## 14<sup>th</sup> INTERNATIONAL SCIENTIFIC CONFERENCE ANIMAL PHYSIOLOGY 2018

# **BOOK OF ABSTRACTS**



13 - 15 June 2018 Kraków, Poland



### 14<sup>th</sup> INTERNATIONAL SCIENTIFIC CONFERENCE

### **ANIMAL PHYSIOLOGY 2018**

### **BOOK OF ABSTRACTS**



13 - 15 June 2018 Kraków, Poland

#### Reviewers

Agnieszka Greń, Ph.D. Grzegorz Formicki, Ph.D. Peter Massányi, D.Sc. Zofia Goc, Ph.D. Renata Muchacka, Ph.D.

#### Editor

Renata Muchacka, Ph.D. Edyta Kapusta, MSc.

© Copyright by Wydawnictwo Naukowe UP, Kraków 2018

#### e-ISBN 978-83-8084-152-9

#### DOI 10.24917/9788380841529

Pedagogical University of Cracow Faculty of Geography and Biology Institute of Biology Poland

Slovak University of Agriculture in Nitra Faculty of Biotechnology and Food Sciences Department of Animal Physiology Slovak Republic

Mendel University in Brno Faculty of Biology Agrisciences Department of Animal Morphology, Physiology and Genetics Czech Republic

> Slovak Academy of Science Institute of Animal Physiology Slovak Republic

#### Scientific Committee of the Conference

#### Laszlo Bardos, Ph.D.

Szent István University, Gödöllő, Hungary

#### Lukasz J. Binkowski, Ph.D.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Martha Valdivia Cuya, Ph.D.

National University of San Marcos, Lima, Peru

#### Štefan Faix, D.Sc.

Institute of Animal Physiology, Slovak Academy of Sciences, Košice, Slovak Republic

#### Zita Faixová, Ph.D.

University of Veterinary Medicine and Pharmacy in Košice, Slovak Republic

#### Grzegorz Formicki, Ph.D.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Agnieszka Greń, Ph.D.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Zdeněk Havlíček, Ph.D.

Department of Animal Morphology, Physiology and Genetics, Faculty of Agronomy, Mendel University in Brno, Czech Republic

#### Monika Martiniaková, Ph.D.

Constantine the Philosopher University, Nitra, Slovak Republic

#### Adriana Kolesárová, Ph.D.

Department of Animal Physiology, Faculty of Biotechnology and Food Science, Slovak University of Agriculture in Nitra, Slovak Republic

#### Jaroslav Kováčik, Ph.D.

Department of Animal Physiology, Faculty of Biotechnology and Food Science, Slovak University of Agriculture in Nitra, Slovak Republic

#### Norbert Lukáč, Ph.D.

Department of Animal Physiology, Faculty of Biotechnology and Food Science, Slovak University of Agriculture in Nitra, Slovak Republic

#### Peter Massányi, D.Sc.

Department of Animal Physiology, Faculty of Biotechnology and Food Science, Slovak University of Agriculture in Nitra, Slovak Republic

#### Radoslav Omelka, Ph.D.

Constantine the Philosopher University, Nitra, Slovak Republic

#### Aleš Pavlík, Ph.D.

Department of Animal Morphology, Physiology and Genetics, Faculty of Agronomy, Mendel University in Brno, Czech Republic

#### Krzysztof Piksa, Ph.D.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Barbara Pinto, Ph.D.

University of Pisa, Italy

#### Shubhadeep Roychoudhury, Ph.D.

Department of Life Science and Bioinformatics, Assam University, Silchar, India

#### Robert Stawarz, Ph.D.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Waldemar Szaroma, Ph.D.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Krzysztof Tokarski, Ph.D.

Department of Physiology, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

#### Francesco Vizzarri, Ph.D.

University of Molise, Campobasso, Italy

#### Michal Zeman, D.Sc.

Department of Animal Physiology and Ethology, Faculty of Natural Science, Comenius University in Bratislava, Slovak Republic

#### **Organizing Committee**

#### Krzysztof Piksa, Ph.D. – Chair

Institute of Biology, Pedagogical University of Cracow, Poland

#### Robert Stawarz, Ph.D.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Grzegorz Formicki, Ph.D.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Agnieszka Greń, Ph.D.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Łukasz J. Binkowski, Ph.D.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Zofia Goc, Ph.D.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Renata Muchacka, Ph.D.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Bartłomiej Zyśk, Ph.D.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Włodzimierz Wojtaś, Ph.D.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Tomasz Łaciak, Ph.D.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Tomáš Slanina, Ph.D.

Faculty of Biotechnology and Food Science, Slovak University of Agriculture in Nitra, Slovak Republic

#### Edyta Kapusta, MSc.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Marta Batoryna, MSc.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Łukasz M. Kołodziejczyk, MSc.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Katarzyna Stachańczyk, MSc.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Tomasz Brzuskowski, MSc.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Filip Tirpák, MSc.

Faculty of Biotechnology and Food Science, Slovak University of Agriculture in Nitra, Slovak Republic

#### Secretary

#### Zofia Goc, Ph.D.

Institute of Biology, Pedagogical University of Cracow, Poland

#### Renata Muchacka, Ph.D.

Institute of Biology, Pedagogical University of Cracow, Poland

#### CONTENT

CADMIUM INHIBITS CELL GROWTH AND FUNCTION THROUGH ALTERED GENE EXPRESSION IN HUMAN OSTEOBLASTS Adamkovicova M., Lukacova M., Mondockova V., Kovacova V., Sarocka A., Babosova R., Martiniakova M., Omelka R
EXPOSURE TO PYRETHROID INSECTICIDES DISRUPTS DEVELOPMENT OF MICE PREIMPLANTATION EMBRYOS Babel'ová J., Šefčíková Z., Čikoš Š., Špirková A., Kovaříková V., Pisko J., Koppel J., Fabian D
EFFECT OF DRIED POMEGRANATE EXTRACT ON HUMAN OVARIAN GRANULOSA CELLS Baldovská S., Halenár M., Michalcová K., Carbonell-Barrachina A.A., Kolesárová A
EMBRYO-MATERNAL COMMUNICATION DURING PREIMPLANTATION DEVELOPMENTAL PERIOD: CELL RECEPTORS TRANSDUCING SIGNALS FROM MATERNAL ENVIRONMENT Čikoš Š., Špirková A., Kubandová J., Kovaříková V., Šefčíková Z., Fabian D., Koppel J
THE EFFECT OF MUSHROOM EXTRACTS ON <i>STAPHYLOCOCCUS AUREUS</i> FROM IXODID THICKS Čuvalová A., Strapáč I., Handrová L., Kmeť V
THE INFLUENCE OF DIFFERENT WAVELENGTHS OF LIGHT DURING INCUBATION ON PINEAL MELATONIN LEVELS IN CHICK EMBRYO Drozdová A., Okuliarová M., Zeman M
EMBRYOTOXIC POTENTIAL OF VARIOUS TYPES OF INSECTICIDES Fabian D., Babel'ová J., Šefčíková Z., Kovaříková V., Čikoš Š., Špirková A., Koppel J
THE EFFECT OF ASCORBIC ACID ON THE LIPID PEROXIDATION AND REDUCED GLUTATHIONE LEVEL IN PANCREAS OF MICE WITH INDUCED OBESITY Goc Z., Kapusta E., Greń A., Muchacka R., Formicki G., Szaroma W., Massanyi P
THE IMPACT OF ECOLOGICAL AND CONVENTIONAL FARMING ON LIVESTOCK PRODUCTIVITY Gogaľová Z., Poráčová J., Konečná M., Sedlák V., Mydlárová Blaščáková M., Nagy M
THE INFLUENCE OF SEX ON MORPHOLOGICAL PARAMETERS OF WHITE BLOOD CELLS OF JUVENILE WHITE STORK <i>(CICONIA CICONIA)</i> Grandtke M., Ciepliński M., Durajski A., Burda E., Jerzak L., Kasprzak M., Siekiera J
CONCENTRATION DEPENDENT EFFECTS OF HYDROGEN PEROXIDE ON TM3 LEYDIG CELLS Greifová H., Jambor T., Zbyňovská K., Lukáč N
TRADITIONAL USES, PHARMACOLOGICAL EFFICACY, AND PHYTOCHEMISTRY <i>PUERARIA LOBATA</i> (KUDZU) AND SOYA Greń A., Muchacka R., Goc Z., Kapusta E., Kołodziejczyk Ł.M., Formicki G., Szaroma W., Massanyi P22
EFFECT OF ZINC SUPPLEMENTATION ON MINERAL STATUS OF FARM ANIMALS Grešáková Ľ., Holodová M., Čobanová K
ANALYSIS OF MINERAL PROFILE OF SHAGYA-ARAB HORSES IN ENDURANCE Halo M., Mlyneková E., Halo M. Jr., Kovačik A24
PREVALENCE OF ANTIMICROBIAL RESISTANCE IN ENTEROCOCCI FROM WILD LIVING ANIMALS IN SLOVAKIA Hamarová Ľ., Kopčáková A., Javorský P., Pristaš P
THE RELATIONSHIP BETWEEN BIOFILM FORMATION, GENES OF VIRULENCE AND IRON METABOLISM IN <i>ESCHERICHIA COLI</i> Handrová L., Kmeť V., Čuvalová A
MINERAL AND ANTIOXIDANT STATUS OF PIGLETS FED TWO Zn AND FIBRE SOURCES Holodová M., Čobanová K., Barszcz M., Taciak M., Tuśnio A., Grešáková Ľ

/ /////////////////////////////////////	Animal	Ph	vsioloa	/ 2018
---	--------	----	---------	--------

EFFECT OF INCLUSION OF STRAWBERRY LEAVES IN RABBIT FEED ON MEAT QUALITY Kalafova A., Emrichová J., Kovacik J., Bucko O., Lubomír O., Rastislav J., Lubica C., Schneidgenova M., Capcarova M
DEPOSITION OF IMMUNOACTIVE SUBSTANCES INTO THE EGG AND IMMUNE RESPONSE OF YOUNG JAPANESE QUAIL SELECTED FOR SHAPE OF GROWTH CURVE Kankova Z., Drozdova A., Klobetzova Z., Lichovnikova M., Zeman M.
THE IMPACT OF UDN ON SELECTED BLOOD PARAMETERS OF FEMALE SEA TROUT <i>SALMO TRUTTA</i> M. <i>TRUTTA</i> (L.) SPAWNERS Kasprzak M., Ciepliński M., Grandtke M., Steliga A., Kamiński P., Jerzak L
CELLULASE ACTIVITY OF BEETLE SPECIES AT DIFFERENT TEMPERATURES AND SUBSTRATE CONCENTRATION Kaźmierczak S., Szentner K., Waśkiewicz A., Wojciechowicz T., Wasielewski O31
DOES BENZO[ <i>A</i> ]PYRENE AFFECT THE HEART EMBRYONIC DEVELOPMENT? Kołodziejczyk Ł.M., Puzik M., Greń A., Batoryna M., Formicki G., Kapusta E
BIOCHEMICAL MARKERS OF LIVESTOCK HEALTH STATUS Konečná M., Poráčová J., Sedlák V., Mydlárová Blaščáková M., Gogaľová Z., Babejová A., Nagy M., Zahatňanská M., Majherová M
DRAFT GENOME SEQUENCE OF <i>ENTEROCOCCUS FAECIUM</i> 8S3, LACTIC ACID–PRODUCING BACTERIUM FROM BRYNDZA CHEESE Kopčáková A., Dubíková K., Kisková J., Javorský P., Pristaš P
METABOLIC ADAPTATIONS OF DAIRY COWS AT THE BEGINNING LACTATION Kováčik J., Massányi P., Capcarová M., Kalafová A
EFFECTS OF AMYGDALIN ON GENE ACTIVITY IN CULTIVATED HUMAN OSTEOBLASTS Kováčová V., Lukáčová M., Adamkovičová M., Šarocká A., Šranko P., Omelka R., Kolesárová A., Martiniaková M
THE EFFECT OF GLUTAMATE ON DEVELOPMENT OF MOUSE PREIMPLANTATION EMBRYOS Kovaříková V., Babeľová J., Šefčíková Z., Špirková A., Fabian D., Koppel J., Čikoš Š
DETECTION OF TAURINE EFFECT ON THE STRUCTURE OF RABBIT KIDNEY Kročková J., Massányi P., Ondruška Ľ., Macho T
ORGAN TOXICITY OF DIETHYLNITROSAMINE AND CAPSAICIN IN MICE – <i>IN VIVO</i> STUDY Kuchařová V., Daněk O., Škorič M., Veselá I., Tomenendálová J
ALCOHOL ADMINISTRATION AFFECTS COMPACT BONE STRUCTURE OF MICE AFTER ONE REMODELING CYCLE
Martiniakova M., Sarocka A., Babosova R., Kapusta E., Goc Z., Greń A., Formicki G., Omelka R
THE PILOT STUDY: EVALUATING EFFECTS OF FLAVONOID ISOQUERCITRIN ON THE VIABILITY AND STEROIDOGENESIS OF HUMAN GRANULOSA CELLS HGL-5 Michalcová K., Halenár M., Baldovská S., Sanisló Ľ., Křen V., Kolesárová A
DECOMPOSITION OF THE BODIES OF FARM ANIMALS DURING THE WINTER MONTHS BY NECROPHAGOUS SPECIES Mifkova T., Urban T., Horakova J
<i>IN VITRO</i> AND <i>IN VIVO</i> EFFECT OF MEDICINAL PLANTS IN LAMBS WITH ENDOPARASITE INFECTION
INTRACAROVA D., DADJAK IM., KONIGOVA A., PISARCIKOVA J., KISIGAYOVA S., VAGIEJCH J., VARADY M., VARADYOVA Z. 43
HERITAGE BREEDS Muchacka R., Sosnówka-Czajka E., Skomorucha I., Kapusta E., Greń A., Goc Z

HOW SELECTION FOR CONTRASTING YOLK TESTOSTERONE LEVELS AFFECTED REPRODUCTIVE AXIS IN MALE JAPANESE QUAIL Okuliarova M., Meddle S.L., Zeman M
ELIMINATION OF DEATH CELLS IN MICE BLASTOCYTS PRODUCED <i>IN VIVO</i> AND <i>IN VITRO</i> Pisko J., Kovaříková V., Fabian D
ASSESSING OF MOUFLON BIOCHEMICAL PARAMETERS DEPENDING ON GENDERS Pošiváková T., Hromada R., Veszelits Laktičová K., Vargová M., Pošivák J., Švajlenka J47
ALTERATION OF HAEMATOLOGICAL PARAMETERS AFTER SINGLE DOSE OF MYCOTOXINS Schneidgenova M., Kalafova A., Capcarova M
MICE PREIMPLANTATION EMBRYO LOSSES CAUSED BY FIPRONIL Šefčíková Z., Babeľová J., Kovaříková V., Čikoš Š., Špirková A., Koppel J., Fabian D49
INVOLVEMENT OF TRANSCRIPTION FACTORS IN CONTROL OF OVARIAN FUNCTIONS Sirotkin A
EFFECT OF MIXED HERB EXTRACT ON SELECTED STRESS PARAMETERS IN BROILER CHICKENS OF THREE GENETIC LINES Skomorucha I., Sosnówka-Czajka E
MINERAL PROFILE OF RABBIT BLOOD AFTER ZEOLITE ADMINISTRATION Slanina T., Tirpák F., Herc P., Zbyňovská K., Halo M., Vizzarri F., Ožvold M., Kováčik A
DIET SUPPLEMENTATION WITH FLAXSEED STIMULATES GUT METABOLISM IN MICE Sopková D., Vlčková R., Andrejčáková Z., Gancarčíková S., Ondrašovičová S., Petrilla V53
SELECTED BLOOD PARAMETERS IN ORGANICALLY RAISED HENS FED WITH PURPLE CONEFLOWER SUPPLEMENTED DIET Sosnówka-Czajka E., Skomorucha I
IDENTIFICATION OF GLUCOCORTICOID RECEPTOR TRANSCRIPTS IN MOUSE OOCYTES AND PREIMPLANTATION EMBRYOS Špirková A., Babeľová J., Kovaříková V., Šefčíková Z., Fabian D., Koppel J., Čikoš Š
AGE-RELATED CHANGES IN BONE MICROSTRUCTURE OF MICE Sranko P., Sarocka A., Kovacova V., Babosova R., Mondockova V., Uhrin P., Omelka R., Martiniakova M56
EFFECT OF SUBSTANCES USED IN "SMART DRUGS" ON SELECTED PARAMETERS OF SPERMATOZOA MOTILITY Stachańczyk K
PERIODICITY OF CHANGES IN FUNCTIONAL INDICES IN ANIMALS AND HUMANS Strashko S., Bilyk V
VIABILITY ASSESSMENT OF CHICKEN PGCS BY TRYPAN BLUE EXCLUSION AND FLUORESCENCE LABELLING TECHNIQUE Svoradová A., Makarevich A., Čurlej J., Chrenek P
CAN XYLENE AND QUERCETIN DIRECTLY AFFECT BASIC OVARIAN CELL FUNCTIONS? Tarko A., Štochmaľová A., Hrabovszká S., Vachanová A., Harrath A.H., Grossman R., Sirotkin A.V60
TWO SIDES OF NON-IONIZING RADIATION IN DAILY LIFE USE – REPRODUCTIVE APPROACH Tirpak F., Slanina T., Halo M. Jr, Mamrakova R., Massanyi P61
EFFECT OF <i>TARAXACUM OFFICINALE</i> ROOT EXTRACT ON MURINE FIBROSARCOMA CELLS <i>IN</i> <i>VITRO</i>
Iomenendalova J., Korbášová M., Kuchařová V., Veselá I

ALTERATIONS IN SELECTED PARAMETERS OF HORMONAL PROFILE IN DAIRY COWS DURING TRANSITION PERIOD
Vargová M., Veszelits Laktičová K., Pošiváková T., Hromada R., Kováč G
DIFFERENT MACS SORTING STRATEGIES FOR THE ENRICHMENT OF LIN <sup>-</sup> (CD34 <sup>+</sup> CD45 <sup>-</sup> ) HEMATOPOIETIC PROGENITOR CELLS: PRELIMINARY STUDY Vašíček J., Baláži A., Parkányi V., Bauer M
DOES AERUGINOSIN-865 HAVE THE ADVERSE EFFECT ON TUMOUR CELL LINES? Veselá I., Celá Kolísková P., Kuchařová V., Tomenendálová J., Řeháková K., Hrouzek P., Cheel J66
DIETARY SUPPLEMENTATION WITH ALGAE AND POLYPHENOLS IN RABBIT MALE: EFFECTS ON SEMEN QUALITY TRAITS Vizzarri F., Palazzo M., Casamassima D., Corino C., Chiapparini S., Ondruska L., Knizatova N., Massanyi M., Tirpak F., Massanyi P
RELEASE OF OVARIAN HORMONES AND THEIR RESPONSE TO FOLLICLE STIMULATING HORMONE BY THE OVARIES ISOLATED FROM MICE FED FLAXSEED Vlčková R., Andrejčáková Z., Sopková D
THE CONCENTRATION OF MERCURY IN ORGANS OF WHIPFIN SILVER BIDDY ( <i>GERRES</i> <i>FILAMENTOSUS</i> CUVIER, 1829) AND FLATHEAD GREY MULLET ( <i>MUGIL CEPHALUS</i> LINNAEUS, 1758) IN COASTAL CENTRAL VIETNAM Vo Van T., Binkowski Ł.J., Stawarz R
VARIABILITY STUDY OF MHC GENES REGION IN <i>CAMELUS DROMEDARIUS</i> USING MICROSATELLITE ANALYZE Wijacki J., Knoll A
AMYGDALIN AFFECTED IMMUNE RESPONSE OF HUMAN ENDOTHELIAL CELLS Zbyňovská K., Halenár M., Greifová H., Jambor T., Kolesárová A., Lukáč N
LIGHT IN POULTRY PRODUCTION. FROM MANAGEMENT TOOL TO PHYSIOLOGICAL MECHANISMS LIGHT QUALITY – FROM PHYSIOLOGY TO A MANAGEMENT TOOL IN POULTRY PRODUCTION Zeman M., Kankova Z., Drozdova A., Okuliarova M
INDEX

#### CADMIUM INHIBITS CELL GROWTH AND FUNCTION THROUGH ALTERED GENE EXPRESSION IN HUMAN OSTEOBLASTS

Adamkovicova M.<sup>1</sup>, Lukacova M.<sup>1</sup>, Mondockova V.<sup>1</sup>, Kovacova V.<sup>2</sup>, Sarocka A.<sup>2</sup>, Babosova R.<sup>2</sup>, Martiniakova M.<sup>2</sup>, Omelka R.<sup>1</sup>

<sup>1</sup>Department of Botany and Genetics, <sup>2</sup>Department of Zoology and Anthropology, Faculty of Natural Sciences, Constantine the Philosopher University in Nitra, Slovak Republic

The bone is a sensitive target of cadmium (Cd) toxicity. Osteoporosis, increased bone fractures, decreased bone mineral density are common findings in populations exposed to Cd. This study was aimed to investigate the effects of Cd on growth and function of human bone cells. For that, the primary human osteoblasts (PromoCell) were exposed to 0, 0.1, 2 and 5 µM of Cd for 48h. Cell proliferation, cytotoxicity and alkaline phosphatase activity were evaluated using colorimetric assays. An RT2 Profiler Custom PCR Array (Oiagen) was used to examine the mRNA levels of COL1A1, ALPL, BGLAP, WNT5A, RUNX2, BAX, CASP1, SOD1, GPX1 genes. The genes are connected with ossification, osteoblast differentiation, bone mineralisation, oxidative stress and apoptosis. In all Cd treated groups, there was a marked reduction of cell size. Cd had varying effect on cell viability, which was decreased in cells exposed to 0.1 µM and 5 µM Cd. The 5 µM Cd dose inhibited cell growth and caused morphological changes such as rounding, shrinkage, and detachment of the cells. The inhibition of alkaline phosphatase activity and gene expression indicates diminished bone mineralisation at 5 µM Cd. At the highest dose, Cd also decreased collagen production by reduced type I collagen gene expression. Furthermore, the expression of antioxidant enzyme glutathione peroxidase 1 was also down-regulated by 5 µM Cd. Overall, results showed direct inhibitory Cd effects on osteoblastic cells which may lead to decreased bone formation and higher oxidative stress sensitivity.

Keywords: cadmium, osteoblasts, gene expression

Acknowledgments: The study was supported by the projects VEGA 1/0505/18 and KEGA 031UKF-4/2016. This article was written during realization of the project CEGEZ No. 26220120073 supported by the Operational Programme Research and Development funded from the European Regional Development Fund.

#### EXPOSURE TO PYRETHROID INSECTICIDES DISRUPTS DEVELOPMENT OF MICE PREIMPLANTATION EMBRYOS

Babeľová J., Šefčíková Z., Čikoš Š., Špirková A., Kovaříková V., Pisko J., Koppel J., Fabian D.

Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences, Slovak Republic

The aim of this study was to evaluate the potential toxicity of pyrethroid insecticides (permethrin, deltamethrin, fenvalerate,  $\lambda$ -cyhalothrin) and commercially available pyrethroid products TOP SPOT ON STRONGER (permethrin mixture), DECIS EW 50 (deltamethrin mixture) on development and guality of mouse preimplantation embryos. During *in vitro* tests, 2-cell stage embryos isolated from superovulated mice dams were cultured in media with addition of listed chemicals until blastocysts formation. Stereomicroscopic examination of *in vitro* collected embryos showed that all pyrethroids negatively affected embryonic development at the highest tested concentration (1000 µM). In case of deltamethrin, harmful impact on embryo development was detected also at 100 µM concentration. As shown by fluorescence staining, such obtained blastocysts displayed significantly lower number of blastomeres in permethrin and fenvalerate treated embryos. Addition of fenvalerate and  $\lambda$ -cyhalothrin (1000 µM) increased incidence of death cells in obtained blastocysts. Lower concentrations of pyrethroids had no effect on any of evaluated parameters. On the other hand, the presence of TOP SPOT ON STRONGER in medium at concentration containing 100 µM of permethrin resulted in impaired blastocysts formation with lower total cell counts, while presence of DECIS EW 50 at concentration containing 100 uM of deltamethrin caused massive degenerations of all embryos. The obtained results indicate that embryotoxic potential of active substances of pyrethroid insecticides is relatively low. On the other hand, commercial pyrethroid products have the potential to impair mouse preimplantation embryo development at even lower concentration than their main compounds.

Keywords: pyrethroids, preimplantation embryos, mouse

Acknowledgments: Study was supported by the Slovak Research and Development Agency under contract APVV 14-0763.

#### EFFECT OF DRIED POMEGRANATE EXTRACT ON HUMAN OVARIAN GRANULOSA CELLS

Baldovská S.1\*, Halenár M.1, Michalcová K.1, Carbonell-Barrachina A.A.2, Kolesárová A.1

<sup>1</sup>Slovak University of Agriculture in Nitra, Slovakia <sup>2</sup>University of Miguel Hernández, Orihuela (Alicante), Spain \* sbaldovska@gmail.com

In these times, several studies investigate bioactive components as a means to associate them with a specific beneficial effect and develop future products and therapeutic applications. Pomegranate (*Punica granatum*) has great interest from consumers due to its reported benefits to human health. Various parts of this plant have been widely used in traditional medicine. Pomegranate fruit is a rich source of polyphenolic compounds like flavonoids, anthocyanins, flavanols and specially ellagitannins (including punicalagins), which release ellagic acid when hydrolyzed. However, the synergistic action of the pomegranate bioactive components appears to be superior when compared to individual constituents. In addition, this fruit present strong anti-inflammatory, antioxidant, antibacterial and antiobesity properties. Pomegranates have been shown to exert antiproliferative and proapoptotic effects on cancer cells, thus leading to an increased popularity as a functional food and nutraceutical source. Ovarian granulosa cells are the sites of estrogen and progesterone production, making them critical in studying steroid biosynthesis. The aim of our study was to determine the effect of dry extract of pomegranate fruit (at the doses 0; 2.5; 5; 10; 20 and 40 ug/ml; for 24h) on the viability of human ovarian granulosa cell line (HGL-5) and secretion of steroid hormones (17 $\beta$ -estradiol, progesterone). The metabolic activity was evaluated by *alamarBlue*<sup>TM</sup> cell viability assay, the release of steroid hormones was assayed by ELISA methods. The number of viable HGL-5 cells significantly ( $P \le 0.001$ ) increased after addition of pomegranate extract at the concentrations 2.5; 5; 10; 20 µg/ml compared to the control. Moreover, our results indicated a significant ( $P \le 0.05$ ;  $P \le 0.001$ ) increase of the 17 $\beta$ -estradiol levels by pomegranate extract at the concentrations 5; 10; 20 and 40 µg/ml in comparison to control. On the other hand, progesterone secretion was not significantly (P≥0.05) affected at all used concentrations. Our study shows dosedependent effects of dried pomegranate extract on steroidogenesis in the human granulosa cells. This *in vitro* cell model system is important tool for the study of ovarian function. The results from our study support the fact that pomegranate fruit is indeed a source of biologically active compounds with beneficial properties.

Keywords: pomegranate, granulosa cells, steroid hormones, viability

Acknowledgment: This work was supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic projects VEGA 1/0039/16, KEGA 011SPU-4/2016, APVV-15-0543, APVV-16-0170 and EU project no. 26220220180: Building Research Centre "AgroBioTech".

#### EMBRYO-MATERNAL COMMUNICATION DURING PREIMPLANTATION DEVELOPMENTAL PERIOD: CELL RECEPTORS TRANSDUCING SIGNALS FROM MATERNAL ENVIRONMENT

Čikoš Š., Špirková A., Kubandová J., Kovaříková V., Šefčíková Z., Fabian D., Koppel J.

Institute of Animal Physiology, Centre of Biosciences of the Slovak Academy of Sciences, Košice, Slovakia

Results of animal studies and observations in humans have shown that maternal physiological condition (nutritional status, body condition, metabolic and other disorders, stress) and external environmental factors (e.g. various xenobiotics that can contaminate food) can significantly influence preimplantation embryo development, and disturbances at this developmental period can have long-term consequences, including unsuccessful gravidity or impaired offspring health. These data indicate that communication between the embryo and its environment takes place already in very early developmental stages. In our study, we examined expression of catecholamine receptors during preimplantation stage of mammalian embryo development. We found that several types of catecholamine receptors are expressed in mouse, rabbit and bovine preimplantation embryos. Interestingly, expression of receptor subtypes which couple primarily to G proteins with opposing actions on adenylyl cyclase activity was found in ovulated oocytes and/or preimplantation embryos of all three examined species. Results of our *in vitro* functional tests suggest that signaling pathways activated by these receptors could converge to regulate early embryo development. Our results also revealed that maternal physiological status can influence expression of some catecholamine receptors in early embryos.

Keywords: preimplantation embryo, embryo-maternal communication, catecholamine receptors

Acknowledgments: This work was supported by the Slovak Research and Development Agency project APVV-14-0763.

### THE EFFECT OF MUSHROOM EXTRACTS ON *STAPHYLOCOCCUS AUREUS* FROM IXODID THICKS

Čuvalová A.<sup>1\*</sup>, Strapáč I.<sup>2</sup>, Handrová L.<sup>1</sup>, Kmeť V.<sup>1</sup>

<sup>1</sup>Institute of Animal Physiology, Centre of Biosciences of the SAS, Kosice, Slovak Republic <sup>2</sup>Department of Chemistry, Biochemistry and Biophysics, Institute of Pharmaceutical Chemistry, University of Veterinary Medicine and Pharmacy, Kosice, Slovak Republic \* cuvalova@saske.sk

Mushrooms have long been appreciated for their taste, flavour, desirable aroma, texture, nutraceutical and medicinal attributes [1]. Also, they are a renowned source of products with an array of bioactivities, from antibacterial to antiviral, cytotoxic, antiinflammatory, antifeeding, antifungal or antioxidant and might be a valuable resource in the search of new bioactive extracts to inhibit biofilm production [2]. Mushrooms might be a valuable resource in the search of new bioactive compounds to inhibit biofilm production [3]. We demonstrate the effect of five mushroom water extracts Macrolepiota procera, Pleurotus ostreatus, Hirneola auricula Judae, Armillaria mellea and Laetiporus sulphurous on biofilm formation of four Staphylococcus aureus strains isolated from ixodid ticks and ewe's milk. The PCR was used for detection of virulence genes (*hla-* alphahemolysin, isdA, B- iron regulated surface determinants A, B, bbp- bone sialoprotein- binding protein, sirB-siderophore, fnbpA- fibronectin- binding protein A, sdrE- serine-aspartate repeat proteins E and agr II - accessory gene regulator). The ability of biofilm formation and anti-biofilm activity was assessed in a quantitative crystal violet assay. The effect on staphylococcal biofilm formation were tested by addition of water extracts from mushrooms. The biofilm formation of Staphylococcus aureus strains was significantly reduced by all mushrooms extracts (p < 0.001). We showed that more significant anti-biofilm effect of the extracts were of *Staphylococcus aureus* isolated from ixodid ticks in comparison to Staphylococcus aureus isolated from ewe's milk. In the present study, A. mellea, P. ostreatus, L. sulphurous, H. auricula Judae and M. procera extracts inhibited biofilm formation by 70.87 %, 67 %, 64.14 %, 62.77 % and 47.71 %, respectively. The results suggest that compounds in mushrooms extracts might be useful to control and handle detrimental infections caused by animal and human pathogens.

Keywords: biofilm, fungi, Staphylococcus aureus, ixodid ticks, milk

#### References

Strapáč, I. et al.: Antioxidant activity of honey mushrooms (*Armillaria Mellea*). Folia Veterinaria, 2016, 60, 37-41.
Martín-Rodríguez, A. J.: Inhibition of Bacterial Ouorum Sensing by Extracts from Aguatic Fungi: First Report from

[2] Martin-Kooriguez, A. J.: Inhibition of Bacterial Quorum Sensing by Extracts from Aquatic Fungi: First Report from Marine Endophytes. *Marine Drugs*, 2014, 12, 5503-5526.

[3] Alves, M.J. et al: Wild Mushroom Extracts as Inhibitors of Bacterial Biofilm Formation. *Pathogens*, 2014, *3*, 667-679.

Acknowledgments: This study was supported by the Slovak projects APVV 14-0274 and VEGA 2/0085/18.

#### THE INFLUENCE OF DIFFERENT WAVELENGTHS OF LIGHT DURING INCUBATION ON PINEAL MELATONIN LEVELS IN CHICK EMBRYO

#### Drozdová A., Okuliarová M., Zeman M.

Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic

Our previous studies proved that the pineal gland exhibits a rhythmic melatonin biosynthesis under in vivo and in vitro conditions. The circadian rhythms melatonin production in chick embryo can be entrained during the last third of embryonic life by the light-dark and partially by temperature cycles. Light is a dominant environmental factor controlling melatonin biosynthesis, with high levels at night and low levels during the light phase. All previous studies were performed with polychromatic white light and little is known about the effect of different wavelengths of light during incubation on melatonin biosynthesis. Therefore, the purpose of this study was to examine effects red, blue, green and white light on rhythmic melatonin production in the pineal gland of 20-dayold chick embryos. The study was performed with Ross 308 broiler equals (n = 450), incubated under the light-dark cycle 12L:12D using white, red, green or blue light during the light phase. Pineal glands were sampled over 24hour cycle and immediately frozen at -80°C. Pineal glands were extracted in absolute methanol and melatonin was measured by radioimmunoassay. Pineal melatonin levels in chick embryos exhibited circadian rhythms under all four wavelengths and the highest concentrations were found in chick embryos incubated under red and white light and the lowest levels under blue light. The incubation under green light induced intermediate results. Melatonin production increased significantly after hatching. In 2-day-old chicks we did not find differences in melatonin levels between white and green incubated light. Our results showed for the first time a different sensitivity of chick embryos pineal gland to different wavelengths of monochromatic light and showed the red light is the most efficient in stimulation of rhythmic melatonin production in embryos.

Keywords: chick embryos, pineal gland, melatonin, incubation, wavelengths of light

Acknowledgments: This study was supported by a grant VEGA 1/0686/15.

#### EMBRYOTOXIC POTENTIAL OF VARIOUS TYPES OF INSECTICIDES

Fabian D., Babeľová J., Šefčíková Z., Kovaříková V., Čikoš Š., Špirková A., Koppel J.

Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences, Slovak Republic

Despite the declared minimal danger for mammalian cells and organs, previous studies have demonstrated that insecticides could adversely affect the mammalian reproductive system. In current study, the sensitivity of undifferentiated pluripotent mammalian embryonic cells to three widely-used types of insecticides as well as their related commercial products was analyzed on mouse model. Results of *in vitro* and *in vivo* tests showed that the potential embryotoxicity of evaluated insecticides rises in the following order: pyrethroids > neonicotinoids > phenyl-pyrazoles. The presence of active components of neonicotinoids and phenyl-pyrazoles at relatively low doses in the microenvironment of embryo negatively affected both developmental capacities and quality of preimplantation embryos. Besides, detrimental effects of several secondary ingredients of commercial preparations were recorded as well. These findings make insecticides one of important environmental factors with potential to negatively affect the continuance of early pregnancy in pets, livestock and wild-living animals, whose are frequently exposed to them either at high single dose (veterinary pests control) or at low doses repeated for a long time (consumption of contaminated vegetables, fruits or crops).

Keywords: neonicotinoids, pyrethroids, phenyl-pyrazoles, preimplantation embryos, mouse

Acknowledgments: Study was supported by the Slovak Research and Development Agency under contract APVV 14-0763.

#### THE EFFECT OF ASCORBIC ACID ON THE LIPID PEROXIDATION AND REDUCED GLUTATHIONE LEVEL IN PANCREAS OF MICE WITH INDUCED OBESITY

Goc Z., Kapusta E., Greń A., Muchacka R., Formicki G., Szaroma W., Massanyi P.

Institute of Biology, Pedagogical University of Cracow, Poland

The objective of the study was to determine the effect of ascorbic acid (AA) supplementation on malonyldialdehyde (MDA) and reduced glutathione (GSH) level in pancreas of mice fed a high-fat diet (HFD). Forty male Swiss mice were divided into four groups. First group of mice (control group) was fed on standard pelleted diet containing 16.6% of energy as protein, 73.1% of energy as carbohydrate and 10.3% of energy as lipid by dry weight. The second group of animals was fed on a high-fat diet in order to generate a diet-induced obesity model. The third group of animals was supplemented with a daily dose of ascorbic acid (750 mg kg<sup>-1</sup> mouse day<sup>-1</sup>) mixed into the standard food. The forth group received HFD contains ascorbic acid at the same dose. Animals had ad libitum access to water during the trial. At the end of the experimental period (12 weeks), mice were sacrificed by decapitation and pancreas were immediately collected to determine the level of MDA and GSH. The data were evaluated using one-way Anova followed by Tukey's post-hoc test. The obtained results showed that feeding mice on HFD caused significant (p>0.05) elevation of the body weight, increase of MDA level and decrease of GSH concentration. On the contrary, a high fat diet supplemented with ascorbic acid exhibited a significant reduction in mass accumulation over time when compared to the HFD group. Furthermore, resulted in a significant increase of GSH level when compared to the high fat diet alone. In conclusion, the current study demonstrated that dietary ascorbic acid supplementation inhibited development of obesity in high fat fed and supported the antioxidant system.

Keywords: obesity, ascorbic acid, high-fat diet

#### THE IMPACT OF ECOLOGICAL AND CONVENTIONAL FARMING ON LIVESTOCK PRODUCTIVITY

Gogaľová Z.<sup>1</sup>, Poráčová J.<sup>1</sup>, Konečná M.<sup>1</sup>, Sedlák V.<sup>1</sup>, Mydlárová Blaščáková M.<sup>1</sup>, Nagy M.<sup>2</sup>

<sup>1</sup>The University of Prešov, Faculty of Humanities and Natural Sciences, Department of Biology, Prešov, Slovak Republic \* zuzana.gogalova@smile.unipo.sk

<sup>2</sup>J. Selye University, Faculty of Education, Komárno, Slovak Republic

Ecological farming products are currently of great interest. The popularity of organic and natural foods is constantly rising. Milk is one of the most important commodities in the world. Consequently, the focus is on the benefits of milk obtained from ecological farmer. The work deals with an evaluation and comparison of milk quality in conventional and ecological breeding of Holstein cattle. In both breeds, the feed and the effect of the season were monitored. These factors can affect the taste of milk, its composition and can improve the health benefits of milk. The amount and composition of the milk was monitored for one year at an interval of each month. In milk samples were analyzed parameters such as proteins, lactose and fats. The work also took into account the number of somatic cells and fat-free solids in milk. Based on statistical data processing, ecological farming has shown lower yields than traditional farming in breed. In milk from ecological farming, however, we recorded higher fat and protein content. These findings can contribute to the knowledge of the ecological farming of the Holstein breed in view of the breed's popularity and growing interest in ecological farming and its products.

Keywords: ecological farming, holstein, milk, utility

Acknowledgements: The work was supported by project: Biochemical, physiological and haematological status in selected species of hunting game, VEGA 1/0783/18.

#### THE INFLUENCE OF SEX ON MORPHOLOGICAL PARAMETERS OF WHITE BLOOD CELLS OF JUVENILE WHITE STORK (CICONIA CICONIA)

Grandtke M.<sup>1\*</sup>, Ciepliński M.<sup>1</sup>, Durajski A.<sup>1</sup>, Burda E.<sup>1</sup>, Jerzak L.<sup>1</sup>, Kasprzak M.<sup>1</sup>, Siekiera J.<sup>2</sup>

<sup>1</sup> Poland Faculty of Biological Sciences, University of Zielona Gora, Poland \* mgrandtke@wp.pl <sup>2</sup> Zvwocice, Poland

Blood samples were taken from 53 juvenile White Storks. The research was conducted in southern Poland (around Krapkowice, near Opole city). The blood samples were collected from the cutaneous ulnar for further analysis. Blood smears were prepared immediately and were stained using the May Grunwald method. Evaluation of avian leukocytes morphology included observation of the cells in a monolayer x1000 field. Leukocytes in bird blood include lymphocytes, monocytes and granulocytes. The granulocytes are further classified as heterophils, eosinophil and basophils. Lymphocytes (37%) of White Storks may be identified as typically round cells with dark purple nonlobed, eccentrically positioned nucleus. They were small and well differentiated with an average of  $8,10 \pm 0.66 \ \mu\text{m}$  in diameter for large lymphocytes and  $5.31 \pm 0.65 \ \mu\text{m}$  for small lymphocytes. Monocytes (8%) were typically the largest leukocytes present in the blood film of White Stork. measuring  $13,40 \pm 0.97 \,\mu\text{m}$  in diameter. The cytoplasm is abundant and it stained blue-gray, very often contained vacuoles. Heterophils (41%) were the largest of granular leukocytes. They were round and  $11.14 \pm 0.65$  µm in diameter. The nucleus of heterophil was lobed, usually with two or three lobes. The cytoplasm contained brick-red, elongated (rod or spiculate shaped) granules. Eosinophils (9,4%) were similar to heterophils due to their size,  $10,72 \pm 0,49$  µm in diameter. The nucleus was lobed and mostly stained clear blue and contained red-orange round or rod-shaped granules. Basophils (1,8%) were round and contained dark granules, with average of  $9,56 \pm 0,78$ µm in diameter. The nucleus was usually nonlobed. Here, we present data on a suite of commonly measured blood parameters from 53 White Stork Ciconia ciconia chicks of molecularly known sex. We examine whether sex contributed to variation in some blood parameters. There were no significant differences in levels of leukocytes between male and female.

Keywords: blood cell size, white stork, hematology, morphometric, morphology

#### CONCENTRATION DEPENDENT EFFECTS OF HYDROGEN PEROXIDE ON TM3 LEYDIG CELLS

Greifová H.\*, Jambor T., Zbyňovská K., Lukáč N.

Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic

\* hgreifova@gmail.com

Endogenous reactive oxygen species such as hydrogen peroxide  $(H_2O_2)$  have long been studied as destructive molecules contributing to the development of oxidative stress in cells. Currently, there is a growing body of evidence indicating that  $H_2O_2$  at lower physiological concentrations could act as an intracellular signaling molecule. The objective of present study was to evaluate the role of  $H_2O_2$  in Levdig cell function at both low and high levels during 24 h of cultivation. Our *in vitro* experiment was focused on viability, intracellular production of superoxide radical and biosynthesis of testosterone and androstenedione by TM3 Levdig cells after cultivation with 20, 50, 100, 300 and 600  $\mu$ M of H<sub>2</sub>O<sub>2</sub>. The cell viability was measured by the MTT (metabolic activity) assay, the nitroblue-tetrazolium (NBT) test was applied to quantify the intracellular superoxide formation and the level of both hormones was evaluated from the culture medium using enzyme-linked immunosorbent assay (ELISA). The MTT test showed a significant increase of viable cells in the case of experimental groups treated with concentrations 20 and 50  $\mu$ M H<sub>2</sub>O<sub>2</sub> (p < 0.01), and also in cells exposed to 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> (p < 0.05). On the other hand, higher concentrations of H<sub>2</sub>O<sub>2</sub> (300 and 600  $\mu$ M) led to a significant (p < 0.001) decline of cell viability after 24 h of cultivation. NBT test revealed increasing tendency of intracellular superoxide production beginning at the concentration 50  $\mu$ M H<sub>2</sub>O<sub>2</sub> with significant (p < 0.001) higher values in samples treated with 300  $\mu$ M H<sub>2</sub>O<sub>2</sub>. Steroidogenesis was significantly impaired in cells exposed to 300  $\mu$ M H<sub>2</sub>O<sub>2</sub> (p < 0.001) in the case of androstenedione, and in cells exposed to 300 and 600  $\mu$ M H<sub>2</sub>O<sub>2</sub> (p < 0.05) in the case of testosterone.

Keywords: hydrogen peroxide, Leydig cells, MTT, NBT, steroidogenesis

Acknowledgments: This work was financially supported by the projects APVV 15-0543, KEGA 009SPU-4/2017 and European Community under project no. 26220220180: Building Research Centre "AgroBioTech".

#### TRADITIONAL USES, PHARMACOLOGICAL EFFICACY, AND PHYTOCHEMISTRY *PUERARIA LOBATA* (KUDZU) AND SOYA

Greń A., Muchacka R., Goc Z., Kapusta E., Kołodziejczyk Ł.M., Formicki G., Szaroma W., Massanyi P.

#### Institute of Biology, Pedagogical University of Cracow, Poland

Plants play a vital role in cultural, social, religious, environmental, and nutritional aspects. Among all other purposes, the use of plants as medicine for human health originated  $\sim$ 60,000 years ago in the mid-Paleolithic age. Sova beans are an abundant source of bioactive compounds of which isoflavonoids have received significant attention due to their potential anticarcinogenic and antiproliferative effects and possible role in many signal transduction pathways. There are two major kinds of isoflavones in sovbeans: alvcosides, including daidzin and genistin and aglycons. Genistein (4', 5, 7-trihydroxyisoflavone) is naturally present in plants of the soy family and is known to have various pharmacological activities, such as anti-cancer, anti-diabetic, anti-oxidant, etc. Kudzu (Pueraria lobata) is a perennial and leguminous vine native to Southeast Asia. Kudzu root has a long history of medicinal use for fever, diarrhea, diabetes, and hangover in China, Japan and Korea. Not only the root but also the flower has been used for alcohol intoxication. Most biological studies have been focused on the kudzu root and flower. In addition, kudzu leaves are edible and used in various foods, as are the root and flower. Kudzu leaves contain kakkalide, genistin, rutin, robinin (kaempferol-3-O-robinoside-7-O-rhamnoside), nicotiflorin (kaepmferol-3-O-rutinoside), and kaikosaponin III. So, the aim of the present review is to provide comprehensive information from recognized sources on the traditional uses, pharmacological efficacy and phytochemistry of the medicinal plants: Pueraria lobata (kudzu) and soya (genistin). The information provided in this review will be very useful for further studies to develop novel therapeutic drugs.

**Keywords:** *Pueraria lobata* (kudzu), soya, genistin, medicinal plant, traditional uses, pharmacology, phytochemistry

#### EFFECT OF ZINC SUPPLEMENTATION ON MINERAL STATUS OF FARM ANIMALS

Grešáková Ľ.\*, Holodová M., Čobanová K.

Institute of Animal Physiology, Centre of Biosciences of the Slovak Academy of Sciences, Košice, Slovak Republic \* gresakl@saske.sk

Feed supplementation with zinc can affect the mineral absorption from the gastrointestinal tract and it could result in different tissue deposition of the trace elements in animals. The nutrient composition and zinc dietary source in feedstuffs have a great effect on mineral absorption and availability. Moreover, the different Zn supplements can influence the expression and levels of transporter proteins responsible for metal ion transport in cells. We investigated the concentration Zn, Cu, Fe and Mn in plasma, liver and kidney of lambs, broiler chickens and rabbits fed diets supplemented with different Zn sources up to maximal total Zn content allowed in the EU. Intake of diets supplemented with zinc either from zinc sulphate or organic sources as Zn-chelate of glycine hydrate (ZnGly) and Zn-chelate of protein hydrolysates (ZnPro) increased Zn concentration in plasma of broiler chickens and in liver tissue of rabbits, regardless of Zn dietary source. The highest Zn liver concentrations were determined in broilers fed the ZnPro diet, while the highest Cu content was measured in liver of rabbits from the same dietary treatment. On the other hand, decreased Cu levels in plasma and liver tissue were determined in lambs supplemented with the ZnPro enriched diet. Feeding of the Zn supplemented diet from ZnGly elevated plasma Zn levels in lambs; however, the increased Zn content in liver tissue was only determined in lambs given ZnSO<sub>4</sub>. Changes in mineral tissue concentrations (Mn, Fe) were not recorded in any animals. Given the different Zn and Cu tissue uptake, it seems that Zn supplementation affect the different metal transport systems depending on Zn dietary source, animal tissue and animal species as well.

Keywords: zinc; mineral tissue uptake; ruminants; broiler chickens; rabbits

Acknowledgement: This study was supported by the Slovak Grant Agency VEGA 2/0069/17.

#### ANALYSIS OF MINERAL PROFILE OF SHAGYA-ARAB HORSES IN ENDURANCE

Halo M.<sup>1\*</sup>, Mlyneková E.<sup>1</sup>, Halo M. Jr.<sup>2</sup>, Kovačik A.<sup>2</sup>

<sup>1</sup>Department of Animal Husbandry, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra <sup>2</sup>Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra \* marko.halo@uniag.sk

In our work was analysed mineral profile in blood serum of Shagya-arab horses, which were used in endurance. All analysed horses were tested in endurance races on distances of 90 kilometres, in age of 8 – 10 years, 4 mares and 4 geldings. The calcium content corresponded with references values from 2.5 mmol.1<sup>-1</sup> to 3.3 mmol.1<sup>-1</sup> in all tested horses. All horses had normal level of phosphorus in blood serum, excepting horse number 7. We consider, that increased level of phosphorus was caused by Periodic Ophthalmia (Moon Blindness), because phosphorus content was 1.56 mmol.I<sup>-1</sup>, whereby in other horses level were in range 0.5 mmol.I<sup>-1</sup> to 1.5 mmol.I<sup>-1</sup>. Magnesium content complied in all horses according to supposed references from 0.7 mmol.<sup>1-1</sup> to 1.1 mmol.1<sup>-1</sup>. Concentration of sodium in horse number 7 was again different from other tested horses, where level of sodium was in references values and in horse number 7 was slightly lower 124.1 mmol.1-1. Final concentrations of potassium in blood serum of examined horses were on top values and in horse number 7 was value of potassium extremely high on level 18.39 mmol.I<sup>-1</sup>. This above average is as consequence increased proteins in blood, what repeatedly pointing out inflammation of the conjunctiva and subsequent blindness. Chlorine is one of the important element during formation of hydrochlorid acid in stomach and participate on decomposition of proteins in stomach. In each blood sample was determined, that chlorine content was in normal values from 98 mmol.I<sup>-1</sup> to 109 mmol.I<sup>-1</sup> and there were no significant deviations. All examined values of mineral profile indicate on good quality and good nutrients balanced feeding dose. At the same time can be stated, that occupational load did not cause any changes in mineral profile of tested horses.

Keywords: mineral profile, blood serum, endurance, Shagya-arab, mineral substances

Acknowledgement: Supported by the Cultural and Educational Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic (KEGA) (Project No. 021SPU-4/2017).

#### PREVALENCE OF ANTIMICROBIAL RESISTANCE IN ENTEROCOCCI FROM WILD LIVING ANIMALS IN SLOVAKIA

Hamarová Ľ.<sup>1</sup>, Kopčáková A.<sup>1</sup>, Javorský P.<sup>1</sup>, Pristaš P.<sup>2</sup>

<sup>1</sup>Institute of Animal Physiology, Centre of Biosciences of the SAS, Košice, Slovak Republic <sup>2</sup>Institute of Biology and Ecology, Pavol Jozef Šafárik University in Košice, Slovak Republic

Resistance to antibiotics has been rising in recent decades not only in hospital environments and among pathogenic bacteria but resistant bacteria can be found in nearly all milieus. In our study, enterococci from the gastrointestinal tract of wild animals were analysed for antibiotic resistance. Samples were collected from faeces of 31 animal species (20 mammals and 11 birds). The enterococci were isolated by cultivation on selective media and identified using MALDI-TOF mass spectrometry. Six species of enterococci with highest incidence of *E. faecalis* were detected. All enterococci (283) were tested against selected antibiotics by disc diffusion method. The resistance to at least one antibiotic was found in 36 % isolates. The most frequent antibiotic resistance patterns of enterococci was tetracycline (29.3 %) followed by resistance to erythromycin (15.9 %). ampicillin (9.2 %) and vancomvcin (5.7 %). Among all resistant isolates 8.8 % had multidrugresistant phenotype. The majority of tetracycline resistant isolates (90.8 %) were positive for tetM gene, 55.2 % for tetL gene. ermB gene (91.1 %) dominated over metA determinant (35.6 %) in erythromycin resistant isolates. Observed resistance patterns of wild animals enterococci are similar to resistance patterns detected in farm animals but are occurred in lower frequencies. This fact indicating that farm environment should affect the spreading of resistance genes among wild animals microbiota. On other hand, our results suggest that faecal contamination by wild animals may represent a risk in terms of the spread of antibiotic resistance in environment.

Keywords: antimicrobial resistance, enterococci, wild animals

Acknowledgement: This work was supported by APVV-16-0171.

#### THE RELATIONSHIP BETWEEN BIOFILM FORMATION, GENES OF VIRULENCE AND IRON METABOLISM IN *ESCHERICHIA COLI*

Handrová L.\*, Kmeť V., Čuvalová A.

Institute of Animal Physiology, Centre of biosciences of the SAS, Kosice, Slovak Republic \* handrova@saske.sk

Escherichia coli is known as one of the bacterial species with the widest adaptability to variety of niches either within organisms or outside in environment. Most strains of *E. coli* are of low virulence and associated with opportunistic infections, whereas others are highly virulent [1]. The success of E.coli in colonizing such a wide range of hosts and environments is basically due to a noticeable ductility in exploiting the available resources. It is becoming increasingly clear that biofilms have an enormous impact on medicine [2] because since 65% of animal and human bacterial infections involve biofilms [3]. In present study, we isolated strains of *E. coli* from animals, 19 interesting isolates were selected and tested by PCR amplification to virulence - iutA, cvac, iss, tsh, papC, kps, iha and iron metabolism genes - sitA, feoB, irp2, fvuA, iroN, ireA. The ability of biofilm formation was assessed in a quantitative assay using a microtiter-plate test. Bacterial strains were grown on BHI. We divided isolates of *E.coli* into four classes (modification of method and classification Stepanovic et al. [4]): very weak (63%), weak (10,5%), moderate (10,5%) and strong (16%) biofilm producers. Representation genes of virulence were highly in isolates from very weak biofilm producers- from 7 genes were 6 highly, only pape (P fimbrial adhesin) was low. Genes of iron metabolism were different. Genes - sitA, fyuA, ireA were represented higher in better isolates producing biofilm and feoB, irp2, iroN in weak producers. The results show possible relation between presence virulence factor and low biofilm formation.

Keywords: biofilm, virulence genes, iron metabolisms genes

#### References

[1] MARABITO S.: Patogenic *Escherichia coli*, Molecular and Cellular Microbiology. EU Reference Laboratory for *E.coli*. Instituto Superiore si Sanita, Rome, Italy. 2014, p.2-3.

[2] MAH, TF.; O'TOOLE GA.: Mechanisms of biofilm resistance to antimicrobial agents. Trends in Microbiology Volume 9, Issue 1, 1 January 2001, Pages 34-39.

[3] LABBATE, M.; QUECK SY.; KOH KS.; RICE SA.; GIVSKOV M., KJELLEBERG S.: Quorum-sensing-controlled biofilm development in Serra- tia liquefactions MG1. J. Bacteriol. Vol. 186, 2004, p. 692–698.

[4] STEPANOVIC et al.: Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. Journal Compilation APMIS, 2007, vol. 115, p. 891–9. ISSN 0903-4641.

Acknowledgement: This work was supported by Slovak grant VEGA 2/0085/18.

#### MINERAL AND ANTIOXIDANT STATUS OF PIGLETS FED TWO Zn AND FIBRE SOURCES

Holodová M.1\*, Čobanová K.1, Barszcz M.2, Taciak M.2, Tuśnio A.2, Grešáková Ľ.1

<sup>1</sup>Institute of Animal Physiology, Centre of Biosciences of the Slovak Academy of Sciences, Košice, Slovak Republic <sup>2</sup>The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland \* holodova@saske.sk

The source of zinc and fibre can affect the absorption of minerals from the gastrointestinal tract results in altered mineral status of animals. The aim of study was to compare the effect of two dietary fibre types and zinc sources on zinc concentration and activity of superoxide dismutase (SOD) in plasma, liver, kidney and pancreas of piglets, and also the concentrations of the metallotransporters: zinc metallothionein (Zn-MT1) and Zn-transporter (ZnT1) in liver tissue. The experiment was performed on 24 castrated male Danbred x Duroc piglets after weaning with initial body weight about 11 kg. The piglets were randomly allocated to four dietary groups and fed a cereal-based diet (BD) supplemented with either 1.7% of cellulose or 5% potato fibre, which corresponded to 1% addition of crude fibre, and 120 mg Zn/kg from Zn different sources: ZnSO<sub>4</sub> and Zn chelate of glycine hydrate (ZnGly). During a 4-week feeding period the piglets fed the experimental diets: the BD containing the cellulose and Zn from either ZnSO<sub>4</sub> (C) or ZnGlv (C+ZnGly), and the BD supplemented with the potato fibre and  $ZnSO_4$  (PF) or ZnGly (PF+ZnGly). Intake of the experimental diets affected the Zn concentration in neither plasma nor the tissues. The activity of total SOD and Cu/Zn SOD in the liver was only increased in the piglets fed both PF diets (PF, PF+ZnGly). Similarly, the liver concentration of ZnT1 was the highest in the PF and PF+ZnGly groups, while Zn-MT1 concentration was not altered. Our results indicated that intake of feed containing PF could improve Zn and antioxidant status of young pigs due to increased ZnT1 level and SOD activity in liver tissue.

Keywords: zinc, fibre, metallotransporters, superoxide dismutase, pigs

Acknowledgement: This study is based upon work from COST Action (PiGutNet), supported by COST (European Cooperation in Science and Technology) and was supported by the Slovak Grant Agency VEGA 2/0069/17.

#### EFFECT OF INCLUSION OF STRAWBERRY LEAVES IN RABBIT FEED ON MEAT QUALITY

Kalafova A.<sup>1\*</sup>, Emrichová J.<sup>1</sup>, Kovacik J.<sup>1</sup>, Bucko O.<sup>3</sup>, Lubomír O.<sup>2</sup>, Rastislav J.<sup>2</sup>, Lubica C.<sup>2</sup>, Schneidgenova M.<sup>1</sup>, Capcarova M.<sup>1</sup>

<sup>1</sup>Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic

<sup>2</sup>National Agricultural and Food Center, Research Institute of Animal Production Nitra, Slovak Republic

<sup>3</sup>Department of Animal Husbandry, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, Nitra,

Slovak Republic

\* anna.kalafova@uniag.sk

Feed containg no chemical additives are increasingly used in rabbit nutrition. Herbs and natural feed additives are being investigated as natural feed additives are being investigated as natural sources biological important major substances. This study adresses the influence of technological parameters meat by enriching the diet of rabbits with strawberry leaves. Fifteen rabbits of broiler line Californian were used in this experiment. Rabbits were divided into four groups, one control group C (n=4) and three experimental groups E1, E2 and E3 (n=4 in each group). Rabbits were fed with a granular feed mixture (FM) with strawberry leaves in various doses; E1- 0.5%; E2 -1.0 % and E3-1.5% for 21 days. Meat guality was analysed from a sample of *Musculus longissimus* dorsi (50 g) for parameters technological parameters (of electric conductivity, pH, colour). The value of pH (24 hour post mortem) was detected by portable battery acidometer OP-109. Instrumental colour measurements were recorded for L (lightness;0: black and 100: white) using a spectrophotometer CM-2600d (Osaka, Japan). The electrical conductivity defined locations of muscles were evaluated using PMV 51 (mS.cm<sup>1</sup>). Slight decrease of pH 24 in the groups E2  $(5.74\pm0.08)$  and E3  $(5.78\pm0.05)$  with higher concentration of strawberry leaves in comparison to the others groups E1 (5.83 $\pm$ 0.09) and C (5.81 $\pm$ 0.14) was found. In group E1 decrease of electric conductivity (P>0.05) when compared to other groups were measured. The values of color L were not affected by application of strawberry leaves.

#### Keywords: rabbit, strawberry leaves, meat quality

Acknowledgments: This study was supported by the KEGA 024 SPU-4/2018.

#### DEPOSITION OF IMMUNOACTIVE SUBSTANCES INTO THE EGG AND IMMUNE RESPONSE OF YOUNG JAPANESE QUAIL SELECTED FOR SHAPE OF GROWTH CURVE

Kankova Z.<sup>1</sup>, Drozdova A.<sup>1</sup>, Klobetzova Z.<sup>1</sup>, Lichovnikova M.<sup>2</sup>, Zeman M.<sup>1,3</sup>

<sup>1</sup>Department or Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University in Bratislava, Slovak Republic

<sup>2</sup>Department of Animal Breeding, Mendel University of Agriculture and Forestry, Brno, Czech Republic

<sup>3</sup>Institute of Animal Biochemistry and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Bratislava, Slovak Republik

Selection for growth parameters is widely used in commercial boiler breeding in order to improve efficiency of meat production. Selection strategies aimed at body weight differences are connected with many health problems in poultry breeding. Therefore, there is a need to establish new ways of selection, which would take into account the need for maximisation of breeding effectivity as well as animal health. In our experiment we used Japanese quail (*Coturnix japonica*) selected at Mendel University of Agriculture and Forestry, Brno (Czech Republic) for high (HG) and low relative weight gain (LG) between 11 and 28 days of age. Faster growth is often connected with trade-offs with other energy consuming systems, especially development and reactivity of the immune system. Since these two lines strongly differ in growth pattern during first 30 days of their development, we choose three time points during the first month of their life (11, 22 and 33 days of age) in order to evaluate the acute phase of immune response. Quail were intraperitoneally injected with lipopolysaccharide (LPS) in a dose 1.5 mg/kg of body weight. Three hours after LPS injection blood was collected for quantification of differential white blood cell counts and concentration of plasma immunoglobulins (Iq). Bursa of Fabricius (FB) was dissected and weighted. Quails were tested also for intensity of cell-mediated immune (CMI) response during the rapid growth period and after this phase. CMI was evaluated as thickness of wing web before and after subcutaneous injection of phytohemagqlutinine (PHA). Birds were regularly weighted during the whole experiment. Moreover, we measured deposition of maternal antibodies and immunoactive substance lysozyme into the eggs of both lines. The two lines did not differ in body weight at hatch, but from day 5 the LG line was almost two times heavier than the HG line. This difference slowly disappeared, since the growth of the HG line was faster during the period between 11 and 22 days and body weight did not differ between lines after day 22. The relative weight of FB did not differ between lines during development. In day 11 animals of LG line showed higher heterophil/lymphocyte (He/Ly) ratio, but lower concentration of Ig in plasma in LPS-injected group. In the second time point (day 22), during the rapid growth phase, no differences in He/Ly ration and concentration of plasma immunoglobulins between groups after LPS injection were found. CMI was not affected by line at the age of 22 day. However, we observed higher CMI in HG line in comparison with LG line at 33 days of age. At the same age, we found again higher He/Ly ratio in LG line in comparison with HG line. Considering maternal investment, egg weight did not differ between the two lines. However, we found higher concentrations of egg lysosome in LG line in comparison with HG line. Our results suggest, that changes in growth rate caused by selection for relative weight gain between 11 and 28 days of age might influence immune response of guail. especially in very early stages of their development. This study was supported by the research grant VEGA 1/0686/15.

Keywords: selection for shape of growth curve, Japanese quail, immune response

#### THE IMPACT OF UDN ON SELECTED BLOOD PARAMETERS OF FEMALE SEA TROUT SALMO TRUTTA M. TRUTTA (L.) SPAWNERS

Kasprzak M.<sup>1\*</sup>, Ciepliński M.<sup>1</sup>, Grandtke M.<sup>1</sup>, Steliga A.<sup>2</sup>, Kamiński P.<sup>3,4</sup>, Jerzak L.<sup>5</sup>

<sup>1</sup>University of Zielona Góra, Faculty of Biological Sciences, Department of Zoology, Zielona Góra, Poland <sup>2</sup>Department of Health Sciences Pomeranian University in Slupsk, Poland

<sup>3</sup>University of Zielona Góra, Faculty of Biological Sciences, Department of Biotechnology, Zielona Góra, Poland <sup>4</sup>Nicolaus Copernicus University in Toruń, Collegium Medicum in Bydgoszcz, Faculty of Medicine, Department of Medical Biology, Department of Ecology and Environmental Protection, Poland

<sup>5</sup>University of Zielona Góra, Faculty of Biological Sciences, Department of Environmental Protection, Zielona Góra,

Poland

\* m.kasprzak@wnb.uz.zgora.pl

Blood tests were performed on 162 female sea trouts *Salmo trutta* m. *trutta* (L.) during four spawning seasons (2014-2017). Fish were caught on a Polish Angling Association trapping point, the Słupia River, Słupsk, northern Poland. The blood for analysis was drawn from caudal vein of 56 healthy and 96 UDN (Ulcerative Dermal Necrosis) - infected females. Fish were divided into three groups: (1) healthy, with no visible signs of UDN, (2) sick, with up to 10% skin damage and (3) agonal, where more than 10% of body surface was infected. Statistically important decrease in red blood cell count (RBC), hemoglobin concentration (HGB) and hematocrit (HCT) were found between sick and agonal fish groups. Concentration of total plasma protein and albumin decreased, in relation to fish health deterioration. Urea had an inverse proportionality to total plasma protein and albumin concentration. Based on decrease of RBC and HCT due to UDN symptoms development a decrease in fish condition was observed. Significantly higher urea concentrations observed in agonal fish indicates respiratory and excretory systems failure.

Keywords: blood, fish, Ulcerative Dermal Necrosis (UDN), hematology, urea

### CELLULASE ACTIVITY OF BEETLE SPECIES AT DIFFERENT TEMPERATURES AND SUBSTRATE CONCENTRATION

Kaźmierczak S.<sup>1</sup>, Szentner K.<sup>2</sup>, Waśkiewicz A.<sup>2</sup>, Wojciechowicz T.<sup>3</sup>, Wasielewski O.<sup>1</sup>

<sup>1</sup>Institute of Zoology, Poznań University of Live Sciences, Poland <sup>2</sup>Department of Chemistry, Poznań University of Live Sciences, Poland <sup>3</sup>Department of Animal Physiology and Biochemistry, Poznań University of Live Sciences, Poland

Efficient and eco-friendly degradation of lignocellulosic biomass is crucial for the biofuel production. Therefore, scientists all over the world make great efforts to identify new approaches which can improve this process. There is growing evidence indicating that the insects including the beetles can be utilised in this area. They produce endogenous and exogenous cellulases which hydrolyse cellulose into the substrates which can be used in biofuel production. Therefore, the aim of this study was to determinate the enzymatic activity of gut cellulases extracted from *Tenebrioide* family species (*Alphitobius diaperinus, Tenebrio molitor* and *Zophobas morio*) including imago and larvae. Cellulase activity was assessed using 3,5-Dinitrosalicylic acid (DNSA) method and carboxymethyl cellulose (CMC) as substrate. Furthermore, the influence of substrate concentration (3, 2 and 0,5% CMC) and incubation temperature (40, 50 and 70°C) on cellulase activity were evaluated. The results of this study indicated that cellulases activity of *Alphitobius diaperinus* larvae at the concertation of 2% CMC at 70°C was the highest among all tested species. In conclusion this study found that celluloses from *Alphitobius diaperinus* larvae have the highest ability to hydrolyse cellulose suggesting their potential utilisation in biofuel production.

#### Keywords: cellulase, Tenebrioide, DNSA

Acknowledgments: The study was funded by National Science Centre, Poland, grant No. 2014/13/B/NZ9/02442.

#### DOES BENZO[A]PYRENE AFFECT THE HEART EMBRYONIC DEVELOPMENT?

Kołodziejczyk Ł.M.\*, Puzik M., Greń A., Batoryna M., Formicki G., Kapusta E.

Institute of Biology, Pedagogical University of Cracow, Department of Animal Physiology and Toxicology, Cracow, Poland \* lukasz.kolodziejczyk@up.krakow.pl

Benzo[a]pyrene is a polycyclic aromatic hydrocarbon well known as carcinogen, teratogen and neurotoxin widely present in urban air pollution, smoke and certain kinds of foods. There are many reports confirmed its toxicity for mice and other mammals, however knowledge on its impact on the birds is still limited. The effect of benzopyrenes on the developing heart of the vertebrates is unknown. For above reasons we used chicken embryos to verify the influence of benzo[a]pyrene on selected parameters of the heart muscle. The benzo[a]pyrene in the organic oil solution was injected in ovo at 6<sup>th</sup> day of the incubation into the yolk, respecting doses of 1 mg/kg weight of eggs: 0.5 mg/kg w. e. and 0.1 mg/kg w. e. The intacts and eggs injected with the organic oil were used as control groups. At 14<sup>th</sup> day of the incubation eggs were opened in order to examine embryos and achieve tissues for further analyses. We performed the electrocardiography of embryos and estimated the weight of heart *post mortem*. We determined also the concentration of reduced glutathione (GSH) and malonylodialdehyde (MDA) in heart tissue. The increased weight of the heart muscle was observed in the embryos treated with dose of 1 mg/kg w. e. In groups intoxicated with lower doses of benzo[a]pyrene heart weight did not differ from the control and intacts. Electrocardiography does not showed any important changes of the heart rhythm in relation to controls. However, we determined a statistically significant increase of the GSH concentration in the heart tissue from the group injected with 1 mg/kg w. e. In the case of the lower benzol alpyrene doses, the level of GSH was similar to the controls. Differences in the MDA concentrations in all experimental groups were not statistically significant in relation to the controls. We conclude that the subacute dose of benzo[a]pyrene is a stress factor, which strongly activates the glutathione – dependent antioxidative defense and probably do not affect the heart conducting system of the chicken embryo. Whereas, the influence of this substance on the morphology and biochemistry of the developing heart requires further examination.

Keywords: heart, benzo[a]pyrene, embryo, ecg, GSH, MDA.

#### **BIOCHEMICAL MARKERS OF LIVESTOCK HEALTH STATUS**

Konečná M.<sup>1\*</sup>, Poráčová J.<sup>1</sup>, Sedlák V.<sup>1</sup>, Mydlárová Blaščáková M.<sup>1</sup>, Gogaľová Z.<sup>1</sup>, Babejová A.<sup>1</sup>, Nagy M.<sup>2</sup>, Zahatňanská M.<sup>3</sup>, Majherová M.<sup>4</sup>

<sup>1</sup>The University of Prešov, Faculty of Humanities and Natural Sciences, Department of Biology, Prešov, Slovak Republic \* maria.konecna@unipo.sk

<sup>2</sup>J. Selye University, Faculty of Education, Komárno, Slovak Republic

<sup>3</sup>The University of Prešov, Faculty of Humanities and Natural Sciences, Department of Pedagogy, Prešov, Slovak Republic

<sup>4</sup>The University of Prešov, Faculty of Humanities and Natural Sciences, Department of Physics, Mathematics and Technology, Prešov, Slovak Republic

Ecological farming has been recognized not only in Europe but also in Slovakia as a recognized alternative to the conventional management model. The main principles of ecological farming are long-term sustainability, consideration not only for nature, but above all for farmed animals. The metabolic and enzyme profile test allows for timely detection of animal health disorders that may affect the production and economy of the breed. In our work, we focused on the analysis and evaluation of biochemical markers (AST, ALT, ALP, GMT, GLU, CHOL, TGL and BIL) of the Aberdeen-Angus cattle in ecological (n = 20) and conventional breeding. We compared the obtained results between conventional breeding and ecological breeding and statistically evaluated. The selected biochemical parameters were within the range of the reference values within both breeds. However, the results show that the mean values of selected biochemical markers were significantly lower in ecological farming compared to conventional farming. Low and maximum bilirubin in conventional breeding [0.63 - 5.15 µmol / L] and organic stock [0.12 - 4.32 µmol / L] and glucose in conventional breeding [1.98-3.99 nmol / L] and organic stock [2.10-3.62 nmol / L]. Minimum and maximum concentration of cholesterol and triacylolycerol in conventional breeding [2.63 - 5.12 mmol / I: 0.36 - 0.86 mmol / I] ecological breeding [2.62 - 4.85 mmol / I; 0.21 to 0.62 mmol / I]. Enzymatic activity of ALT [0.26 µkat / L; 0.32 µkat / l], AST [0.30 µkat / L; 0.39 µkat / L] and LP [0.47 µkat / L; 0.61 ukat / L1 was significantly lower in cattle in ecological farming compared to conventional breeding. Selected biochemical parameters were determined on a fully automatic biochemical analyzer, Cobas Integra 400 plus (Roche, Switzerland).

**Keywords:** biochemical analysis, conventional breeding, ecological farming, Aberdeen-Angus, metabolic profile.

Acknowledgements: The work was supported by project: Biochemical, physiological and haematological status in selected species of hunting game, VEGA 1/0783/18.

#### DRAFT GENOME SEQUENCE OF *ENTEROCOCCUS FAECIUM* 8S3, LACTIC ACID-PRODUCING BACTERIUM FROM BRYNDZA CHEESE

Kopčáková A.<sup>1</sup>, Dubíková K.<sup>2</sup>, Kisková J.<sup>3</sup>, Javorský P.<sup>1</sup>, Pristaš P.<sup>1,3</sup>

<sup>1</sup>Institute of Animal Physiology, Centre of Biosciences of the SAS, Košice, Slovak Republic <sup>2</sup>P. J. Šafarik University, Faculty of Natural Science, Department of Biochemistry, Košice, Slovak Republic <sup>3</sup>Institute of Biology and Ecology, Faculty of Natural Science, P. J. Šafarik University in Košice, Slovak Republic

We report here the draft genome sequence of the lactic acid producing bacterium *Enterococcus faecium* strain 8S3, isolated from bryndza traditional cheese produced from unpasteurised ewe milk in Slovakia. The draft genome sequence consists of 2.8 Mbp, with a mean G+C content of 38.2% and show high similarity to other *E. faecium* genomes sequenced. A total of 2.833 coding sequences, including 62 structural RNAs (3 rRNA and 59 tRNA) were predicted. Comparative genomic data indicate that prophages and bacteriophage remnants are the main source of diversity among *E. faecium* genomes. The draft genome sequence and comparative genomic studies of this bacterial strain provides new genetic data that support its biotechnological potential.

Keywords: Enterococcus faecium, lactic acid bacteria, raw ewe milk, genome analysis

#### METABOLIC ADAPTATIONS OF DAIRY COWS AT THE BEGINNING LACTATION

Kováčik J., Massányi P., Capcarová M., Kalafová A.

Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic

During the reproduction and production period, dairy cows experience complex anatomical and physiological changes that reflect differences in metabolism intensity and nutritional and breeding requirements. Lactation is a particularly demanding period with a critical culmination of the first 6-8 weeks. With lactogenesis a complex of very complex metabolic adaptations stat, which occur not only in the milk gland, but in the whole organism. The most significant adaptations occur during lactation in the cardiovascular system, the liver, the kidney, in the epithelium of the rumen, and the skeletal muscle. Lactation induces gastrointestinal hyperfagia and endocrine changes that activate intestinal transport of nutrients into the blood. Critical organ of dairy cows at the start of lactation is liver, that is heavily loaded with metabolic and detoxification functions. Its weight is increasing by up to 40% and the activity of the carbohydrate and lipid metabolism enzymes intensify. During lactation, glucose production in the liver increases up to threefold in order to synthesize lactose in the mammary gland. Since glycogen stores in the liver are insignificant in terms of their needs, gluconeogenesis is the main process of glucose production. Dairy cows are unable to maintain a positive energy balance at the start of lactation; they must mobilize body reserves and get to a negative energy balance that reaches the maximum during 1st to 3<sup>rd</sup> week. Therefore, in this period, the major metabolic adaptations of dairy cows are characterized by increased lipolysis and the use of fatty acids as a significant source of energy as well as intensive gluconeogenesis in the liver from propionate and glucoplastic amino acids. An important place in metabolic adaptations has regulating mechanisms of dairy cows, where endocrine regulation plays a major role in maintaining metabolic homeostasis. Increased energy demands at the onset of lactation are an impulse for the activity of significant metabolic hormones - somatotropin (STH) and insulin. Somatotropin increases milk production, increases metabolism in the anterior sections of the gastrointestinal tract through hypertrophy and higher resorption. It reduces the sensitivity of peripheral tissues to insulin and directs the flow of glucose into the mammary gland, thereby reducing its irreversible losses. Hormone insulin is a significant metabolic regulator that is in direct relationship to the energy balance. Its reduced level at the onset of lactation is a significant stimulus to trigger lipolysis and intensive gluconeogenesis - the most important metabolic adaptations at the onset of lactation. These metabolic adaptations, along with changes in their regulatory systems, may lead to the production diseases of dairy cows. Energy deficiency, intense gluconeogenesis and lipolysis are reflected in parameters of the metabolic profile of blood, urine and milk. Accompanying phenomena of predominant catabolic processes are significant changes in the mineral, acid-base and nitrogen profiles, which are considered as a risk factor for the development of metabolic and reproductive disorders of dairy cows.

**Keywords:** lactation, metabolic adaptations, hormonal regulation, gluconeogenesis, energy balance

Acknowledgement: The work was financially supported by KEGA 024SPU-4/2018.
#### EFFECTS OF AMYGDALIN ON GENE ACTIVITY IN CULTIVATED HUMAN OSTEOBLASTS

Kováčová V.<sup>1\*</sup>, Lukáčová M.<sup>2</sup>, Adamkovičová M.<sup>2</sup>, Šarocká A.<sup>1</sup>, Šranko P.<sup>2</sup>, Omelka R.<sup>2</sup>, Kolesárová A.<sup>3</sup>, Martiniaková M.<sup>1</sup>

<sup>1</sup>Department of Zoology and Anthropology, Constantine the Philosopher University, Nitra, Slovak Republic <sup>2</sup>Department of Botany and Genetics, Constantine the Philosopher University, Nitra, Slovak Republic <sup>3</sup>Department of Animal Physiology, Slovak University of Agriculture, Nitra, Slovak Republic \* veroni.kovacova@qmail.com

Amygdalin, one of the most common cyanogenic glycoside, can be found abundantly in the kernels of bitter almonds and apricots. It has been used for the treatment of cancer because of a release of toxic hydrogen cyanide. Its effect on bone cells has not been investigated to date. The objective of this in vitro study was to determine an impact of amygdalin on osteoblasts. Primary human osteoblasts were incubated with amygdalin at concentrations 100, 1 000 and 10 000 µg.ml<sup>-1</sup> for 72 hours and compared to the control without amvodalin administration. We determined cell viability by CCK8 assay, osteoblast morphology, and expression of ten selected genes connected with ossification, osteoblast differentiation, bone mineralization, oxidative stress and apoptosis by realtime PCR. Our results revealed adverse impact of amygdalin at the highest concentration on viability of osteoblasts. We also observed significantly (P<0.05) lower values of mean area and perimeter of bone cells. Our data showed that expression of osteocalcin (BGLAP), TNFSF11 and WNT5A was significantly (P < 0.05) increased in osteoblasts exposed to the highest concentration of amygdalin. On the contrary, we found a significant (P < 0.05) downregulation of collagen type I a1 (COL1A1; at concentrations 100 and 10 000 µg.ml<sup>-1</sup>) and alkaline phosphatase (ALPL; at concentration 10 000 µg.ml<sup>-1</sup>). Levels of RUNX2, BAX, CASP1, SOD1 and GPX1 expression did not change in all experimental groups. The middle concentration of amygdalin (1 000 µg.ml<sup>-1</sup>) did not show significant differences in the expression of any analysed gene. In conclusion, amygdalin is capable of inhibiting collagen production and bone mineralization and stimulating bone resorption. On the other hand, it had no effect on oxidative stress and cells apoptosis.

Keywords: amygdalin, osteoblast, viability, morphology, gene expression

Acknowledgments: The study was supported by the projects VEGA 1/0653/16 and KEGA 031UKF-4/2016. This work was supported by Research Center AgroBioTech built in accordance with the project Building Research Centre "AgroBioTech" ITMS 26220220180.

#### THE EFFECT OF GLUTAMATE ON DEVELOPMENT OF MOUSE PREIMPLANTATION EMBRYOS

Kovaříková V., Babeľová J., Šefčíková Z., Špirková A., Fabian D., Koppel J., Čikoš Š.

Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences, Slovak Republic

L-glutamate is most commonly used food additive. There is no evident effect of orally administered glutamate on fetal development, because glutamate, even in higher doses doesn't cross placenta and doesn't reach fetal circulation. *In vitro* experiments indicated that addition of specific amino acids may have stimulatory or inhibitory effect on preimplantation embryo. The aim of our experiment was to examine the effect of increased concentration of glutamic acid on development and quality of mouse preimplantation embryos *in vitro*. Two-cell embryos were cultured in KSOM medium for 72 hours with or without addition of glutamic acid at final concentrations 1mM, 2 mM, 5 mM and 10 mM (no significant osmotic stress or ammonium increase should be induced in these conditions). Glutamate (at all tested concentrations) decreased the proportion of higher-stage embryos *in* that reached the blastocyst stage showed decreased cell number in glutamate-treated embryos (the difference was statistically significant at 2 mM concentration. Analysis of dead cell per blastocyst showed increased numbers of dead cells in embryos treated with 5 mM and 10 mM glutamic acid. The results of this experiment indicated that higher concentrations of glutamic acid could influence development and quality of preimplantation embryos.

Keywords: glutamic acid, preimplantation embryos

Acknowledgements: This work was supported by the Slovak Research and Development Agency project APVV-14-0763.

#### DETECTION OF TAURINE EFFECT ON THE STRUCTURE OF RABBIT KIDNEY

Kročková J., Massányi P., Ondruška Ľ., Macho T.

Slovak University of Agriculture in Nitra, Slovak Republic

The current situation in the world is set by examining in detail the most diverse substances that are or could be part of human nutrition. In addition, this global mood is driven by an increasing group of people who cares about healthy nutrition and by what substances we get into our bodies. These conditions also meet the taurine. From a chemical point of view, taurine is a  $\beta$ -amino acid, the synthesis of which occurs through the amino acid cysteine. It has strong antioxidant effects and engages in tissue and organ development. In our study we focused on its effects on morphometric parameters of the kidneys. The kidneys are part of the excretory system, where they form a functional basis. The excretory system serves to filter blood, remove waste matter from the body, and maintain electrolyte homeostasis. The aim of the work was to experimentally prove the effect of taurine on the rabbit kidney in vivo. In the experiment, we compiled 4 groups of rabbits according to the daily dose of taurine (1st experimental group - 321.675 mg, 2nd experimental group - 643.350 mg, 3rd experimental group - 965.025 mg, control without taurine dose) administered to them in drinking water for 4 weeks. Following the killing of the rabbits, we removed the organs for the creation of permanent microscopic specimens. Using a microscope and analytical software, we evaluated speciments where we focused on monitoring these parameters - the diameter of the renal corpuscle (Bowman's sac, glomerulus) and the tubules (epithelium, lumen), the renal particles content, the number of cells at 10.000 µm2 area and the number of kidney particles at surface area of 630.000 µm2. Different groups varied in the parameters only minimal, the greatest differences were found between the 3rd experimental group and the control group. In the 3rd experimental group, increased values of renal corpuscules were observed, the glomerulus diameter was 125.41 µm while 96.75 µm in the control. We also noticed a significant change in the renal body content (11 620.20 µm 2 in the 3rd group vs. 6830.60 µm2 in the control). These values indicate a positive effect of taurine on kidney structure, predominantly on size of renal corpuscules, which could also affect its function (more efficient filtration). However, even after the statistical analysis we did not find any demonstrable change.

#### Keywords: taurine, kidney, rabbit, mophometry

Acknowledgements: This work was financially supported by the projects APVV 15-0543 and APVV 16-0289.

#### ORGAN TOXICITY OF DIETHYLNITROSAMINE AND CAPSAICIN IN MICE – IN VIVO STUDY

Kuchařová V.1, Daněk O.1, Škorič M.2, Veselá I.1, Tomenendálová J.1

<sup>1</sup>Department of Physiology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic <sup>2</sup>Department of Pathological Morphology and Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

Diethylnitrosamine (DEN) is proven to be toxic to kidneys and liver and to act as a potent carcinogen mainly in liver. Capsaicin (CAP) is an alkaloid produced by *Capsicum* genus plants and is considered to be a protective agent against toxicity and carcinogenicity of many substances including DEN. The aim of this study was to assess the organ toxicity of DEN and CAP in mice. A total of 51 female CD-1 mice were randomly divided into five groups. The experiment started after two weeks of acclimatization and was conducted according to the Czech animal welfare protection legal guidelines. The experimental design is shown in Fig. 1.

Group	Week of experiment							
	1	2	3	5	7	9	11	13
Contr.			1 % ethanol					
CAP			CAP	CAP	CAP	CAP	CAP	
n=10			1,5mg/kg	1,5mg/kg	0,75kg/kg	0,75kg/kg	0,75kg/kg	sia.
CAP-DEN	CAP	DEN	0,9 %	0,9 %	0,9 %	0,9 %	0,9 %	na:
n=10	1,5mg/kg	25mg/kg	NaCl	NaCl	NaCl	NaCl	NaCl	Ę.
DEN-CAP		DEN	CAP	CAP	CAP	CAP	CAP	Eut
n=10		25mg/kg	1,5mg/kg	1,5mg/kg	0,75kg/kg	0,75kg/kg	0,75kg/kg	
DEN		DEN	0,9 %	0,9 %	0,9 %	0,9 %	0,9 %	
n=10		25mg/kg	NaCl	NaCl	NaCl	NaCl	NaCl	

Figure 1. Experimental design; Contr. = control group, CAP was dissolved in 1% ethanol solution, DEN was dissolved in 0,9% NaCl solution, all applications were intraperitoneal

At the end of the experiment the mice were sacrificed and selected methods assessing the organ toxicity of DEN and CAP were used.

There was no statistically significant difference in body and organ weight between control and experimental groups. The histopathological examination of the liver revealed multifocal lymphoplasmacytic reaction in parenchyma in DEN treated group. CAP used as both preventive and therapeutic agent caused reduction in number and extent of lesions. In CAP group mitotic figures were found indicating xenobiotic-induced hepatotoxicity or regenerative changes. In the kidneys DEN revealed also multifocal lymphoplasmacytic reaction that has been mitigated by CAP. Moreover histopathological observation of the kidney in DEN group has revealed granular dystrophy of the renal tubules which has not been presented in CAP treated mice. Levels of ALT, AST activity, total protein and albumin concentration was not statistically different among control and experimental groups. In this study mild protective effect of CAP on DEN-induced hepatotoxicity and nephrotoxicity was shown only in histopathological changes. The toxicity of CAP itself is questionable and further studies should be performed to verify its chemopreventive potential.

Keywords: capsaicin, diethylnitrosamine, hepatotoxicity, mice, nephrotoxicity

Acknowledgement: This work was supported by grant IGA VFU Brno 114/2016/FVL.

#### ALCOHOL ADMINISTRATION AFFECTS COMPACT BONE STRUCTURE OF MICE AFTER ONE REMODELING CYCLE

Martiniakova M.<sup>1</sup>, Sarocka A.<sup>1</sup>, Babosova R.<sup>1</sup>, Kapusta E.<sup>3</sup>, Goc Z.<sup>3</sup>, Gren A.<sup>3</sup>, Formicki G.<sup>3</sup>, Omelka R.<sup>2</sup>

<sup>1</sup>Department of Zoology and Anthropology, <sup>2</sup>Department of Botany and Genetics, Faculty of Natural Sciences, Constantine the Philosopher University in Nitra, Slovak Republic <sup>3</sup>Department of Animal Physiology and Toxicology, Institute of Biology, Faculty of Geography and Biology, Pedagogical

Department of Animal Physiology and Toxicology, Institute of Biology, Faculty of Geography and Biology, Pedagogical University in Cracow, Poland

Chronic alcohol consumption is detrimental to many organs and tissues, including bones. However, its impact on microscopic structure of the bone after one remodeling cycle is not known yet. In our study, ten clinically healthy 12 weeks-old mice (males) were randomly divided into two groups. Animals from experimental (E) group (n=5) drank a solution composed of 15 % ethanol and water (1.7 g 100 % ethanol/kg bw per day) for 14 days (one remodeling cycle). The group without ethanol administration served as a control (C) group (n=5). In mice from E group, primary vascular radial bone tissue was observed in *pars posterior* of endosteal border. Also, more secondary osteons (about 46 %) and more resorption lacunae (four times more) mainly in *pars anterior* were found which corresponds with a lower level of serum ALP and decreased GSH level in alcohol fed mice. All variables of primary osteons' vascular canals (P<0.05) were increased and those for Haversian canals and secondary osteons (P<0.05) were decreased in mice from E group. Alcohol significantly decreased relative bone volume with and without marrow cavity, bone mineral density of the compact bone. However, it had no effect on trabecular bone microstructure. Liver function test showed higher levels of ALT, AST in the E group suggesting advanced liver disease. In summary, only changes in compact bone microstructure of mice after one remodeling cycle were observed due to both direct and indirect effects of alcohol.

Keywords: alcohol, compact bone, trabecular bone, mice, microstructure

Acknowledgement: The study was supported by the projects VEGA 1/0653/16 and KEGA 031UKF-4/2016. This work was supported by Research Center AgroBioTech built in accordance with the project Building Research Centre "AgroBioTech" ITMS 26220220180.

#### THE PILOT STUDY: EVALUATING EFFECTS OF FLAVONOID ISOQUERCITRIN ON THE VIABILITY AND STEROIDOGENESIS OF HUMAN GRANULOSA CELLS HGL-5

Michalcová K.<sup>1\*</sup>, Halenár M.<sup>1</sup>, Baldovská S.<sup>1</sup>, Sanisló Ľ.<sup>2</sup>, Křen V.<sup>3</sup>, Kolesárová A.<sup>1</sup>

<sup>1</sup>Slovak University of Agriculture in Nitra, Slovak Republic <sup>2</sup>St. Elizabeth Cancer Institute Hospital, Bratislava, Slovak Republic <sup>3</sup>Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic \* kmichalcova86@gmail.com

Ovarian granulosa cells are of critical importance during oocyte development. Their function, namely their secretory profile, is of paramount importance for final oocyte maturation, folliculogenesis, ovulation and luteogenesis. Modern scientific research has revealed that the wide variety of dietary and medicinal functions of flavonoid compounds can be attributed to the isoquercitrin (IO). IO is a pale-yellow crystalline glucoside, occurring widely in medicinal herbs, fruits, vegetables and plant-derived foods and beverages. There are many in vitro and in vivo studies indicated strong anti-inflammatory and anticarcinogenic potential of IO as a medicament. The objective of the current research work was to evaluate the effect of flavonoid IO at the doses 5, 10, 25, 50, 100 µg/mL on the viability and secretion of selected steroidogenic hormones by human granulosa cell line HGL-5. IQ used in this study was prepared by selective derhamnosylation of rutin using recombinant  $\alpha$ -L-rhamnosidase from A. terreus (D. Gerstorferová et al., 2012). HGL-5 were cultivated without (control) or with IQ at the different doses for 24 h. The cell viability was determined by *alamarBlue*<sup>™</sup> reagent and the release of progesterone and 17-β-estradiol was assayed by ELISA method. The obtained results showed that IQ did not induce a statistically significant ( $P \ge 0.05$ ) changes in cell viability in comparison to control. Progesterone secretion was not significantly ( $P \ge 0.05$ ) influenced by IO at all used concentrations. Similarly, IO at the concentrations 5, 10, 25, 50, 100 μg/mL did not affect 17-β-estradiol secretion by HGL-5. In conclusion, this in vitro study did not suggest any significant implication of isoquercitrin into the process of steroidogenesis/cellular processes. Nevertheless, we suppose that further studies are needed to evaluate this association.

Keywords: isoquercitrin, progesterone, viability, granulosa cell line

Acknowledgment: This work was supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic projects VEGA 1/0039/16, KEGA 011SPU-4/2016, APVV-15-0543, APVV-16-0170, Czech Science Foundation 18-00121S and EU project no. 26220220180: Building Research Centre "AgroBioTech".

#### DECOMPOSITION OF THE BODIES OF FARM ANIMALS DURING THE WINTER MONTHS BY NECROPHAGOUS SPECIES

Mifkova T.\*, Urban T., Horakova J.

Department of Morphology, Physiology and Animal Genetics, Faculty of AgriSciences, Mendel University in Brno, Czech Republic

\* tamaramifkova@gmail.com

We studied the decomposition of vertebrate bodies (pig and chicken) during the winter months. Necrophagous insects play an important role, especially in forensic practice in determining the time of death. These necrophagous species were identified with anatomical-morphological and molecular genetic methods, which have been compared to each other. For this purpose, the mitochondrial DNA was isolated from samples and specific segments with cytochrome oxidase I gene were amplified by the PCR method and sequenced. The experiment was performed from September 2016 to March 2017 and from October 2017 to March 2018. Monitoring of cadavers was based on the ongoing decomposition and the collection of necrophagous fauna was subsequently carried out in connection with current climatic conditions. During the first experiment, 11 species of necrophagous fauna were found on cadavers, of which 5 were of the order Diptera and 6 of the order Coleoptera. In the second experiment the same species are expected which will be verified by molecular genetic methods.

**Keywords:** cadaver decomposition, necrophagous insects, cytochrome oxidase I, mitochondrial DNA

Acknowledgements: research was supported by internal grant agency FA IP-2018/077.

# *IN VITRO* AND *IN VIVO* EFFECT OF MEDICINAL PLANTS IN LAMBS WITH ENDOPARASITE INFECTION

Mravčáková D., Babják M., Königová A., Pisarčíková J., Kišidayová S., Vadlejch J., Várady M., Váradyová Z.

Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences, Kosice, Slovak Republic

The use of medicinal plants containing bioactive compounds is an alternative strategy to control gastrointestinal (GI) parasitic nematodes in sheep. Traditional medicinal plants common in central Europe were used: Althaea officinalis, Petasites hybridus, Inula helenium, Malva sylvestris, Chamomilla recutita, Plantago lanceolata, Rosmarinus officinalis, Solidago virgaurea, Fumaria officinalis, Hyssopus officinalis, Melissa officinalis, Foeniculum vulgare and Artemisia absinthium. In vitro and in vivo experiments were carried out. The anthelmintic activities of aqueous and methanolic extracts from the plants on GI nematode Haemonchus contortus in egg hatch tests and larval development tests were determined in vitro. Eggs for both tests were obtained from fecal samples of lambs experimentally infected with 5000-6000 third-stage larvae of an anthelminticsusceptible strain of H. contortus. Plant bioactive compounds (gallic acid, rutin, diosmin, hesperidin, quercetin and kaempferol) were quantify in the methanolic extracts by UPLC/MS/MS. Aqueous extracts generally had a greater effect than methanolic extracts on the L3 stages of H, contortus in both tests. Each plant was also incubated in vitro in ruminal fluid to assess its effect on parameters of ruminal fermentation (pH, ammonia, gas, methane and short-chain fatty acids). Variables differed after 24 h of ruminal incubation of the individual medicinal plants, but the plants had no negative effect on the fermentation patterns. Mixture of these plants was used determine the effect of dietary supplementation on hematological, parasitological and inflammatory parameters of lambs infected experimentally with H. contortus. Twenty-four female lambs were divided into four groups: infected animals, infected animals supplemented with plant mixture, uninfected control animals and uninfected animals supplemented with plant mixture. The mean eggs per gram counts were significantly lower between day 32 and day 50 of infection and between day 32 and day 60 for the infected animals supplemented with plant mixture. Mean cumulative gain in live weight was higher in infected animals supplemented with plant mixture as compared to infected animals. The evaluation of red blood cells identified clinical signs of haemonchosis, such as anemia, in infected animals. The concentration of serum calprotectin differed significantly in infected animals vs. infected animals supplemented by plant mixture. The supplementation by plant mixture can slow the dynamics of infection. Parallel in vivo and in vitro experiments are useful for finding promising chemotherapeutic alternatives.

**Keywords:** gastrointestinal nematode, plant extracts, plant treatments, lambs, ruminal fermentation in vitro

Acknowledgement: This study was supported by funds from the Slovak Research and Development Agency (APVV 14-0169).

#### ANTIOXIDANT ENZYMES ACTIVITY, GSH AND MDA LEVEL IN EGGS FROM HENS OF THREE HERITAGE BREEDS

Muchacka R.1\*, Sosnówka-Czajka E.2, Skomorucha I.2, Kapusta E.1, Greń A.1, Goc Z.1

<sup>1</sup>Department of Animal Physiology and Toxicology, Institute of Biology, Pedagogical University of Cracow, Poland <sup>2</sup>Department of Poultry Breeding, National Research Institute of Animal Production, Balice n. Cracow, Poland \* renata.muchacka@up.krakow.pl

The objective of the study was to determine the activity of antioxidant enzymes and the level of GSH and MDA in yolk and albumen of hen's eggs. The experiment was carried out with 99 hens of three heritage breeds (Greenleg Partridge - group I, Sussex – group II, Rhode Island Red – group III). Layers were reared in the litter system and had outdoor access. Birds were fed *ad libitum* standard diets based on concentrates for laying hens and had free access to water throughout the experiment. All the groups were managed under uniform environmental (air humidity and temperature, lighting programme) and feeding conditions. At 48 weeks of age, 6 eggs from each group were collected. In the samples of egg yolks and albumens the concentration of GSH and MDA, and activity of SOD, GPx and CAT were estimated. The results were statistically analysed by one-way analysis of variance and significant differences were estimated with Duncan's multiple range test. The highest activity of SOD, the highest level of GSH and the lowest level of MDA were characterized by eggs from Sussex hens, which may indicate their best quality. Based on the obtained results, it can be concluded that the activity of antioxidant enzymes (mainly SOD) and the levels of GSH and MDA which occur in eggs depend on the breed of hens.

Keywords: antioxidant enzymes, eggs, GSH, hens, MDA

#### HOW SELECTION FOR CONTRASTING YOLK TESTOSTERONE LEVELS AFFECTED REPRODUCTIVE AXIS IN MALE JAPANESE QUAIL

Okuliarova M.<sup>1</sup>, Meddle S.L.<sup>2</sup>, Zeman M.<sup>1,3</sup>

<sup>1</sup>Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic

<sup>2</sup>The Roslin Institute, The Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush, UK <sup>3</sup>Institute of Animal Biochemistry and Genetics, Center of Biosciences, Slovak Academy of Sciences, Bratislava, Slovak Republic

Variability of maternal testosterone (T) levels in the egg yolk has been associated with developmental programming of avian embryos and consequent effects on physiological and behavioural phenotype across all life stages. In our study, we focused on genetically determined variability of such hormone-mediated maternal effects using two lines of Japanese quail divergently selected for low (LET) and high (HET) volk T concentrations. Previously we showed that although yolk T deposition and reproductive performance are regulated by the same neuroendocrine mechanisms, high yolk T concentrations are not limited by the costs on female's reproduction due to permanently increased T levels in the maternal circulation. However, it is not clear, whether there is a link between genetically determined yolk T levels and neuroendocrine mechanisms controlling reproductive performance in males. Therefore, we examined main indicators of reproductive status in the LET and HET males from puberty until the age of 12 months. During maturation, at the age of 4 weeks, HET males displayed a lower index of cloacal gland size (length × width) as well as proportion of individuals producing foam than LET males, while no line differences were found in these reproductive characteristics at the age of 5 weeks. Nonetheless, relative testes weight and basal plasma T concentrations were lower in HET as compared to LET males at both the age of 5 weeks and 12 months (there was a tendency to lower plasma T). At the age between 4 and 6 months, lines did not differ in these parameters but luteinizing hormone response to gonadotropin releasing-hormone challenge was higher in HET than LET males with no line differences in gonadal response. In conclusion, genetic selection for high yolk T levels delayed the onset of sexual maturity and accelerated reproductive decline in male quails. Therefore, our data imply that genetically determined high yolk T deposition can be associated with decreased reproductive performance experienced by males.

Keywords: yolk hormones, sex steroids, maternal effects, reproduction, males

Acknowledgement: Supported by the grant VEGA 1/0686/15 to MO and grant funding from the BBSRC to SLM.

# ELIMINATION OF DEATH CELLS IN MICE BLASTOCYTS PRODUCED IN VIVO AND IN VITRO

Pisko J., Kovaříková V., Fabian D.

Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences, Slovak Republic

It has been shown that phagocytosis is a physiological event occurring during early embryo development. Aim of our study was to identify the physiological capacity of blastomeres to eliminate apoptotic cells by phagocytosis in *in vivo* and *in vitro* obtained blastocysts. Expanded blastocysts were obtained from ICR mice stimulated by hormonal treatment. In vivo developed blastocysts were freshly isolated from fertilized females on Day 4 of pregnancy (168 h post eCG). In vitro blastocysts were derived from the culture of 2-cell embryos in KSOM medium for 72 h at standard conditions. The incidence and localization of phagocytosis was evaluated by fluorescence morphological staining followed by confocal laser scanning microscopy. Nuclei showing apoptotic features (i.e. fragmented nuclear morphology visualized by Hoechst 33342 DNA staining and/or specific DNA fragmentation visualized by positive TUNEL labeling) localized in blastocoele cavity or *perivitelline space* were classified as extruded apoptotic cells, which escaped phagocytosis, Apoptotic nuclei localized within the cytoplasm of neighboring cells (surrounded by continuous plasmatic membrane visualized by F-actin staining) containing normal nucleus as well were classified as phagocytized apoptotic cells. Apoptotic nuclei surrounded by plasmatic membrane of three normal neighboring cells were classified as non-phagocytized apoptotic cells. All other cases were assigned dubious. In vitro derived blastocysts showed significantly higher number of apoptotic cells per blastocyst (6.88 at average) when compared to *in vivo* developed ones (5.18 at average) (P<0.01). In *in vitro* derived blastocysts higher rate of extruded cells (15,58%) than in *in vivo* developed blastocysts (8,97%) was noticed (P<0.05). Predominant majority of extruded cells was localized in blastocoele cavity. In both in vivo and in vitro developed blastocysts, the incidence of phagocytized apoptotic cells reached approximately 25%. Phagocytized apoptotic cells were regularly distributed (50:50) in both embryonic lines (trophoblast and embryoblast). The results indicate, that in blastocysts developed in *in vitro* conditions, higher rate of apoptotic cells escape phagocytosis than in naturally developed ones.

Keywords: phagocytosis, apoptosis, preimplantation embryos, mouse

Acknowledgement: Study was supported by the Slovak Research and Development Agency under contract APVV 14-0763.

#### ASSESSING OF MOUFLON BIOCHEMICAL PARAMETERS DEPENDING ON GENDERS

Pošiváková T.1\*, Hromada R.1, Veszelits Laktičová K.1, Vargová M.1, Pošivák J.2, Švajlenka J.3

<sup>1</sup>Department of the environment, veterinary legislation and economy, University of Veterinary Medicine and Pharmacy in Kosice, Slovak Republic

<sup>2</sup>Clinic for ruminants, University of Veterinary Medicine and Pharmacy in Kosice, Slovak Republic <sup>3</sup>Department of epizootiology and parasitology, University of Veterinary Medicine and Pharmacy in Kosice, Slovak

Republic

<sup>3</sup>Department of Construction Technology and Management, Technical Universities in Kosice, Slovak Republic \* terezia.posivakova@uvlf.sk

The aim of our study was to evaluate the selected biochannel parameters depending on gender. For research were used thirty mouflons of both sexes with approximate age in winter season. Blood samples for biochemical analysis were taken from *vena jugularis* for determination of selected biochemical parameter. Biochemical indicators were measured using the standard automatic analyser. The results of statistical testing of selected biochemical parameters in the experimental group of animals confirmed differences between the genders and at the selected biochemical parameters. Our objective was to find the significant differences in the activity of selected biochemical parameters in experimental group of animals.

Keywords: biochemical status, female, male, laboratory analyses, mouflon

### ALTERATION OF HAEMATOLOGICAL PARAMETERS AFTER SINGLE DOSE OF MYCOTOXINS

## Schneidgenova M., Kalafova A., Capcarova M.

Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic

The aim of the present study was to determine the effect of single dose of mycotoxins (deoxynivalenol DON and T-2 toxin) on selected haematological parameters of rabbit's blood. Mycotoxins are produced by fungi that evoke a toxic response when introduced in low concentrations to animals and humans. These compounds can cause a wide range of acute and chronic systemic effects that cannot be attributed to fungal growth within the host or allergic reactions to foreign proteins. The immune system is designed to protect the host from pathogens and to eliminate disease. Activation of immune system is generally believed to require processing of the antigen by the phagocytic cells such as macrophages, monocytes, or related cells. Experimental group of rabbits received intramuscularly mycotoxins (Romer Labs Division Holding GmbH, Tulln, Austria) at dose 0.08 mg per kg of body weight 72 hours before slaughter. Whole experiment lasted 90 days. In whole blood selected haematological parameters (WBC total white blood cell count, MID medium size cells count, GRA granulocytes count, RBC red blood cell count, HGB haemoglobin, HCT haematocrit, PLT platelet count, MPV mean platelet volume, and PDWc platelet distribution width) were measured using haematology analyser Abacus junior VET (Diatron<sup>®</sup>, Austria). In conclusion, DON and T-2 toxin in single dose 0.08 mg had no effect on rabbit blood: however our previous studies revealed that various secondary metabolites exhibit a wide range of immunomodulating activity. High doses of deoxynivalenol in porcine blood in vitro influenced lymphocyte count. It is known that trichothecenes have adverse effects on dendritic cells and dendritic cell maturation process. Results of this study provide a foundation for further analysis and researches on mycotoxins impact on blood cells and the system of possible protection against its effects as well as evaluation of various dose dependencies on haematological parameters.

Keywords: mycotoxins, haematological parameters, immunomodulating activity

Acknowledgments: This work was financially supported by VEGA scientific grant 1/0760/15.

### MICE PREIMPLANTATION EMBRYO LOSSES CAUSED BY FIPRONIL

Šefčíková Z., Babeľová J., Kovaříková V., Čikoš Š., Špirková A., Koppel J., Fabian D.

Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences, Slovak Republic

The aim of this study was to evaluate the possible toxicity of phenylpyrazole fipronil and the related commercial product FIPRON spot-on on the developmental capacities and qualitative parameters of mice preimplantation embryos. During in vitro tests, isolated two-cell stage embryos were cultured in media with addition of different concentration of fipronil (0, 0.1, 1, 10 and 100 µM) or its commercial product FIPRON spot-on (containing 0.1 and 10 µM of active substance fipronil) until blastocysts formation. During in vivo tests, fipronil at concentration of 0.009 mg/kg BW (corresponding to acute reference dose) and 0.9 mg/kg BW were administered to fertilized female mice by oral gavage from days 1 to 3 of pregnancy. Embryos were isolated from dams on day 4 of pregnancy. Stereomicroscopic evaluation of *in vitro* produced embryos showed that fipronil at 1 µM and higher concentration negatively affected embryonic development. Fluorescence staining revealed that the obtained blastocysts displayed lower numbers of blastomeres at 10 µM concentrations and elevated incidence of cell death from 1 µM concentration. The presence of FIPRON spot-on at a concentration equivalent to 10 µM of fipronil caused massive degeneration of all embryos. During *in vivo* tests in embryos collected from treated mouse females, significantly elevated incidence of cell death was observed even at the acute reference dose. Fipronil impaired the development and quality of mouse preimplantation embryos in both in vitro and in vivo tests and showed embryotoxicity even at the acute reference dose.

Keywords: preimplantation embryo, mouse, in vitro, in vivo, fipronil, FIPRON spot-on

Acknowledgments: This study was supported by the Slovak Research and Development Agency under contract APVV 14-0763.

#### INVOLVEMENT OF TRANSCRIPTION FACTORS IN CONTROL OF OVARIAN FUNCTIONS

#### Sirotkin A.

Constantine the Philosopher University and Animal Production Research Center, Nitra, Slovak Republic

We are the first who studied the control and the role of transcription factors in the regulation of reproductive functions. Our presentation represents 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, STAT1, CREB1), 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. Comparison of cDNA constructs encoding phosphorylable and non-phosphorylable CREB1 showed no substantial differences in their action on the majority of analysed ovarian functions. 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 phosphorylation of some transcription factors is not necessary for their action.

Keywords: transcription factor, ovary, proliferation, apoptosis, hormone, phosphorylation

#### EFFECT OF MIXED HERB EXTRACT ON SELECTED STRESS PARAMETERS IN BROILER CHICKENS OF THREE GENETIC LINES

Skomorucha I.\*, Sosnówka-Czajka E.

Department of Poultry Breeding, National Research Institute of Animal Production, Balice, Poland \* iwona.skomorucha@izoo.krakow.pl

The aim of the study was to determine the effect of adding a mixture of anti-stress herbs to drinking water on stress hormone levels and the heterophil to lymphocyte ratio in the blood of intensively reared broiler chickens of three genetic lines. The experiment was performed with Ross 308, Cobb 500 and Hubbard Flex broiler chickens, which were divided into three experimental groups. Birds were maintained on litter for 42 days at a stocking density less than 33 kg/m<sup>2</sup>. All the groups were provided with the same environmental and feeding conditions. In each group from 21 to 35 days of rearing, water drinkers were supplemented for 5 h/day (08:00-13:00 hours) with an alcoholic extract from mixed herbs (30% chamomile, 10% oregano, 10% yarrow, 10% knotgrass, 10% valerian, 20% inflorescence of large-leaved lime) at 2 ml/l water. At 21, 28, 35 and 42 days of rearing, blood was collected from 10 birds per group to determine corticosterone, noradrenaline and adrenaline levels. The heterophil to lymphocyte ratio (H:L) was also calculated. On day 42 of the study, 500 broiler chickens were characterized by the lowest noradrenaline concentration and a lower H:L ratio compared to Ross 308 and Hubbard Flex ( $p \le 0.05$ ) and Ross 308 chickens  $(p \le 0.01)$ , respectively. It was concluded from the study that the herb extract had the most favourable effect on relieving the body's physiological response to stress, and thus on improving welfare in Cobb 500 compared to Ross 308 and Hubbard Flex broilers. It can therefore be considered that active substances in herbs have varying effects on the body of broiler chickens of different origin.

Keywords: adrenaline, broiler chickens, corticosterone, H:L, mixed herb extract, noradrenaline

#### MINERAL PROFILE OF RABBIT BLOOD AFTER ZEOLITE ADMINISTRATION

Slanina T.1\*, Tirpák F.1, Herc P.1, Zbyňovská K.1, Halo M.1, Vizzarri F.2, Ožvold M.1, Kováčik A.1

<sup>1</sup>Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic

<sup>2</sup>Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy \* tomas.slanina@uniag.sk

Zeolite is a tectosilicate with three-dimensional structure consisting of SiO<sub>4</sub> and AlO<sub>4</sub> tetrahedrons linked through the shared oxygen atoms. It is porous material, characterized by the ability to lose and gain water reversibly. It is able to adsorb molecules either as adsorbent or molecular sieve and to exchange their constituent cations without major change of their structure. The administration of zeolite as a feed supplement potentially enhance feed utilization. The aim of the study was to determine influence of zeolite on mineral profile of rabbit blood. Animals (Castorex, adult male rabbits, body weight  $4 \pm 0.5$  kg; n=12) were divided into two groups: the control group (C) without addition of zeolite and the experimental group (Z) which received zeolite peroral in concentration 0.2 g per kg of body weight. During the experiment three collections of blood was obtained from vena auricularis (after 4, 8 and 12 weeks of the experiment). Blood serum was obtained by centrifugation of coagulated blood for 20 min at 3000 rpm and stored at -20 °C until analyses at the Department of Animal Physiology, Biochemical parameters of mineral profile were analysed. Concentrations of calcium, magnesium, and phosphorus in blood serum were determined on device Rx Monza (Randox Laboratories Ltd., United Kingdom). Sodium (Na), potassium (K) and chloride (CI) ions were analysed using EasyLite analyser (Medica, Bedford, MA, USA) provided with an ionselective electrode. The statistical evaluation was performed using statistical program GraphPad Prism 6.01 (t-test). The obtained results showed that zeolite has non-significant ( $P \ge 0.05$ ) effect on monitored parameters. The content of chlorides, potassium and calcium in the blood had increasing tendency in both control and experimental group. On the other hand decreasing concentration of phosphorus in rabbit blood we observed during the experiment. Higher values of calcium, phosphorus, sodium, potassium and chlorides ( $P \ge 0.05$ ) after 12 weeks of experiment in experimental group were observed in comparison with the control group. Concentration of magnesium was insignificantly lower ( $P \ge 0.05$ ) in experimental group in comparison with the control group. This study showed that zeolite has not significantly negative effect on mineral profile of rabbit blood after 12 weeks of administration and it could be used as a supplement in feed mixtures without potential risk.

Keywords: clinoptilolite, feed, rabbits, blood serum, biochemistry

Acknowledgments: This work was supported by the Slovak Research and Development Agency under the contract no. APVV-16-0289.

#### DIET SUPPLEMENTATION WITH FLAXSEED STIMULATES GUT METABOLISM IN MICE

Sopková D.\*, Vlčková R., Andrejčáková Z., Gancarčíková S., Ondrašovičová S., Petrilla V.

Department of Anatomy, Histology and Physiology, Institut of Physiology, University of Veterinary Medicine and Pharmacy, Košice, Slovak Republic \* drahomira.sopkova@uvlf.sk

Essential polyunsaturated fatty acids (PUFAs) in the feed may affect the gastrointestinal microbiota. The present study investigated the effect of 35-day supplementation of mice diet with 5 % concentration of high- $\omega$ -3 PUFAs in flaxseed with focus on intestinal metabolism of mice. Capillary isotachophoresis method was used for the assessment of the level of organic acids in the gut material and faeces. Supplementation of diet with flaxseed (FA) caused a significant increase in the level of organic acids in mice caecum (acetic acid, P<0,01; propionic, butyric and valeric acids P<0,001) in comparison with group K. Examination of faeces of flaxseed-fed mice showed increased concentrations of lactic acid on days 7 and 28 (P<0.01), acetic acid on days 14 (P<0.05), 21 and 28 (P<0.01) and butyric acid on days 14 (P<0.001), 21 and 28 (P<0.01) of supplementation in comparison with corpus K. The most pronounced effect of supplementation of flaxseed on the level of all investigated organic acids was observed on day 28 of the feeding experiment (lactic, acetic and butyric acids P<0.01). On day 35 of the experiment, we observed a pronounced decrease in the concentration of acetic (P<0.001), lactic (P<0.01) and butyric (P<0.05) acids in the fortified group. The investigated additive had a stimulatory effect on intestinal metabolism and fermentation activity of beneficial bacteria.

Keywords: flax seed, intestine, mice, microbiota, organic acids

Acknowledgement: The study was supported by the project VEGA No. 1/0476/16.

#### SELECTED BLOOD PARAMETERS IN ORGANICALLY RAISED HENS FED WITH PURPLE CONEFLOWER SUPPLEMENTED DIET

#### Sosnówka-Czajka E.\*, Skomorucha I.

Department of Poultry Breeding, National Research Institute of Animal Production, Krakow, Poland \* ewa.sosnowka@izoo.krakow.pl

The aim of the study was to determine the effect of dietary addition of dried purple coneflower (Echinacea purpurea (L.) Moench) on selected blood parameters of chicks and hens of the native Greenleg Partridge breed (Z-11), maintained on a certified poultry farm according to organic farming principles. Birds were divided into two groups according to diet: organic layer diet (group 1), and the same diet supplemented with certified purple coneflower (*Echinacea purpurea* (L.) Moench) from 20 wks of age at 10 g/kg feed (group 2). Layers were fed the certified layer diet (17% protein and 11.1 MJ ME/kg diet) following organic farming principles. Experimental incubations were performed at 34 wks of age. Blood was collected from laving hens at 34 wks and from newly hatched chicks at one day of age to determine selected blood parameters. The organically raised Greenleg Partridge hens and chicks responded to the purple coneflower supplement by changes in blood picture, which are important indicators of avian health and condition. As in Z-11 chicks, purple coneflower supplemented to the layer diet reduced the H:L ratio and induced a tendency for higher level of the immunoglobulin complex and IgG in blood, which shows their better living comfort and higher immunity. Dietary purple coneflower was observed to have no effect on the level of hematocrit, erythrocytes, leukocytes and hemoglobin. High individual variation occurred for the immunoglobulin complex and IaG in the organically raised Greenleg Partridge hens.

Keywords: hens, chicks, purple coneflower, blood parameters, organic production, immunity

Acknowledgement: Carried out based on Ministry of Agriculture and Rural Development decision no. HOR.re.027.5.2017

#### IDENTIFICATION OF GLUCOCORTICOID RECEPTOR TRANSCRIPTS IN MOUSE OOCYTES AND PREIMPLANTATION EMBRYOS

Špirková A., Babeľová J., Kovaříková V., Šefčíková Z., Fabian D., Koppel J., Čikoš Š.

Institute of Animal Physiology, Centre of Biosciences of the Slovak Academy of Sciences, Košice, Slovak Republic

Available data suggest that glucocorticoids can affect very early stages of reproductive process. including oocyte maturation, fertilization and preimplantation embryo development. Actions of natural and synthetic glucocorticoids in target tissues are mediated by glucocorticoid receptor (GR). and a number of receptor subtypes arising from alternative processing of the GR gene have been discovered in recent years. We examined expression of known and potential glucocorticoid receptor transcripts in ovulated mouse oocytes and preimplantation embryos at various developmental stages. Using RT-PCR with specific oligonucleotide primers we obtained PCR products with the sizes corresponding to two GR splice variants (in oocytes, 4-cell embryos and 8- to 16-cell embryos) or to four splice variants (in blastocysts). To confirm the identity of the amplified sequences, PCR products obtained from oocytes and preimplantation embryos were sequenced. We found 100% identity between the nucleotide sequences of PCR products amplified with GRa, GRB and GRy primers and the corresponding mouse glucocorticoid receptor mRNA sequences deposited in gene databases. In embryos at the blastocyst stage, we identified a transcript orthologous to the human GR-P subtype. Our results indicate that the GR-P transcript is formed in the same way as in human and porcine orthologs. However, the predicted mouse GR-P protein contains significantly more amino acids coded by the new (GR-P-specific) exon sequence than the human protein. These results suggest that glucocorticoids can directly act in cells of preimplantation embryos, and their effects can depend on the glucocorticoid receptor subtype expressed in the cells.

Keywords: preimplantation embryos, glucocorticoid receptor, GR splice variants

Acknowledgement: This work was supported by the Slovak Academy of Sciences project VEGA 2/0039/15.

#### AGE-RELATED CHANGES IN BONE MICROSTRUCTURE OF MICE

Sranko P.<sup>1</sup>, Sarocka A.<sup>2</sup>, Kovacova V.<sup>2</sup>, Babosova R.<sup>2</sup>, Mondockova V.<sup>1</sup>, Uhrin P.<sup>3</sup>, Omelka R.<sup>1</sup>, Martiniakova M.<sup>2</sup>

<sup>1</sup>Department of Botany and Genetics, <sup>2</sup>Department of Zoology and Anthropology, Faculty of Natural Sciences, Constantine the Philosopher University, Nitra, Slovak Republic <sup>3</sup>Department of Vascular Biology and Thrombosis Research, Center for Biomolecular Medicine and Pharmacology,

Department of Vascular Biology and Thrombosis Research, Center for Biomolecular Medicine and Pharmacology, Medical University of Vienna, Vienna, Austria

The current study was performed to analyse possible age-related changes in bone microstructure of 129 S/v Swiss mice. Ten healthy 129 S/v Swiss mice (males) were divided by different age in two groups: juvenile mice (E1 group) at age 87 days and adult mice (E2 group) at age 355 days. Modern 2D and 3D imaging methods were used to determine femoral bone microstructure in detail. On the basis of morphometrical 2D evaluations of the compact bone, it can be concluded that with growing age, the sizes of the primary osteons vascular canals, Haversian canals and secondary osteons increased considerably (P<0.05). 3D imaging methods revealed that relative bone volume and bone surface of the compact bone were not affected by different age. However, bone mineral density (BMD) was significantly higher in adult mice in comparison to the E1 group (P<0.05). The number of secondary osteons was also higher in adult individuals, which can be justify by ongoing intracortical (endocortical) bone remodelling. An occurrence of some resorption cavities was observed in the E2 group; whereas these structures were absent in the E1 group. In the trabecular bone, significantly lower values of relative bone volume, trabecular number and trabecular thickness were documented in adult mice (P<0.05). On the contrary, the value for trabecular separation was higher in the E2 group as compared to the E1 group. Our results indicate several changes in femoral bone microstructure of 129 S/v Swiss mice due to different age.

Keywords: compact bone tissue, trabecular bone tissue, age, mice

Acknowledgements: The study was supported by the project VEGA 1/0505/18. This work was supported by Research Center AgroBioTech built in accordance with the project Building Research Centre "AgroBioTech" ITMS 26220220180.

#### EFFECT OF SUBSTANCES USED IN "SMART DRUGS" ON SELECTED PARAMETERS OF SPERMATOZOA MOTILITY

#### Stachańczyk K.

<sup>2</sup>Department of Vertebrate Zoology and Human Biology, Institute of Biology, Pedagogical University of Cracow, Poland \* stachanczyk.katarzyna@gmail.com

The domestic rabbit (Orvctolagus cuniculus f. Domesticus L. 1758) is one of the examples of model organisms widely used in reproductive biology research. Semen of these mammals is successfully used in all experiments seeking to improve methods of in vitro fertilization. "Smart drugs" have recently become a media topic, due to the revealed cases of serious health complications after taking them. In Poland, the first store offering "smart drugs" was estabilished in 2008. In other European countries, mostly in Great Britain, Germany and the Netherlands, they have been operating for several years. So far, these stores have not been banned in any of the European Union countries. The denomination "smart drugs" is not a scientific term [...], and knowledge about the risks associated with their use is very limited [...] [1]. One of the substances used in these drugs is  $\beta$  - phenylethylamine. There is a supposition that this compound in sufficiently large amounts has a psychoactive effect, similar to the action of amphetamine. Nevertheless, the exact role of this trace amine is not fully understood. The aim of the experiment was to verify the effect of  $\beta$  - phenylalanine on selected rabbit semen parameters. The ejaculate samples were stored at 5 °C. Each of them was analyzed using the CASA Sperm Vision ™ program. During the experiment, the following parameters were considered: MOT: Percentage of mobile spermatozoa (%), PRO: percentage of sperm with progressive movement (%), VCL: mean sperm speed after the real path of movement (u / s). ALH: amplitude of lateral head displacement (um) and BCF: frequency of lateral deviations of the sperm head (Hz). The obtained data was analyzed using the GraphPad Prism 6 program.

Keywords: rabbit, spermatozoa, phenylethylamine, CASA

#### References

[1] B. Bukowska, M. Kidawa, D. Chojecki: Dopalacze, Remedium nr 5 (195), maj 2009 s. 30.

#### PERIODICITY OF CHANGES IN FUNCTIONAL INDICES IN ANIMALS AND HUMANS

Strashko S.\*, Bilyk V.

Department of medico-biological and valeologic fundamentals of life and health protection, Faculty of Pedagogy and Psychology, National Pedagogical Dragomanov University, Kyiv, Ukraine \* s.strashko@gmail.com

In a series of long-term experiments with duration of up to 100 days, we investigated the dynamics of food and water consumption, body weight gain, body density, body water content, and changes in the hypoxic endurance of rats. These indicators were measured daily at the same time. Studies were carried out on young white rats of the Wistar line of both sexes. Dry mixed fodder balanced for a growing organism was used as feed. The body volume was determined by Renew-Lermontov volumenometer with an accuracy of 0.1 ml. Water content of the body was calculated from the body density by Yu. M. Madievsky's method. The hypoxic endurance was determined by smoothing of the T wave of cardiogram and by convulsive syndrome manifestation when simulating rise to an altitude in a pressure chamber according to V. Ya. Berezovsky's method. There were no significant interindividual differences in all the studied indicators. For instance, the average density of the body was  $1.1222 \pm 0.0148$  g / cm<sup>3</sup>. At the same time, individual density indices fluctuated within  $\pm$  5% from the average value. Variance of indicators in hypoxic resistance of the same animal on different days reached ± 19.3% and were to 7 500 to 11 000 meters above sea level. As a result of processing of the dynamics series by the method of sliding smoothing, were revealed periodic changes of the studied indices, with a length of about two weeks. They are correlatively related to body weight fluctuation that enables to refer them to manifestation of the "Basal rhythm of trophic processes" (BRT) (according to I. S. Kucherov). The analysis of primary data in the study of BRT in the activity of various human physiological systems made possible to establish an average value of the period of this biorhythm, which is 12.6 days. The synchronizer of this rhythm is unclear. However, its stability allows to speak of the presence of an external synchronizer, and, consequently, of the exogenous nature of the BRT and its adaptive role aimed at compensating for changes in environmental parameters.

Keywords: basal rhythm of trophic processes, exogenous biorhythm

#### VIABILITY ASSESSMENT OF CHICKEN PGCs BY TRYPAN BLUE EXCLUSION AND FLUORESCENCE LABELLING TECHNIQUE

Svoradová A.<sup>1\*</sup>, Makarevich A.<sup>2</sup>, Čurlej J.<sup>3</sup>, Chrenek P.<sup>2,3</sup>

<sup>1</sup>Constantine the Philosopher University, Faculty of Natural Sciences, Nitra, Slovak Republic <sup>2</sup>Research Institute for Animal Production in Nitra, National Agricultural and Food Centre, Lužianky, Slovak Republic <sup>3</sup>Slovak University of Agriculture, Faculty of Biotechnology and Food Science, Nitra, Slovak Republic \* svoradovaandrea1@gmail.com

In the present study, we compare a classical trypan blue exclusion (TBE) and fluorescence labelling (FL: propidium iodide staining) techniques to evaluate viability (live/dead cells) of fresh and frozen/thawed chicken primordial germ cell (PGCs). Six-day old embryos of ROSS 308 chicken breed line were used in the experiment. Gonads were recovered from chicken mesonephros using forceps, scissors and needles. The PGCs were isolated from the gonads by disaggregation in 0.25% trypsin-0.02% EDTA in HBS solution and subsequently re-suspended in a freezing medium composed of MEME medium, 20% fetal bovine serum and 10% DMSO. Aliquots of cell suspension were transferred into cryovials, cooled in a BICELL commercial freezing device and kept in a freezer at -80 °C overnight before placing into LN<sub>2</sub> for storage. Following 7 day-storage, the cell samples were thawed by placing the cryovials into a water bath at 37 °C until the disappearance of ice crystals. For the live/dead cell assessment, TBE and FL techniques were performed. We observed significant differences (p < 0.05) between TBE and FL techniques in detectable percentages of dead cells in fresh (14.14  $\pm$  2.54 vs. 7.16  $\pm$  2.08, resp.) and frozen-thawed (56.08  $\pm$  2.96% vs. 35.45  $\pm$  3.21%, resp.) samples. These differences may be due to methodological features of each technique tested. In particular, although TBE is obviously more fast and simple than FL, when evaluation time is longer than 10 min, trypan blue enters also live cells, thus giving an overestimated value. Therefore, the choice of proper technique for a cell viability evaluation should be done carefully.

Keywords: chicken, PGCs, viability, trypan blue, fluorescence microscopy

Acknowledgement: This study was financially supported by the Slovak Research and Development Agency (No. APVV-14-0043) and VEGA 1/0611/15.

#### CAN XYLENE AND QUERCETIN DIRECTLY AFFECT BASIC OVARIAN CELL FUNCTIONS?

Tarko A.<sup>1\*</sup>, Štochmaľová A.<sup>1</sup>, Hrabovszká S.<sup>1</sup>, Vachanová A.<sup>1</sup>, Harrath A.H.<sup>2</sup>, Grossman R.<sup>3</sup>, Sirotkin A.V.<sup>1#</sup>

<sup>1</sup>Department of Zoology and Anthropology, Constantine the Philosopher University, Slovak Republic <sup>2</sup>Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia <sup>3</sup>Department of Functional Genomics and Bioregulation, Friedrich Loeffner Institute, Mariensee, Neustadt, Germany \* adam.tarko@ukf.sk, # asirotkin@ukf.sk

Exposure to xylene is associated with the dysfunction of mammalian female reproduction. Ouercetin present in vegetables contribute significantly to their role as health-promoting foods. The effects of both xylene and guercetin on ovarian cell function, their interrelationships, and mechanisms of action are insufficiently studied. In this in vitro study, we examined the effects of xylene, guercetin, and xylene/guercetin combination on basic bovine ovarian cell functions, such as proliferation, apoptosis, and hormone release. Furthermore, we examined the protective effect of guercetin against the potential negative effects of xylene. Proliferation and apoptosis were assessed via immunocytochemistry using PCNA and BAX markers. The release of progesterone, testosterone, and insulin-like growth factor (IGF-I) was analysed by RIA. Xylene stimulated proliferation and IGF-I release, but inhibited progesterone and testosterone release. Ouercetin inhibited proliferation, apoptosis, and release of IGF-I, progesterone, and testosterone. When administered with xylene, guercetin prevented the action of xylene on proliferation and IGF-I release, induced the stimulatory action of xylene on apoptosis, and promoted the effect of xylene on release of progesterone but not testosterone. Our results demonstrated the actions of both xylene and guercetin on ovarian cell functions. Xylene promoted ovarian cell proliferation and IGF-I release but inhibited progesterone and testosterone release. When administered alone, guercetin inhibited all the examined ovarian cell functions. Moreover, quercetin prevented some effects of xylene (proliferation and IGF-I release), while inducing/promoting other effects (progesterone release and stimulatory effect on apoptosis), suggesting that guercetin could be used for the prevention of certain effects of xylene on reproductive processes.

Keywords: xylene, quercetin, proliferation, apoptosis, hormones, ovary

Acknowledgments: The authors would like to thank Ing. Ž. Kuklová and Mrs. K. Tóthová for their technical assistance. This study was funded by the grants APVV–0854–11, APVV–0404–11, APVV–15–0296, VEGA–1/0392/17, as well as grants from the UGA VIII/26/2017. " and the International Scientific Partnership Program ISPP at King Saud University for funding this research work through ISPP# 0013.

#### TWO SIDES OF NON-IONIZING RADIATION IN DAILY LIFE USE - REPRODUCTIVE **APPROACH**

Tirpak F.\*, Slanina T., Halo M. Jr, Mamrakova R., Massanyi P.

Department of Animal Physiology; Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic

\*filip.tirpak@gmail.com

Non- ionizing radiation (NIR) is nowadays widely used in all aspects of humanity. Ubiguitousness of NIR makes the everyday human activities much easier so its adverse effects are often forgotten or underestimated. One of the most important and irreplaceable uses of NIR is found in protection of public danger. Security screenings arised from the multiple terrorist activities. However the positive outcome of NIR use in this way is very clear, harmful effects have not been thoroughly studied vet. Freshly collected boyine semen was radiated with hand-held metal detector in various exposition times (10 seconds, 15, 30 and 60 minutes) and the spermatozoa were subjected to motility analyses. After an hour of incubation radiated spermatozoa showed significantly decreased total and progressive motility. Velocity parameters were significantly increased right after radiation. Cryopreserved semen was radiated (10 seconds, 1 and 5 minutes) prior to / post freezing to determine the potential risk of airport security screening. Significant changes were monitored in spermatozoa velocity parameters of semen radiated prior to cryopreservation. In semen radiated following the freezing process, the spermatozoa motility was significantly affected after 60 minutes of incubation, while spermatozoa velocity was enhanced in both measured intervals. Current study presents the potential risk arising from NIR, which is commonly used in everyday life, with undesirable impact on reproduction of animals and the mankind.

Keywords: non-ionizing radiation, sperm, bull, motility, cryoconservation

Acknowledgment: We would like to thank MSc. Zofia Ballonova, for her assistance related to samples collection and analyses. This work was funded by the Slovak Research and Development Agency Grants no. VEGA 1/0539/18, VEGA 1/0760/15, APVV-16-0289 and APVV-15-0544.

#### EFFECT OF TARAXACUM OFFICINALE ROOT EXTRACT ON MURINE FIBROSARCOMA CELLS IN VITRO

Tomenendálová J.1\*, Korbášová M.2, Kuchařová V.1, Veselá I.1

<sup>1</sup>Department of Physiology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic <sup>2</sup>Department of Pharmacology and Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

\* tomenendalovaj@vfu.cz

The aim of this study was to investigate effect of *Taraxacum officinale* root extract (TOE) on the viability of mice fibrosarcoma cells (FSC), compared to physiological 3T3 fibroblasts (3T3), describe mechanisms of TOE activity and evaluate impact of season, when plant was collected. The shred TO root was macerated in methanol for 48 hours, and in chloroform for 24 hours. Both extracts were reduced in vacuum rotary evaporator (VRE) and hydrophilic compounds were separated by dissolving in water and reducing in VRE. Non-tumorous mice fibroblasts 3T3 and mice fibrosarcoma cells WEHI-13VAR (ATTC<sup>®</sup> CRL-2148<sup>™</sup>) were incubated with TOE diluted in medium (25, 50, 100, 250 and 500times [TOE 25x, 50x, 100x, 250x, 500x]) and with medium only as a control. The test of viability (TACS MTT Cell Proliferation Assays [Trevigen, USA]), detection of caspase 3 (Colorimetric Caspase 3 Assay Kit [Sigma-Aldrich, USA]) and test of production of ROS (Total ROS/Superoxide Detection Kit [Enzo Life Sciences, USA]) have been performed. FSCs showed significantly lower viability (P<0.01) compared to 3T3 when incubated in TOE 25x-250x. Viability of both lines did not differ significantly when incubated in TOE 500x. TOE of the plants harvested after flowering (autumn) shows a lower cytotoxic effect than the TOE from plants harvested in the spring before flowering. This is particularly evident in FSC (P<0.01). Production of ROS was significantly higher in both FSC and 3T3 incubated in TOE 50x compared to untreated control cells. Activity of caspase 3 was significantly higher in FSC, compared to 3T3 (P<0.01; TOE 50x) and in TOE 50x incubated FSC compared to untreated FSC (P<0.0001). There was no difference compared TOE 50x treated 3T3 fibroblasts and untreated 3T3. Taraxacum officinale root extract shows cytotoxic effect to mice fibrosarcoma cells, increases production of ROS in both physiological and tumor cells and increases activity of caspase 3 in fibrosarcoma cells.

Keywords: dandelion, Taraxacum officinale, fibrosarcoma, fibroblast, viability, caspase 3

Acknowledgement: This work was supported by grant IGA VFU Brno 114/2016/FVL.

#### **RABBIT ADIPOSE TISSUE AS A SOURCE OF MESENCHYMAL STEM CELLS**

Tomková M.1\*, Kulíková B.2, Vašíček J.1,2, Baláži A.2, Chrenek P.1,2

<sup>1</sup>Faculty of Biotechnology and Food Science, Slovak University of Agriculture, Nitra, Slovak Republic <sup>2</sup> NPPC - Research Institute for Animal Production Nitra, Lužianky, Slovak Republic \* majkat.93@gmail.com

Adipose tissue is composed mostly of adipocytes and stromal vascular fraction. This fraction includes fibroblasts, endothelial cells and preadipocytes. Currently, this tissue also appears as a good and easily accessible source of stem cells. The aim of our study was to optimize the methodology of isolation and cultivation of stem cells from rabbit adipose tissue. Rabbits of New Zealand White line were subjected to the study at age of 3 – 6 months. Visceral fat samples were collected from the humanely sacrificed animals. Adipose tissue was minced and digested by 0.075% collagenase type I at 37°C during 90 minutes of continual shaking. Stem cells sedimented on the bottom of the 50 ml centrifugation tube. The pellet of separated cells was resuspended in 1 ml of culture medium and consequently transferred into culture flasks. Cells were cultivated in aMEM medium supplemented with 20% FBS and 1% penicillin and streptomycin at 37°C and 5% CO<sub>2</sub>. When cells reached 80 – 90% confluency they were dissociated using 0.05% trypsin and reseeded again. Cells from the third passage were used for the flow cytometry analyses. In addition to the cell viability, surface markers (CD29, CD34, CD44 and CD45) were observed to confirm the phenotype of mesenchymal stem cells. In the first days of primary culture, single cells with spindle shape morphology were monitored. With respect to the morphology, the cells were spreading along with the increased length of cultivation and confluency. The positive expression of CD29 and CD44 confirmed the mesenchymal origin of the adipose derived stem cells. Hematopoietic lineage markers CD34 and CD45 were not detected. In conclusion, rabbit adipose tissue showed to be a promising source of stem cells that could be preserved as a genetic resource in animal gene bank.

Keywords: rabbit; adipose tissue; stem cells; flow cytometry

Acknowledgments: This study was funded by the grants APVV-14-0043 coordinated by the Slovak Research and Development Agency and VEGA 1/0611/15 and VEGA 1/0160/18.

#### ALTERATIONS IN SELECTED PARAMETERS OF HORMONAL PROFILE IN DAIRY COWS DURING TRANSITION PERIOD

Vargová M.1\*, Veszelits Laktičová K.1, Pošiváková T.1, Hromada R.1, Kováč G.2

<sup>1</sup>Department of the environment, veterinary legislation and economy, University of Veterinary Medicine and Pharmacy, Košice, Slovak Republic

<sup>2</sup>Clinic for Ruminants, University of Veterinary Medicine and Pharmacy, Košice, Slovak Republic \* maria.vargova@uvlf.sk

The majority of all diseases occur during three weeks before parturition to three weeks after parturition, in the transition period (Bauman 2000). These diseases include the fatty liver syndrome, ketosis, alkalosis, oxidative stress, laminitis, mastitis, milk fever, retained placenta, metritis, infertility. A number of metabolic hormone concentrations also change over this critical period. Changes in insulin play an important role in the metabolic adaptation of cattle to changes in weight and body condition (León et al. 2004). Genetic selection for milk production has been associated with a decline in circulating insulin levels in dairy cows (Taylor et al. 2003) and insulin concentrations tend to fall in early lactation. Changes in the plasma concentration of leptin, could also be an important adaptation, particularly given the role of white adipose tissue (WAT) in support of early lactation in dairy cattle. Leptin act to regulate food intake, energy expenditure and homeostasic body weight. Ghrelin has a role in signaling the deposition of fat tissue by increasing food intake and reducing fat utilization (Bradford and Allen 2008). The aim of this study was to evaluate dynamics of parameters of hormonal profile on 15 dairy cows of the Slovak Pied Cattle from 3 weeks (wk) before parturition (a.p.) to 9 weeks after parturition (p.p.). The concentrations of leptin during a.p. increased from  $23.08 \pm 10.58$  ng/ml to  $26.80 \pm 11.47$  ng/ml then gradually decreased (P>0.05). The highest value of ghrelin was recorded 6 weeks after parturition (35.94  $\pm$ 16.85 pg/ml). In the case of insulin, the concentrations before calving were higher (from 580.8  $\pm$ 66.3 to 625.5  $\pm$  174.9 IU/ml) than after calving (from 483.3  $\pm$  289.0 to 388.7  $\pm$  172.5 IU/ml). These data provide evidence of the ability of dairy cows to cope with nutritional and metabolic challenges during the transition from non-lactation gestation period to lactation non-gestation period.

Keywords: hormonal profile, transition period, dairy cows, leptin, ghrelin, insulin

Acknowledgement: This work was supported by KEGA č. 003UVLF-4/2016 from the Ministry of Education.

#### DIFFERENT MACS SORTING STRATEGIES FOR THE ENRICHMENT OF LIN<sup>-</sup> (CD34<sup>+</sup>CD45<sup>-</sup>) HEMATOPOIETIC PROGENITOR CELLS: PRELIMINARY STUDY

Vašíček J.<sup>1,2\*</sup>, Baláži A.<sup>1</sup>, Parkányi V.<sup>1</sup>, Bauer M.<sup>1,3</sup>

<sup>1</sup>Institute for Farm Animal Genetics and Reproduction, Research Institute for Animal Production Nitra, NAFC, Lužianky, Slovak Republic

<sup>2</sup>Department of Biochemistry and Biotechnology, Faculty of Biotechnology and Food Science, Slovak University of Agriculture, Nitra, Slovak Republic

<sup>3</sup>Department of Botany and Genetics, Faculty of Natural Sciences, Constantine the Philosopher University, Nitra, Slovak

Republic

\* jaromir.vasicek@gmail.com

Magnetic-activated cell sorting (MACS) has become a standard method for the isolation of hematopoietic stem/progenitor cells (HSC/HPC) in human or mouse model using CD34 antibodies. However, at the present there is no useable CD34 antibody that could be successfully used for the selection of rabbit HSC/HPC. Therefore, the aim of this preliminary study was to remove all mature cells (CD45<sup>+</sup>) from the heterogeneous mixture of rabbit peripheral blood and bone marrow mononuclear cells (PBMCs and BMMCs) in order to enrich these cell populations for the CD34<sup>+</sup> cells. Briefly, cells were stained with CD45 antibody and proper magnetic Microbeads. Three different MACS sorting strategies were used in the experiment that differed mainly in the sample loading rate and the number of used magnetic columns. Control (unsorted) and sorted cells were assessed for the sorting efficiency (% of double positive cells for CD45 and Labelling Check Reagent – LCR) by flow cytometry and for the relative expression of CD34 antigen by gPCR. According to flow cytometry, Depl025 mode reached the best sorting efficiency in terms of the lowest percentages of CD45<sup>+</sup>LCR<sup>+</sup> cells for rabbit PBMCs as well as BMMCs, aPCR analysis confirmed this mode as the best in terms of the relative CD34 expression for rabbit PBMCs. However, higher relative expression of CD34 in BMMCs were obtained by other mode Posselds. In conclusion, this study indicated possible enrichment of rabbit (CD34<sup>+</sup>) HSC/HPC by the magnetic depletion of mature hematopoietic (CD45<sup>+</sup>) cells.

Keywords: CD34, CD45, flow cytometry, hematopoietic stem cells, MACS, qPCR, rabbit

## DOES AERUGINOSIN-865 HAVE THE ADVERSE EFFECT ON TUMOUR CELL LINES?

Veselá I.<sup>1\*</sup>, Celá Kolísková P.<sup>1</sup>, Kuchařová V.<sup>1</sup>, Tomenendálová J.<sup>1</sup>, Řeháková K.<sup>2</sup>, Hrouzek P.<sup>3</sup>, Cheel J.<sup>3</sup>

<sup>1</sup>Department of Physiology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

<sup>2</sup>Small Animal Clinical Laboratory, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic <sup>3</sup>Centre Algatech, Institute of Microbiology, The Czech Academy of Sciences (CAS) v.v.i., Trebon, Czech Republic \* veselai@vfu.cz

Aeruginosins represent group of tetrapeptides composed of for subunits. They have been isolated from different strains of Cyanobacteria (Nodularia, Microcystis or Planktothrix). These natural substances have been an attractive research topic because of their interesting biological properties such as antioxidant, antiproliferative or cytotoxic activities. Aeruginosin-865 (Aer-865) is a new aeruginosin-type peptide with a unique structure, formula  $C_{41}H_{64}N_6O_{14}$ , and is the first aeruginosin isolated from terrestrial cyanobacterium Nostoc strain. Simultaneously Aer-865 is the first aeruginosin showing anti-inflammatory activity, which makes it an interesting immunomodulatory agent with potential medical applications. In this study we tested a response of animal tumour cell lines to the different range of Aer-865 concentrations. Aer-865 was provided by the Laboratory of Algal Biotechnology, Institute of Microbiology, Czech Academy of Sciences in Trebon. We applied Aer-865 in 10  $\mu$ M, 50  $\mu$ M, 100  $\mu$ M and 200  $\mu$ M concentrations to the mouse tumour fibroblast cell line WEHI-13VAR and compared with the effect on mouse normal fibroblast cell line NIH/3T3. Cell lines were cultured in RPMI-1640 medium and MEM Alpha medium that were supplemented with 10% FBS and 1% Penicillin-Streptomycin and placed in 96-well plates into a 5% CO<sub>2</sub> incubator at 37°C. The effect of Aer-865 was evaluated after 24hours incubation by LDH cytotoxicity assay kit. A positive and a negative control were provided. Statistical analysis was evaluated using MedCalc statistical software. Differences with a  $\rho < 0.05$  were considered statistically significant. The mouse normal fibroblast cell line NIH/3T3 tolerated Aer-865 in all used concentrations without any cytotoxic effects when compared with tumour fibroblast cell line WEHI-13VAR. Aer-865 showed high-dose cytotoxicity on fibrosarcoma cells with statistical significance p < 0.05. Aer-865 differs significantly in its cytotoxic effect on normal and tumour fibroblast cells. Mouse fibrosarcoma cells were more sensitive to the cytotoxic effect of Aer-865, which makes it a potential therapeutic medication. Further studies are necessary to clarify the dissimilar mechanism of action in mouse tumour fibroblast cells in comparison with normal mouse fibroblasts.

Keywords: Aeruginosin-865, cyanobacteria, fibroblast, mouse, cytotoxicity

Acknowledgement: This work was supported by the Internal Grant Agency of the University of Veterinary and Pharmaceutical Sciences Brno, Grant No. 109/2016/FVL.

#### DIETARY SUPPLEMENTATION WITH ALGAE AND POLYPHENOLS IN RABBIT MALE: EFFECTS ON SEMEN QUALITY TRAITS

Vizzarri F.<sup>1</sup>, Palazzo M.<sup>1</sup>, Casamassima D.<sup>1</sup>, Corino C.<sup>2</sup>, Chiapparini S.<sup>2</sup>, Ondruska L.<sup>3</sup>, Knizatova N.<sup>4</sup>, Massanyi M.<sup>4</sup>, Tirpak F.<sup>4</sup>, Massanyi P.<sup>4</sup>

<sup>1</sup>Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy <sup>2</sup>Department of Health, Animal Science and Food Safety, University of Milano, Milano, Italy <sup>3</sup>National Agricultural and Food Centre, Nitra, Lužianky, Slovak Republic <sup>4</sup>Department of Animal Physiology, Slovak University of Agriculture, Nitra, Slovak Republic

In recent years, many studies have been focused on natural substances that can affect health of animals. A mix of different extracts was used as dietary supplement and it consists exclusively of natural products. Its main components are polyphenols from terrestrial and marine origins and plant polysaccharides. The effect of this supplement on reproduction has not been reviewed in the past what is a reason why its effect on the reproduction potential of male rabbits was tested. The aim of the present study is to determine effects of natural mix during 120-days long in vivo experiment on selected reproductive traits of male rabbits. Natural mix was supplemented in two different concentrations (T1 - 0.3 % and T2 - 0.6 %) with the basal ingredients of the conventional rabbit feed in pellet form. In our experiments, emphasis was placed on both the spermatozoa concentration and its motility parameters as well as on the properties of seminal plasma and antioxidant activity. The dietary supplementation with the natural extracts mix positively altered the quality traits of rabbit spermatozoa, but these changes were statistically not significant. In experimental T1 group a significant increase of GPx and FRAP content, both regarding the antioxidant markers profile in seminal plasma was recorded. We can conclude that the supplementation of 0.3 % of natural mix did not significantly negatively affect any of the studied reproductive parameters of male rabbits, but some improvement in several antioxidant parameters was found.

Keywords: extract, rabbit, spermatozoa, motility, seminal plasma, antioxidants

#### RELEASE OF OVARIAN HORMONES AND THEIR RESPONSE TO FOLLICLE STIMULATING HORMONE BY THE OVARIES ISOLATED FROM MICE FED FLAXSEED

Vlčková R.\*, Andrejčáková Z., Sopková D.

Department of Anatomy, Histology and Physiology, Institute of Physiology, University of Veterinary Medicine and Pharmacy in Košice, Slovak Republic \* radoslava.vlckova@uvlf.sk

Popularity of flaxseed consumption in human and animal nutrition increases mainly due to the beneficial effects of active substances that exhibit protective effects on various organ systems. The aim of this preliminary study was to determine the changes in progesterone (P4), oestradiol-178 (E2), and IGF-I levels secreted by the isolated ovaries of mice dietary supplemented with or without flaxseed and their response to the addition of follicle stimulating hormone (FSH). Female mice fed on standard diet (control; n=20) or standard diet supplemented with 10% flaxseed (n=20) for 42 days were used. On day 39 of feeding, all mice were induced to oestrus using the Whitten effect method. Oestrus was confirmed on the third night from the exposition to a litter contaminated with the urine of male mice. Twelve mice from each group were killed by cervical dislocation, and their ovaries were removed for the cultivation of fragments with or without the addition of FSH to medium (0, 30 and 3000 mIU.ml<sup>-1</sup>). Culture medium was analysed for ovarian hormones by using EIA methods. In control animals, the addition of FSH supported the secretion of P4 and E2 in a dose dependent manner. Dietary supplementation with flaxseed had stimulatory effect on the secretion of P4 and E2, but not IGF-I by isolated ovarian fragments compared with controls. Higher dose of FSH added inhibited the P4 output by ovarian fragments, although it did not influence markedly their response to FSH in output at lower dose and the output of E2 and IGF-I.

Keywords: flaxseed, insulin-like growth factor (IGF-I), mice, oestradiol-17β, progesterone

Acknowledgement: This work was supported by the projects of the Ministry of Education, Science, Research and Sport of the Slovak Republic No. 1/0476/16.

#### THE CONCENTRATION OF MERCURY IN ORGANS OF WHIPFIN SILVER BIDDY (GERRES FILAMENTOSUS CUVIER, 1829) AND FLATHEAD GREY MULLET (MUGIL CEPHALUS LINNAEUS, 1758) IN COASTAL CENTRAL VIETNAM

Vo Van T.<sup>1,2\*</sup>, Binkowski Ł.J.<sup>1</sup>, Stawarz R.<sup>1</sup>

<sup>1</sup>Institute of Biology, Pedagogical University of Cracow, Poland <sup>2</sup>Faculty of Agriculture, Forestry and Fisheries, Quang Binh University, Dong Hoi, Quang Binh, Vietnam \* thiep.vo-van@up.krakow.pl

The concentration of mercury (Hg) in fish species has direct consequences to health of man and ecosystem. Thus, in this paper, the accumulation of Hg in gills, liver, and muscles of the two fish species (Whipfin silver biddy - *Gerres filamentosus* and Flathead grey mullet - *Mugil cephalus*) were measured by cold vapor atomic absorption spectrometry. The fish specimens were collected from local markets and direct fishing with the help of fishermen over the period from July to September 2017 in coastal Vietnam. Differences in the total Hg were found both between two species and organs. The concentration of Hg in all organs investigated of Whipfin silver biddy was higher than of Flathead grey mullet (p<0.05). The content of Hg in the muscles of Whipfin silver biddy was higher than in liver and gills (p<0.05), 0.460, 0.245, 0.077  $\mu$ g g<sup>-1</sup> w.w., respectively. Liver of Flathead grey mullet had more Hg accumulated than muscles and gills (0.195, 0.097, 0.046  $\mu$ g g<sup>-1</sup> w.w., respectively). The results revealed that Hg concentrations did not exceed food fish safety limits established for human consumption.

Keywords: Coastal Vietnam, Gerres filamentosus, Mugil cephalus, mercury

## VARIABILITY STUDY OF MHC GENES REGION IN *CAMELUS DROMEDARIUS* USING MICROSATELLITE ANALYZE

Wijacki J.\*, Knoll A.

Department of Animal Morphology, Physiology and Genetics, Faculty of Agrisciences, Mendel University in Brno, Czech Republic

\* jwijacki@gmail.com

Microsatellite markers genotyping represents standard, relatively easy and inexpensive method of assessing genetic diversity of complex genomic regions in various animal species, such as the major histocompatibility complex (MHC). The aim of this study is to find and test number of microsatellites located in MHC region and describe their variability and suitability for future testing and usability for multiplex PCR method. MHC is a genome region containing of genes for immune response and genes causing host and pathogen interactions. These genes are divided into class I and II encoding antigen presenting molecules responsible for detection of peptide on cell surface. This is the reason for the great selection pressure on MHC genes including both positive and negative selection. Microsatellites are used for variability study of specific genome region.

Keywords: camel, MHC, microsatellite, multiplex PCR, variability study

#### AMYGDALIN AFFECTED IMMUNE RESPONSE OF HUMAN ENDOTHELIAL CELLS

Zbyňovská K.\*, Halenár M., Greifová H., Jambor T., Kolesárová A., Lukáč N.

Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic

\* zbynovska.katarina@gmail.com

Cvanogenic glycoside amygdalin can be found in plant families of the *Caprifoliaceae*. *Mimosaceaw*. Oleaceae and Rosaceae [1]. Many studies have reported that amygdalin can be effectively used for prevention and treatment of various diseases [2]. Zymosan has been used in many experiments as inflammation enhancer [3]. In this *in vitro* study the human endothelial cells (HUVECs) were exposed to different concentrations of zymosan (1, 5 and 10 µq/mL) alone or in combination with 10 000 µg/mL of amyadalin to investigate the impact on the cell viability and production of proinflammatory interleukins 6 (IL-6) and -8 (IL-8) after 24h of cultivation. The cells were cultured at 37°C in M199 supplemented with FBS, ECGS, L-glutamine, heparin and antibiotics (penicillin and streptomycin) in an atmosphere of 5 % CO<sub>2</sub>. The cells were seeded in 96-well plates (1 x  $10^4$  cells per well) and after 24 h plating, the cells were treated with the substances for 24 h. The mitochondrial activity and viability of cells was detected by the metabolic (MTT) assay. IL-6 and IL-8 were analyzed in culture media using commercial ELISA Kit (Invitrogen™, Thermo Fisher Scientific, Waltham, USA). Whereas viability of cells was not significantly affected by treatment with zymosan and amyadalin, we observed significant changes in IL-6 and IL-8 concentrations in all experimental groups except for the group with the lowest concentration of zymosan (1 µg/mL) in comparison with the control group. Concentrations of IL-6 and IL-8 significantly dose-dependently increased in experimental groups where zymosan was added in concentrations 5  $\mu$ g/mL (p<0.01 IL-6 and p<0.001 IL-8) and 10 µg/mL (p<0.001 in both IL-6 and IL-8) in comparison with the control group. On the other hand, production of IL-6 and IL-8 significantly dose-dependently decreased in experimental groups where zymosan with combination of amygdalin were added as compared to the control group (p<0.01 in experimental group with 10  $\mu$ g/mL of zymosan with amygdalin; and p < 0.001 in experimental groups with 1 and 5  $\mu$ g/mL of zymosan with combination of amyodalin in both IL-6 and IL-8). Based on our results we can confirm that the HUVECs are sensitive to the influence of zymosan in concentrations  $1 - 10 \,\mu\text{g/mL}$  by production of proinflammatory interleukins IL-6 and IL-8 without effect to viability of cells and amyadalin may be able to inhibit these effects caused by zymosan.

Keywords: amygdalin, zymosan, interleukins, endothelial cells, cell viability

#### References

[1] Fukuda, T., Ito, H., Mukainaka, T., Tokuda, H., Nishino, H., Yoshida, T. Biol Pharm Bull 26 (2003) 271-273.

[2] Zhou, C. G., Qian, L., Ma, H., Yu, X., Zhang, Y., Qu, W., Zhang, X., Xia, W. Carbohydr Polym 90 (2012) 516-523.
[3] Takhavi, M., Mortaz, E., Khosravi, A., Nikaein, D., Barin, A., Vahedi, G., Shokri, H. Comp Clin Pathol 25 (2016) 825-831.

Acknowledgements: This work was financially supported by the projects APVV 15-0543, APVV-0304-12, VEGA 1/0039/16, KEGA 009SPU-4/2017 and European Community under project no. 26220220180: Building Research Centre "AgroBioTech".
## LIGHT IN POULTRY PRODUCTION. FROM MANAGEMENT TOOL TO PHYSIOLOGICAL MECHANISMS LIGHT QUALITY – FROM PHYSIOLOGY TO A MANAGEMENT TOOL IN POULTRY PRODUCTION

Zeman M.<sup>1,2</sup>, Kankova Z.<sup>1</sup>, Drozdova A.<sup>1</sup>, Okuliarova M.<sup>1</sup>

<sup>1</sup>Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius, University, Bratislava, Slovakia <sup>2</sup>Centre of Biosciences, Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia

Environment and nutrition represent two basic prerequisites for manifestation of genetic potential of modern breeds and hybrids in poultry production. Among environmental factors, lighting plays a dominant role. Traditionally, in poultry practice, lighting was considered especially from the point of intensity and duration of light (L):dark (D) phases, e.g. photoperiod. Optimum photoperiod has been extensively investigated and physiological mechanisms are rather understood especially in hens and turkeys, much less in water fowl. Research in this field [1] performed with Japanese quail revealed a fundamental role of thyroid hormone metabolism in the brain controlled by the photoperiod. Subsequently, these mechanisms were shown to be valid and important also for mammals. Based on research and progress in this field, well-accepted lighting programs have been introduced for rising and breeding of pullets and laying hens. More complicated situation is in broiler industry, where continuous lighting was traditionally used for fattening. Novel legislation in the European Union and North America challenged this practice and new impacts from research are expected. Another important stimulus is introduced by new Light Emitting Diods (LED). The LED based lighting systems represent a chance to increase efficiency of poultry production not only due to saving costs for electricity, but also a capacity to modulate behaviour and welfare in poultry. In addition, introducing of LED enables changing of light quality (especially wavelength) to optimize rearing conditions. Moreover, there is a potential to modulate epigenom by embryonic and early postembryonic light treatments and "program" birds to better adapt to most acceptable environmental conditions. For epigenetic programming, the early developmental stages represent the most sensitive period and the chick embryo represents an ideal model offering well defined conditions in the egg and an absence of direct interference of the internal milieu of the mother with development of her progeny.

Keywords: lighting, light wavelength, colours, LED

## References

[1] Yoshimura T, Yasuo S, Watanabe M, Iigo M, Yamamura T, Hirunagi K, Ebihara S.: Light-induced hormone conversion of T4 to T3 regulates photoperiodic response of gonads in birds. Nature. 2003, 426:178-181.

Acknowledgements: Supported by the grant VEGA 1/0686/15

Adamkovičová M. 11, 36 Andreičáková Z. 53, 68 Babeiová A. 33 Babel'ová J. 12, 17, 37, 49, 55 Babiák M. 43 Babosova R. 11, 40, 56 Baláži A. 63, 64 Baldovská S. 13, 41 Barszcz M. 27 Batoryna M. 32 Bauer M. 65 Bilvk V. 58 Binkowski Ł.J. 69 Bucko O. 28 Burda E. 20 Capcarová M. 28, 35, 48 Carbonell-Barrachina A.A. 13 Casamassima D. 67 Celá Kolísková P. 66 Cheel J. 66 Chiapparini S. 67 Chrenek P. 59, 63 Ciepliński M. 20, 30 Čikoš Š. 12, 14, 17, 37, 49, 55 Čobanová K. 23, 27 Corino C. 67 Čurlei J. 59 Čuvalová A. 15, 26 Daněk O. 39 Drozdová A. 16, 29, 72 Dubíková K. 34 Duraiski A. 20 Emrichová J. 28 Fabian D. 12, 14, 17, 37, 46, 49, 55 Formicki G. 18, 22, 32, 40 Gancarčíková S. 53 Goc Z. 18, 22, 40, 44 Gogaľová Z. 19, 33 Grandtke M. 20, 30 Greifová H. 21, 71 Greń A. 18, 22, 32, 40, 44 Grešáková Ľ. 23, 27 Grossman R. 60 Halenár M. 13, 41, 71 Halo M. 24, 52 Halo M. Jr 24, 61 Hamarová Ľ. 25 Handrová L. 15, 26 Harrath A.H. 60 Herc P. 52 Holodová M. 23, 27 Horakova J. 42 Hrabovszká S. 60 Hromada R. 47, 64 Hrouzek P. 66 Jambor T. 21, 71 Javorský P. 25, 34 Jerzak L. 20, 30 Kalafová A. 28, 35, 48

## INDEX

Kamiński P. 30 Kankova Z. 29, 72 Kapusta E. 18, 22, 32, 40, 44 Kasprzak M. 20, 30 Kaźmierczak S. 31 Kišidayová S. 43 Kisková J. 34 Klobetzova Z. 29 Kmeť V. 15, 26 Knizatova N. 67 Knoll A. 70 Kolesárová A. 13, 36, 41, 71 Kołodziejczyk Ł.M. 22, 32 Konečná M. 19, 33 Königová A. 43 Kopčáková A. 25, 34 Koppel J. 12, 14, 17, 37, 49, 55 Korbášová M. 62 Kováč G. 64 Kováčik A. 24, 52 Kováčik J. 28, 35 Kováčová V. 11, 36, 56 Kovaříková V. 12, 14, 17, 37, 46, 49, 55 Křen V. 41 Kročková J. 38 Kubandová J. 14 Kuchařová V. 39, 62, 66 Kulíková B. 63 Lichovnikova M. 29 Lubica C. 28 Lubomír O. 28 Lukáč N. 21, 71 Lukáčová M. 11, 36 Macho T. 38 Maiherová M. 33 Makarevich A. 59 Mamrakova R. 61 Martiniaková M. 11, 36, 40, 56 Massányi P. 18, 22, 35, 38, 61, 67 Massanyi M. 67 Meddle S.L. 45 Michalcová K. 13, 41 Mifkova T. 42 Mlvneková E. 24 Mondockova V. 11, 56 Mravčáková D. 43 Muchacka R. 18, 22, 44 Mydlárová Blaščáková M. 19, 33 Nagy M. 19, 33 Okuliarová M. 16, 45, 72 Omelka R. 11, 36, 40, 56 Ondrašovičová S. 52 Ondruška Ľ. 38, 67 Ožvold M. 52 Palazzo M. 67 Parkányi V. 65 Petrilla V. 53 Pisarčíková J. 43 Pisko J. 12, 46

Poráčová J. 19, 33 Pošivák J. 47 Pošiváková T. 47, 64 Pristaš P. 25, 34 Puzik M. 32 Rastislav J. 28 Řeháková K. 65 Sanisló Ľ. 41 Schneidgenova M. 28, 49 Siekiera J. 20 Sirotkin A. 50, 60 Skomorucha I. 44, 51, 54 Škorič M. 39 Slanina T. 52, 61 Sopková D. 53, 68 Sosnówka-Czajka E. 44, 51, 54 Šranko P. 36, 56 Stachańczyk K. 57 Stawarz R. 69 Steliga A. 30 Štochmaľová A. 60 Strapáč I. 15 Strashko S. 58 Švajlenka J. 47 Svoradová A. 59 Szentner K. 31 Šefčíková Z. 49 Šarocká A. 11, 36, 40, 56 Špirková A. 12, 14, 17, 37, 49, 55 Šefčíková Z. 12, 14, 17, 37, 55 Szaroma W. 18, 22 Sedlák V. 19, 33 Taciak M. 27 Tarko A. 60 Tirpák F. 52, 61, 67 Tomenendálová J. 39, 62, 66 Tomková M. 63 Tuśnio A. 27 Uhrin P. 56 Urban T. 42 Vachanová A. 60 Vadlejch J. 43 Várady M. 43 Váradyová Z. 43 Vargová M. 47, 64 Vašíček J. 63, 65 Veselá I. 39, 62, 66 Veszelits Laktičová K. 47, 64 Vizzarri F. 52, 67 Vlčková R. 53, 68 Vo Van T. 69 Wasielewski O. 31 Waśkiewicz A. 31 Wijacki J. 70 Wojciechowicz T. 31 Zahatňanská M. 33 Zbyňovská K. 21, 52, 71 Zeman M. 16, 29, 45, 72

## e-ISBN 978-83-8084-152-9

DOI 10.24917/9788380841529