ($P < 0.05$) of mean corpuscular haemoglobin concentration (MCHC) in E2 experimental group in comparison with the control group was observed after two weeks and in the E2 and E3 group after four weeks of epicatechin administration. At the end of the experiment MCHC was lower in all experimental groups when compared with the control group but without significant differences ($P > 0.05$). Medium size cell percentage was significantly affected ($P < 0.05$) by epicatechin after two weeks of exposure in the E2 group in comparison with the control group. Epicatechin had no effect on the others analysed haematological parameters. Further investigation with haematological response of animals on epicatechin will be worthy of further investigation.

Keywords: epicatechin, haematology, rabbits blood

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THE POTENTIAL FOR MAINTAIN OF DNA METHYLATION IN PORCINE ENDOMETRIUM HARVESTED ON DAYS 15-16 OF PREGNANCY AND THE ESTROUS CYCLE – THE EFFECT OF THE REPRODUCTIVE STATUS

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ABSTRACT

DNA methylation, which includes maintenance DNA methylation directed by DNA methyltransferase 1 (DNMT1), is important for embryos development and may have a direct impact on success of pregnancy. The mechanisms of the regulation of genes expression in the uterus and embryos may be fundamental for endometrial receptivity during the most important periods of early pregnancy, especially during periimplantation period. Hence, we assumed, that physiological status of female as well as the presence of periimplantation embryos within the uterus, has the potential to induce alternations in expression of genes encoding methyltransferase in the endometrium. In this study, we focused on the potential for maintain DNA methylation in the endometrium, defined as alternation in the endometrial expression of gene encoding DNMT1, during early pregnancy and respective days of the estrous cycle (Days 15 to 16). The endometrial expression of mRNA DNMT1 was estimated using Real-time PCR. Location and activity of DNMT1 protein was analyzed using immunofluorescence staining. The relative expression of DNMT1 in gravid pigs (n=5) on Days 15 to 16 it was statistically lower than in non-gravid pigs (n=5) ($P \leq 0.05$). Accuracy of the amplified DNA sequence in the studied tissues indicated 100% homology with *Sus scrofa* DNA (cytosine-5-)-methyltransferase 1 (DNMT1) (Access No. NM 001032355.1). Furthermore, immunofluorescence staining revealed, that DNMT1 protein is localized in luminal epithelial cells, granular epithelial cells and stromal cells. The activity of DNMT1 protein, as well as gene expression, in pigs during periimplantation embryos (n=5) it was statistically significant lower than in pigs the respective days of the estrous cycle (n=5) ($P \leq 0.05$). We observed a strong positive correlation ($R=0.77$) between the relative expression of DNMT1 and activity of DNMT1 protein during Days 15 to 16 of the estrous cycle. In conclusions, the results have shown, that the reproductive status, as a pregnancy and the presence of embryos in the uterus, may affect on the endometrial expression of DNMT1 on the gene and protein level. During early pregnancy the potential to maintain of DNA methylation in the uterus tissue, through presence of periimplantation embryos, may be significantly decreased. These preliminary study indicates the potential role of DNMT1 in the proper regulation of genes expression of the uterus during early pregnancy and success of embryonic development. Early pregnancy is the period when maternal tracks tissues may be more sensitive for global demethylation. However, further studies are needed to clarify alternations in gene and protein expression, which are responsible for maintain of DNA methylation during early pregnancy.

Keywords: epigenetic, methylation, endometrium, pregnancy, pigs

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