DOI: https://doi.org/10.15414/2024.9788055227443

BOOK OF ABSTRACTS

June 11th – 13th, 2024
Liptovský Ján, Slovakia

ANIMAL PHYSIOLOGY 2024

19th International Scientific Conference

Animal Physiology 2024

inabi

SUA
Slovak University of Agriculture in Nitra

SUA·FBFS
Faculty of Biotechnology and Food Science

DOI: https://doi.org/10.15414/2024.9788055227443

ANIMAL PHYSIOLOGY 2024 - book of abstracts from a scientific conference

Scientific Committee

The conference was realized with the support of the following projects:
APVV-21-0168 From Cell to Organism - From Protectivity to Toxicity
APVV-20-0218 Determination of effect of agricultural xenobiotics on alterations of animal system in vitro
APVV-19-0243 Molecular and clinical manifestation of ethnopharmaceutics effect detected on animal model
CEEPUS Network: SK-1913-01-2425 BioTox
VEGA 1/0571/23 Risk assessment of persistent organic pollutants and microelements for freshwater animals
KEGA 054SPU-4/2024 Innovation of the teaching strategy of the subject "Toxicology of animals" by integrating the digital web-concept e-lab.

DOI: https://doi.org/10.15414/2024.9788055227443

Organizing Committee

Co-funded by the European Union

Erasmus+ KA2

Cooperation partnerships in higher education

Fostering Internationalisation in Higher Education by BioFood Virtual Labs (BioFoodVirLabs)

2021-1-SK01-KA220-HED-000032062

DOI: https://doi.org/10.15414/2024.9788055227443

Organizer

SLOVAK UNIVERSITY OF AGRICULTURE IN NITRA

Faculty of Biotechnology and Food Sciences
Institute of Applied Biology
SLOVAK REPUBLIC

Partners

UNIVERSITY OF THE NATIONAL EDUCATION COMMISSION, KRAKOW

Faculty of Exact and Natural Sciences
POLAND

MENDEL UNIVERSITY IN BRNO

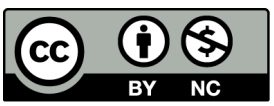
Faculty of Agrisciences
Department of Animal Morphology, Physiology and Genetics
CZECH REPUBLIC

SLOVAK ACADEMY OF SCIENCES

Institute of Animal Physiology
Centre of Biosciences
SLOVAK REPUBLIC

Editors
Anton Kováčik, Ph.D. - Slovak University of Agriculture in Nitra, Slovak Republic
Tomáš Jambor, Ph.D. - Slovak University of Agriculture in Nitra, Slovak Republic

Approved by the Rector of the Slovak University of Agriculture in Nitra on June 6th, 2024 as a Book of Abstracts published online.



ISBN 978-80-552-2744-3

DOI: https://doi.org/10.15414/2024.9788055227443

DOI: https://doi.org/10.15414/2024.9788055227443

Conference Sponsors

LAMBDA LIFE

RANDOX

SUA·ABT RC
AgroBioTech
Research
Centre

DOI: https://doi.org/10.15414/2024.9788055227443

CONTENT 5

Diet supplemented with alpha linolenic acid has beneficial effect on *in vivo* developed mouse preimplantation embryos
Janka Babeľová, Zuzana Šefčíková and Dušan Fabian 11
The effect of enterolactone and n-3 PUFAs on the proliferation and apoptosis in the mouse endometrium
Diana Babjáková, Martina Lecová, Kristína Rodáková, Soňa Gancarčíková, Drahomíra Sopková, Radoslava Vlčková 12
Bioavailability of thymol in the rabbit organism
Kristina Bacova, Karin Zitterl-Eglseer, Gesine Karas Räuber, Iveta Placha 13
Effect of shiitake and glycyrrhizin on growth factor TGF-β2 and its receptor in human ovarian granulosa tumor cells *in vitro*
Olha Balytska, Michal Mihal, Katarina Majerik Behinska, Tibor Lukac, Tatiana Bojnanska, Adriana Kolesarova 14
Morphometric analysis of the omasum of calves fed with a starter combination of muesli and straw.
Andrej Batik, Anna Zuzanová 15
Effect of endocrine disrupting chemicals on expression of ACE2 receptors in TM4 cell line
Denis Bažány, Nikola Kalamenová, Nikola Štefunková, Hana Greifová, Tomáš Jambor, Katarína Tokárová, Lucia Zuščíková, Norbert Lukáč 16
The effect of metformin and sea buckthorn on bone microarchitecture in ZDF rats
Roman Biro, Veronika Kovacova, Vladimira Mondockova, Noemi Penzes, Marcela Capcarova, Anna Kalafova, Radoslav Omelka, Monika Martiniakova 17
Effects of diet supplementation with zinc oxide nanoparticles on plasma mineral and antioxidant status of lambs
Alexandra Bombárová, Ľubomíra Grešáková, Klaudia Čobanová 18
Cultivation of macrophages using 3D cell culture system
Michaela Burvalova, Jana Rehakova, Lucie Jelinkova, Ales Pavlik, Petr Slama 19
The effect of bee pollen on some parameters of antioxidant status of rats
Marcela Capcarova, Anna Kalafova, Monika Schneidgenova, Patricia Simkova, Miroslav Juracek, Milan Simko, Branislav Galik 20
Microsatellite panel optimisation for studying of diversity in bees
Eva Cvingráfová, Petra Bartoňová, Aleš Knoll 21
Evaluation of the Influence of Platinum Nanoparticles on the Motility and Viability of Rabbit Spermatozoa *In Vitro*
Lucia Dianová, Filip Tirpák, Marko Halo Jr., Nikola Štefunková, Tomáš Slanina, Michal Lenický, Klaudia Jaszcza, Iveta Urban, Peter Massányi 22
Irisin affects the transcriptome of the porcine anterior pituitary cells during the mid-luteal phase of the oestrous cycle
Kamil Dobrzyń, Barbara Zarzecka, Marta Kieżun, Grzegorz Kopij, Mariia Himultidinova, Oghuzan Koker, Agnieszka Rak, Tadeusz Kamiński, Nina Smolińska 23

The effect of extremely low-frequency electromagnetic field (ELF-EMF) on the expression of epigenetic mechanism-related genes in the endometrium of pigs during the peri-implantation period
Anita Franczak, Pawel Wydorski, Agata Zmijewska 24
Influence of *Trigonella foenum-graecum* L. microgreens on HUVEC cells
Zofia Goc, Grzegorz Formicki, Agnieszka Greń, Tomas Jambor, Norbert Lukac 25
Effect of different doses of *Hippophae rhamnoides* L. on the concentration of GSH and MDA in the brain of diabetic rats
Zofia Goc, Edyta Kapusta, Agnieszka Greń, Renata Muchacka, Grzegorz Formicki, Marcela Capcarová, Anna Kalafová, Monika Schneidgenová, Nikoleta Šimonová 26
Improving livestock biosecurity trough trace mineral supplementation
Ľubomíra Grešáková, Andrea Lauková, Monika Pogány Simonová, Katarína Tokarčíkova 27
Effect of pentoxifyline on stallion spermatozoa parameters
Marko Halo Jr, Monika Šmondrková, Lucia Dianová, Michal Lenický, Kateryna Vanivska, Martin Massányi, Tomáš Slanina, Jiřina Zemanová, Katarína Tokárová, Eva Mlyneková, Marko Halo, Peter Massányi 28
Impact of Bisphenols on the Secretory Activity of Testosterone in Human Carcinoma NCI-H295R Cells
Marek Helczman, Nikola Štefunková, Tomáš Jambor, Anton Kováčik, Peter Massányi, Norbert Lukáč 29
Determination of selected biogenic amines in common carp (*Cyprinus carpio*)
Silvia Jakabová, Július Árvay, Lucia Benešová, Jozef Golian 30
The potential impact of *Trigonella foenum-graecum L.* microgreens on interleukin-6 release in HUVEC cells *in vitro*
Tomas Jambor, Bazany Denis, Hana Greifova, Nikola Stefunkova, Zofia Goc, Norbert Lukac 31
Effect of *Trigonella foenum-graecum L.* microgreens on bovine spermatozoa motility and cell membrane integrity *in vitro*
Tomas Jambor, Michal Ďuračka, Eva Tvrdá, Norbert Lukac 32
Meat colour evaluation after bee bread supplementation of female Japanese quails
Anna Kalafova, Nikoleta Simonova, Monika Schneidgenova, Patricia Simkova, Emilia Hanusova, Cyril Hrncar, Peter Hascik, Jan Brindza, Renata Mamrakova, Marcela Capcarova 33
Detection of antibiotic resistance in *E. coli* from calves
Lívia Karahutová, Dobroslava Bujňáková 34
Association of chemerin levels with progression of colorectal cancer
Jacek Kiezun, Anna Koprowicz-Wielguszewska, Marlena Gudelska, Katarzyna Kisielewska, Janusz Godlewski, Bartlomiej E. Krazinski 35
Omentin-1 regulates endometrial angiogenesis through the VEGF system expression
Marta Kieżun, Kamil Dobrzyń, Oguzhan Köker, Ewa Zaobidna, Grzegorz Kopij, Marlena Gudelska, Katarzyna Kisielewska, Barbara Zarzecka, Mariia Himultidinova, Nina Smolińska, Tadeusz Kamiński 36
Omentin-1 modulates prostaglandin synthesis in the porcine endometrium: *in vitro* insights from early pregnancy and mid-luteal phase of the oestrous cycle
Oguzhan Köker,Kamil Dobrzyń, Grzegorz Kopij, Marlena Gudelska, Barbara Zarzecka, Mariia Himultidinova, Nina Smolińska, Tadeusz Kamiński, Marta Kieżun 37

Monitoring the effects of glycyrrhizin and shiitake extract on steroid hormone production by human ovarian tumor cells *in vitro*
Adriana Kolesarova, Olha Balytska, Michal Mihal, Katarina Majerik Behinska, Tibor Lukac, Tatiana Bojnanska 38
Virtual laboratories and digital learning environment for internationalization in tertiary education
Adriana Kolesárová, Silvia Jakabová, Tomáš Jambor, Ľubomír Belej, Eva Ďurčanská, Dominik Hollý, Petr Sláma, Grzegorz Formicki, Péter Sipos 39
Barefoot and shod horses, rehabilitation of defects in their hoof capsule
Anežka Kopečková, Kristína Žuffová, Terezie Zavadilová 40
Persistent Organic Pollutants and Oxidative Stress in Freshwater Fish
Anton Kovacik, Marek Helczman, Tomas Jambor, Marian Tomka, Martin Fik, Eva Kovacikova, Peter Massanyi 41
Cemtirestat supplementation does not influence macroscopical and mechanical properties of femoral bone in ZDF rats
Veronika Kovacova, Radoslav Omelka, Vladimira Mondockova, Roman Biro, Noemi Penzes, Piotr Londzin, Joanna Folwarczna, Karol Svik, Marta Soltesova Prnova, Monika Martiniakova 42
Stabilised form of thymol in rabbit diet, its absorption from gastrointestinal tract and effect on intestinal morphology.
Radoslava Kristofova, Karin Zitterl-Eglseer, Maria Teresa Capucchio, Dorotea Ippolito, Francesco Gai, Fardad Firooznia, Ales Franc, Iveta Placha 43
Protective effect of mitochondria-targeted antioxidant Mito-TEMPO on rabbit sperm function during cryopreservation
Lenka Kuzelova, Andrea Svoradova, Jakub Vozaf, Andrej Balazi, Jaromir Vasicek, Peter Chrenek 44
Is Porcine Granulosa Cells' Hormone production influenced by DHA and EPA?
Martina Lecová, Diana Babjáková, Kristína Rodáková, Maria Romerowicz-Misielak, Katarzyna Kozioł, Drahomíra Sopková, Radoslava Vlčková 45
Determination of beehive by-product on motility characteristics of stallion spermatozoa *in vitro*.
Michal Lenický, Ewlina Sidor, Lucia Dianová, Marko Halo, Tomáš Slanina, Denis Bažány, Nikola Štefunková, Małgorzata Dżugan, Peter Massányi 46
Dose-dependent effect of *Trigonella foenum-graecum L*. microgreens on angiogenesis in HUVEC cells *in vitro*
Norbert Lukac, Bazany Denis, Hana Greifova, Nikola Stefunkova, Agnieszka Gren, Tomas Jambor 47
Determination of potential impact of *Trigonella foenum graecum L*. on progressive motility and DNA fragmentation in bovine spermatozoa *in vitro*
Norbert Lukac, Michal Ďuračka, Eva Tvrdá, Tomas Jambor 48
Impact of *Aronia melanocarpa* on the female hormonal profile
Katarina Majerik Behinska, Olha Balytska, Jana Kopcekova, Jana Mrazova, Adriana Kolesarova 49
The effect of culture system on the *in vitro* production of embryos from fresh and vitrified cattle oocytes
Alexander Makarevich, Linda Dujíčková, Lucia Olexiková 50

Is obesity beneficial for bone health?
Monika Martiniakova, Roman Biro, Noemi Penzes, Anna Sarocka, Veronika Kovacova, Vladimira Mondockova, Radoslav Omelka 51
The effect of zinc nanoparticles on the spermatozoa parameters in vitro
Peter Massányi, Barbora Remeňová, Marko Halo, Tomáš Slanina, Lucia Dianová, Michal Lenický, Filip Tirpák, Jaroslav Kováčik 52
Isorhamnetin and its effect on human ovarian *stratum granulosum* tumor cells *in vitro*
Michal Mihal, Katarina Majerik Behinska, Olha Balytska, Adriana Kolesarova 53
The inhibitory effect of alfacalcidol on mineralisation in primary rat osteoblast cell culture
Vladimira Mondockova, Veronika Kovacova, Roman Biro, Noemi Penzes, Monika Martiniakova, Radoslav Omelka 54
Analysis of estrogen receptors expression in the gastrointestinal tract of the domestic chicken at various stages of development
Zuzanna Mucha, Agnieszka Greń, Marcin Lis, Jacek Kieżun, Grzegorz Formicki 55
Evaluation of the antioxidant potential of cannabidiol in reducing oxidative stress in chicken embryonic liver tissue
Renata Muchacka, Edyta Kapusta, Agnieszka Greń, Zofia Goc, Grzegorz Formicki 56
Dose-dependent effect of vitamin K2 on biochemical and microstructural parameters of bone in ovariectomized rats
Radoslav Omelka, Martina Babikova, Veronika Kovacova, Vladimira Mondockova, Roman Biro, Noemi Penzes, Karol Svik, Marta Soltesova-Prnova, Juraj Vozar, Monika Martiniakova 57
Breed-specific blood parameters reference interval variations in cattle
Ales Pavlik, Andrej Batik, Petr Vevera, Petr Slama 58
The impact of metformin and sea buckthorn on biochemical and macroscopical parameters of femoral bone in ZDF rats
Noemi Penzes, Roman Biro, Veronika Kovacova, Vladimira Mondockova, Radoslav Omelka, Anna Kalafova, Marcela Capcarova, Monika Martiniakova 59
Effect of meadow grassland and chicory on growth performance and rumen microbiota in grazing lambs with endoparasites
Daniel Petrič, Klára Mikulová, Dominika Batťányi, Michaela Komáromyová, Marián Várady, Zora Váradyová 60
Effect of fipronil maternal exposure on further development of mouse offspring
Jozef Pisko, Kamila Fabianová, Zuzana Šefčíková, Dušan Fabian 61
Potential role of plants used in traditional medicine in ovarian steroidogenesis
Shubhadeep Roychoudhury, Petr Slama, Adriana Kolesarova 62
The effect of fipronil on the expression of glutathione metobalism transcripts
Laura Rušinová, Veronika Kovaříková, Zuzana Šefčíková, Štefan Čikoš, Alexandra Špirková, Jozef Pisko, Dušan Fabian 63
Alterations in the chemical composition of meat following bee bread supplementation in male Japanese quails
Monika Schneidgenova, Anna Kalafova, Nikoleta Simonova, Patricia Simkova, Emilia Hanusova, Cyril Hrncar, Peter Hascik, Jan Brindza, Renata Mamrakova, Marcela Capcarova 64

DOI: https://doi.org/10.15414/2024.9788055227443

Effects of anabolic steroids on the reproductive system
Kristýna Skoupá, Andrej Bátik, Kamil Šťastný, Zbyšek Sládek 65
Effect of punicalagin on the viability of bovine mononuclear cells
Lucie Polakova, Tina Babackova, Eva Venusova, Adriana Kolesarova, Shubhadeep Roychoudhury, Ales Pavlik, Petr Slama 66
Bee bread as obesity prevention
Patricia Simkova, Anna Kalafova, Monika Schneidgenova, Jan Brindza, Marcela Capcarova 67
***In vitro,* the influence of rose hip on selected motility parameters of turkey spermatozoa**
Tomáš Slanina, Radovan Floriančič, Peter Massányi 68
Effect of irisin on LH and FSH secretion by the porcine anterior pituitary cells through NFκβ signalling pathway
Nina Smolińska, Barbara Zarzecka, Kamil Dobrzyń, Marta Kieżun, Grzegorz Kopij, Mariia Himultidinova, Oghuzan Koker, Cecilia Dall'Aglio, Agnieszka Rak, Tadeusz Kamiński 69
The impact of maternal obesity on expression of genes connected with insulin signaling in mouse blastocysts
Alexandra Špirková, Martina Kšiňanová, Štefan Čikoš, Veronika Kovaříková, Zuzana Šefčíková, Jozef Pisko, Janka Babeľová, Dušan Fabian 70
Evaluation of the effect of ZnO nanoparticles on oxidative balance and viability in H295R cells
Nikola Štefunková, Hana Greifová, Anton Kováčik, Tomáš Jambor, Lucia Zuščíková, Denis Bažány, Norbert Lukáč, Klaudia Jaszcza, Iveta Urban, Peter Massányi 71
Bacterial species composition on the skin of healthy reptiles
Lucia Štempelová, Viola Strompfová 72
A modified method for the isolation/preparation of primary small intestinal epithelial cells as *in vitro* intestinal models
Katarína Tokarčíkova, Ľubomíra Grešáková, Petr Sláma, Andrej Bátik, Petr Vevera, Aleš Pavlík 73
Toxic aspect of nanoparticles at the cellular level
Kateryna Vanivska, Lucia Dianová, Michal Lenický, Marko Halo, Peter Ivanič, Iveta Urban, Nikola Štefunková, Peter Massanyi 74
Laminin enrichment of rabbit of DBA$^+$ primitive spermatogonial cells: a preliminary study
Jaromir Vasicek, Andrea Svoradova, Andrej Balazi, Jakub Vozaf 75
Effect of biologically active compounds in drone brood on bone tissue cells metabolism
Petr Vevera, Michala Steinerova, Katarina Tokarcikova, Andrej Batik, Petr Slama, Ales Pavlik 76
Variability of mercury concentrations within the digestive tract of muskrats.
Izabela Wiśniowska, Łukasz J. Binkowski, Katarzyna Tomczyk, Robert Stawarz 77
The effect of exposure to an extremely low-frequency electromagnetic field on the abundance of endothelial nitric oxide synthase in the endometrium
Pawel Jozef Wydorski, Agata Zmijewska, Anita Franczak 78
Effect of irisin on the transcriptomic profile of the porcine pituitary cells during implantation
Barbara Zarzecka, Kamil Dobrzyń, Marta Kieżun, Grzegorz Kopij, Mariia Himultidinova, Oguzhan Köker, Agnieszka Rak, Tadeusz Kamiński, Nina Smolińska 79
The effect of sage essential oil on rabbit spermatozoa motility *in vitro*
Jiřina Zemanová, Anton Kováčik, Michal Lenický, Eva Kováčiková, Peter Massányi 80

DOI: https://doi.org/10.15414/2024.9788055227443

The effect of kisspeptin on the secretion of progesterone and 17β-estradiol by endometrium of pigs during the peri-implantation period
Agata Zmijewska, Pawel Wydorski, Anita Franczak 81
The effect of acetamiprid TM3 Leydig cells morphology in a 3D system *in vitro*
Lucia Zuscikova, Hana Greifova, Nikola Stefunkova, Norbert Lukac, Iva Sovadinova, Eliska Sychrova, Denis Bazany, Tomas Jambor 82

DOI: https://doi.org/10.15414/2024.9788055227443

Diet supplemented with alpha linolenic acid has beneficial effect on *in vivo* developed mouse preimplantation embryos

Janka Babeľová, Zuzana Šefčíková and Dušan Fabian

Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences, Košice, Slovak republic

Abstract

The aim of our study was to evaluate the effect of diet supplementation by two various concentrations of alpha linolenic acid (ALA) on mouse preimplantation embryo development and quality of *in vivo* derived blastocysts. **Mouse females** (outbred CD-1 strain) were divided into three groups: **control** (fed only standard feed mixture), **ALA I** (fed a diet supplemented with pure alpha linolenic acid at concentration 100 mg/kg body weight) and **ALA II** (fed a diet supplemented with pure alpha linolenic acid at concentration 200 mg/kg body weight). ALA was administered to female mice *per os* for 2 weeks (during approx. 3 estral cycles). Embryos were isolated from dams on day 4 of pregnancy. Stereomicroscopic evaluation of oviduct and uterine content showed that administration of ALA in diet did not significantly affect the average number of isolated embryos per mother. Analysis of developmental capacities showed that more then 90% embryos reached blastocysts stage in each evaluated group (Control: 93.89%; ALA I: 90.38%; ALA II: 91.60%). However, the evaluation of blastocysts quality showed that administration of ALA in diet significantly decreased number of apoptotic cells in blastocysts (ALA I: 4.39±0.27; ALA II: 4.28±0.27 compared to control 5.06±0.27; $P<0.05$).

In conclusion, the obtained results indicate that administration of ALA (at both concentrations) could have beneficial effect on quality of blastocysts in mice.

Keywords: preimplantation embryo; mice; alpha linolenic acid

Acknowledgments

This work was supported by the Slovak Research and Development Agency under contract APVV-22-0071

DOI: https://doi.org/10.15414/2024.9788055227443

The effect of enterolactone and n-3 PUFAs on the proliferation and apoptosis in the mouse endometrium

Diana Babjáková[1], Martina Lecová[1], Kristína Rodáková[1], Soňa Gancarčíková[2], Drahomíra Sopková[1], Radoslava Vlčková[1]

[1]*Department of Biology and Physiology, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak Republic*
[2]*Gnotobiology Laboratory, Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak Republic*

Abstract

Omega-3 (n-3) polyunsaturated fatty acids (PUFA) are essential fatty acids necessary for human health. Studies have shown that the long-chain n-3 PUFAs, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) were associated with a lower risk of endometrial cancer. Enterolactone may protect against certain types of cancer, particularly hormone-sensitive cancers such as breast, endometrial, and prostate, by interfering with the metabolism of sex hormones. Therefore, this study aimed to determine the possible protective, anti-proliferative, and anti-carcinogenic effect of n-3 PUFA (DHA, EPA) and enterolactone on the endometrium of mice. The study was performed on 6 – 8 weeks old female BALB/c mice fed with standard pelleted feed. Mice (n=12) were humanely euthanized and uterine horns (n=24) were collected for tissue culture and the following immunohistochemical analysis. Uterine horns were cultured with or without the addition of DHA (10 mg/ml), EPA (10 mg/ml), and enterolactone (EL; 1 IU/ml). After 24 h the uterine horns were removed and prepared for immunohistochemistry. Expression of the proliferation marker PCNA and the apoptotic marker caspase-3 were analysed and the relative optical density (ROD) was calculated. The expression of PCNA in the superficial epithelium, stroma, and endometrial glands was lower ($P < 0.01$) in all groups (DHA, EPA, EL) compared to the control group of mice. On the other hand, the expression of caspase-3 was stronger for DHA and EL ($P < 0.01$) in all parts of the endometrium while insignificant ($P > 0.05$) for the EPA group in the stroma compared to the control uterine tissue. The results showed that the addition of DHA, EPA, and EL suppressed proliferation and promoted apoptosis of uterine endometrial cells. The results confirmed that n-3 PUFA and enterolactone show an anti-cancer effect precisely through their anti-proliferative and pro-apoptotic actions in the murine uterus.

Keywords: mouse, uterus, proliferation, apoptosis, DHA, EPA, enterolactone

Acknowledgment: This work was supported by the projects VEGA 1/0414/23 and APVV-22-0071.

DOI: https://doi.org/10.15414/2024.9788055227443

Bioavailability of thymol in the rabbit organism

Kristina Bacova[1], Karin Zitterl-Eglseer[2], Gesine Karas Räuber[2], Iveta Placha[1]

[1]*Centre of Biosciences, Slovak Academy of Sciences, Institute of Animal Physiology, Soltesovej 4–6, 040 01 Kosice, Slovakia*

[2]*Centre for Animal Nutrition and Welfare, University of Veterinary Medicine Vienna, Veterinärplatz 1, A-1210 Wien, Austria*

Abstract

The purpose of this study was to investigate the bioavailability of thymol, a major constituent of *Thymus vulgaris* L., in the rabbit organism. Forty-eight rabbits were allocated to control and experimental groups with thymol addition (250 mg/kg feed) for 21 days and then withdrawal for 7 days. Concentration of thymol determined by GC/MS after solid phase microextraction in the intestinal wall (IW) was significantly higher than in plasma (*P=0.0211*) and liver (*P=0.0305*), and in the kidneys it was significantly higher than in plasma (*P=0.0259*) and liver (*P=0.0415*) during the period of thymol addition. Our results show intensive absorption of thymol from the intestine, its accumulation and intensive metabolic processes in the kidneys and its metabolic and excretion processes in the liver. Thymol in fat and muscle was found only in trace amounts, probably due to its biotransformation into hydrophilic metabolites and extensive elimination from the organism. During the period without thymol addition, thymol content was determined only in trace amounts and its concentration was significantly higher in IW than in plasma (*P=0.0035*). Only in faeces was thymol detected above trace amount during both experimental periods, and was significantly higher than in the colon (*P=0.0127, P=0.0023*). The process of caecotrophy, a typical feature of the rabbit digestion, probably supported the biological activity of thymol and its metabolites in the rabbit's organism by repeated biotransformation processes also after its withdrawal from feed. Finally, to our knowledge, this is the first study describing processes of thymol bioavailability in the rabbit organism.

Keywords: bioavailability, thymol, rabbit.

Acknowledgement: This work was supported by VEGA 2/0009/20, 2/0005/24; CNR-SAS-2022-02, Open-Mob-2022-01, COST CA22109, Action Austria-Slovakia ICM-2019-13685.

DOI: https://doi.org/10.15414/2024.9788055227443

Effect of shiitake and glycyrrhizin on growth factor TGF-β2 and its receptor in human ovarian granulosa tumor cells *in vitro*

Olha Balytska[1], Michal Mihal[1], Katarina Majerik Behinska[1], Tibor Lukac[1,2], Tatiana Bojnanska[3], Adriana Kolesarova[1,4]

[1]*Institute of Applied Biology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic*
[2]*One Pharma, s.r.o., Janka Kráľa 5, 974 01 Banská Bystrica, Slovak Republic*
[3]*Institute of Food Sciences, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic*
[4]*AgroBioTech Research Centre, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic*

Abstract

Shiitake (*Lentinula edodes)* and glycyrrhizin (bioactive compound from *Licorice root)* have been used in traditional medicine for centuries and now they are actively studied for the mechanisms of their influence on various systems of the human body. Previous studies reported abut encouraging anti-inflammatory, antioxidant, immunomodulatory and anticancer effects of active biocompounds from *Lentinula edodes* and glycyrrhizin. However, their impact on TGF-β2 signaling pathways is poorly studied. Transforming Growth Factor beta 2 (TGF-β2) is a multifunctional cytokine involved in various physiological processes, including cell growth, differentiation, and immune regulation. Dysregulation of TGF-β2 expression and signaling has been associated with various diseases, including cancer, fibrosis, and autoimmune disorders. Modulating TGF-β2 levels and signaling pathway activity presents a promising therapeutic strategy for managing these conditions. The aim of this *in vitro* study was to investigate the effect of shiitake and glycyrrhizin and extracts separately at concentrations of 10, 50, 100, 250, 500 and 1000 µg/ml during 48-hour cultivation on the production TGF-β2 as well as its receptor expression by ovarian cancerous cells (human granulosa tumor cells COV434). To carry out this experiment, we applied the ELISA method. Our results indicate that Shiitake extract is significant ($P \leq 0.05$) effected on TGF-β2 production only at the highest concentration tested (1000 µg/ml). In contrast, glycyrrhizin significantly ($P \leq 0.05$) reduced TGF-β2 production and its receptor expression, particularly at the concentration of 1000 mg/ml. These findings suggest dose-dependent effects of shiitake and glycyrrhizin extracts on TGF-β2 signaling in COV434 cells. However, further research is needed to confirm our hypothesis.

Keywords: Shiitake, glycyrrhizin, TGF-β2, TGF-β2 receptor, COV434 cell line.

Acknowledgement: The work was supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic project APVV-21-0206, VEGA 1/0620/24 and KEGA 035SPU-4/2023.

DOI: https://doi.org/10.15414/2024.9788055227443

Morphometric analysis of the omasum of calves fed with a starter combination of muesli and straw.

Andrej Batik[1], Anna Zuzanová[2]

[1]Department of Animal Morphology, Physiology and Genetics, Faculty of AgroSciences, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech Republic
[2]Department of Animal Nutrition and Forage Production, Faculty of AgroSciences, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech Republic

Abstract

This study investigates the effects of starting feed, namely the mix of chopped straw and muesli starter, on calf omasum development. Calves' early food consumption has a significant influence on how their gastrointestinal tracts, particularly the rumen and omasum, develop. Using detailed morphometric analysis, we looked into how this specific feed mixture affected glandular growth, surface area, and abomasal tissue structure.

Preliminary findings suggest a clear relationship between the muesli and chopped straw starter and notable morphological changes in the omasum. Stronger omasum structure is encouraged by this combination, which may enhance enzymatic activity and nutrient absorption.

Improved knowledge of the relationship between the elements and physical makeup of starting feed and omasum growth should result in improved calf nutrition techniques. Information regarding enhancing feeding practices to promote omasal health and overall calf wellbeing is provided by this study.

After being weighed and measured at birth, the calves received their first dose of colostrum—two liters—two hours later. By the time they were 4 days old, they were receiving 3 litres of colostrum every feeding, twice a day. After being relocated to outdoor individual pens on the fifth day, the calves were given milk replacers twice a day in amounts that corresponded to their group assignment from buckets equipped with teats. The amounts varied between 2 and 3 litres every feeding. The calves were fed a beginning meal made of straw from the fifth day until they were sacrificed, which occurred at 56 days of age. Following salughtering, omasum samples were obtained for histological examination. Comparing our results to other commercial feeds, we discover that our customized beginning feed considerably improves calf omasum development. For the best adult cow feeding practices and long-term nutritional results, this is essential.

In conclusion, our research provides valuable insight into the impact of early nutrition on calf gastrointestinal development and clarifies the crucial role that starting feed plays in shaping omasum morphometries.

Keywords: calf omasum, starter feed, morphometric analysis, gastrointestinal development

Acknowledgement: Effect of milk and starter diets on the growth performance and development of the digestive tract of dairy calves (AF-IGA2023-IP-037)

DOI: https://doi.org/10.15414/2024.9788055227443

Effect of endocrine disrupting chemicals on expression of ACE2 receptors in TM4 cell line

Denis Bažány[1], Nikola Kalamenová[1], Nikola Štefunková[1], Hana Greifová[1], Tomáš Jambor[1], Katarína Tokárová[1], Lucia Zuščíková[1], Norbert Lukáč[1]

[1]*Institute of Applied Biology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic*

Abstract

The issued study is focused on the determination of the influence of endocrine disruptors (ED) on ACE2 receptor expression in a cellular model. Endocrine-disrupting chemicals are ubiquitous chemical compounds that can now be found in the entire human environment. The most prevalent usage comes from applications for industrial purposes. It is believed that the mechanism of action of EDc´s can affect the formation and ongoing disease's progression. The main focus of this study was on angiotensin-converting enzyme 2, which serves as a functional receptor for coronavirus (SARS-CoV-2). The aims of the analysis were the expression of ACE2 in cell model culture and the effect of ED on ACE2 expression. We used TM4 Sertoli cells, which served as a suitable cellular model. In the experimental part, we investigated the effect and consequences associated with the exposure of TM4 cells to different concentrations (0.1, 0.5, 1; 10; 20; 50; and 100 µM) of BPS in vitro. We used the ab235649 Human ACE2 SimpleStep ELISA® Kit (enzyme-linked immunosorbent assay) to determine the abundance of ACE2 receptors in the cells. In the practical part, we also paid attention to the investigation of the effect of BPS on the viability-mitochondrial and metabolic activity parameters of the cells in a dose-dependent manner by colorimetric MTT, Alamar blue, and neutral red assays. In MTT assays, we concluded that the highest concentration (100 µM) of BPS exerted a cytotoxic effect on the cells; there was a significant decrease ($P<0.01$) in mitochondrial activity. No significant decrease in cell viability was observed in the Alamar blue and neutral red assays. The results obtained by the ELISA method demonstrate a significant decrease ($P<0.01$) in ACE2 activity from the lowest concentration (0.1 µM), and with increasing BPS concentration (0.5; 1; 10; 20 µM), we observed an even more significant decrease ($P<0.001$) in ACE2 activity. However, at the two highest BPS concentrations (50 and 100 µM), we did not record any significant difference in ACE2 representation. Despite the reduced viability, at the highest concentration of BPS (100 µM), which was measured using the mitochondrial activity assay, we did not observe a significant difference in ACE2 expression. Summarizing the obtained results of the effect of BPS on ACE2 protein representation, it can be concluded that ACE2 expression is dependent on the applied BPS concentration. The inhibitory effect was observed at lower concentrations of 0.1, 0.5, 1, 10, and 20 µM; on the contrary, higher concentrations of 50 and 100 µM exerted a rather stimulatory effect.

Keywords: ACE2, SARS-CoV-2, COVID19, endocrine disruptors, bisphenol S

Acknowledgments: This work was financially supported by projects: APVV-20-0218, APVV-19-0243, VEGA 1/0207/23, VEGA 1/0083/21, and the Cultural and Educational Grant Agency of the Slovak Republic - KEGA 054SPU-4/2024 and KEGA 023SPU-4/2022.

DOI: https://doi.org/10.15414/2024.9788055227443

The effect of metformin and sea buckthorn on bone microarchitecture in ZDF rats

Roman Biro[1], Veronika Kovacova[1], Vladimira Mondockova[1], Noemi Penzes[1], Marcela Capcarova[2], Anna Kalafova[2], Radoslav Omelka[1], Monika Martiniakova[1]

[1]*Faculty of Natural Sciences and Informatics, Constantine the Philosopher University in Nitra, Slovak Republic*
[2]*Faculty of Biotechnology and Food Science, Slovak University of Agriculture in Nitra, Slovak Republic*

Abstract

Metformin is one of the most commonly used drugs to treat type 2 *diabetes mellitus* (T2DM). It's positive impact on osteoblast proliferation, type I collagen production, and alkaline phosphatase activity has been demonstrated. Sea buckthorn contains several bioactive substances with antioxidant activity, therefore it could be used as a supplement for T2DM patients. In our study, the effect of metformin and sea buckthorn on quantitative 3D parameters of cortical and trabecular bone tissues was examined using an experimental animal model of T2DM. ZDF rats were divided into 4 groups: C (diabetic control), E1 (metformin supplemented, 150 mg/kg/day), E2 (sea buckthorn supplemented, 500 mg/kg/day), and E12 (simultaneously supplemented, 150 mg/kg/day of metformin + 500 mg/kg/day of sea buckthorn) for 120 days. Our results indicate that simultaneous application of metformin and sea buckthorn significantly increased trabecular BMD versus C group. A slight but a non-significant increase in trabecular bone volume fraction and bone surface, trabecular thickness was determined in E12 group. Considering cortical bone, no significant changes between all groups were observed. The presented results show that simultaneous application of metformin and sea buckthorn has the most positive effect on trabecular bone microarchitecture in ZDF rats.

Keywords: metformin, sea buckthorn, bone microarchitecture, type 2 *diabetes mellitus*, cortical bone, trabecular bone

Acknowledgement: This study was supported by the Ministry of Education, Research, Development and Youth of the Slovak Republic, grant numbers: VEGA 1/0416/22; VEGA 1/0328/24.

DOI: https://doi.org/10.15414/2024.9788055227443

Effects of diet supplementation with zinc oxide nanoparticles on plasma mineral and antioxidant status of lambs

Alexandra Bombárová[1], Ľubomíra Grešáková[1], Klaudia Čobanová[1]

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

Abstract

The use of nanominerals in animal nutrition has shown promising results in enhancing mineral bioavailability due to the small particle size, large surface area to volume ratio, different physicochemical properties, and high reactivity compared to standard mineral sources. The goal of this trial was to evaluate the microelements (Zn, Cu, Fe) level and antioxidant status by determining the activity of antioxidant enzymes and lipid peroxidation in plasma/blood of lambs fed diets supplemented with ZnO nanoparticles (< 30 nm). A total of 28 lambs (Improved Valachian) at the age of 4 months were randomly allotted to one of 4 treatments (n=7). Dietary treatments included the basal diet (BD, 350 g/day of barley and 700 g/day of meadow hay), and the BD enriched with ZnO nanoparticles (40 or 80 mg Zn/kg of diet) or inorganic ZnO (80 mg Zn/kg of diet). On days 35 and 70, jugular blood samples were collected from each lamb for analysis. Intake of zinc at 80 mg/kg diet increased Zn concentration in plasma. Plasma Cu concentration was influenced by treatment with the higher level in lambs fed inorganic ZnO while plasma Fe level was affected by time. The activity of alkaline phosphatase and superoxide dismutase was affected by treatment with the highest activity recorded in lambs fed ZnO nanoparticles at 80 mg Zn/kg. The inclusion of zinc into the diets increased the activity of blood glutathione peroxidase and decreased the concentration of malondialdehyde in plasma. In conclusion, the results of this study suggest that ZnO nanoparticles added to the diet at the dose up to the maximum authorized Zn content in complete feed did not interfere with intestinal absorption of microelements (Cu, Fe) as well as did not induce oxidative stress in ruminants. Dietary zinc intake elevated plasma zinc level and improved the antioxidant status of lambs.

Keywords: zinc, nanoparticles, lamb, antioxidant enzymes

Acknowledgement: This work was supported by the Slovak Research and Development Agency under the contract no. APVV-21-0301, APVV-SK-PL-23-0004, and by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V02-00020.

DOI: https://doi.org/10.15414/2024.9788055227443

Cultivation of macrophages using 3D cell culture system

Michaela Burvalova, Jana Rehakova, Lucie Jelinkova, Ales Pavlik, Petr Slama

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

Abstract

The development and testing of new 3D models for macrophage culture is important for a proper understanding of the functions of many diseases. The practical part of this work deals with testing the system of 3D culture for cultivation of macrophages. For that purpose, there were used nanofiber scaffolds with various concentration of polycaprolactone and fibroin. The results of the experiments showed that monocytes and the developing macrophages from them thrived and developed best on nanofibers with increasing fibroin content. While 100% polycaprolactone with random fibre arrangement showed the lowest cell number attached to fibres.

Keywords: 3D cell culture, polycaprolactone, fibroin, monocyte, macrophage

Acknowledgement: This work was supported by the Ministry of Education, Youth and Sport of the Czech Republic, project number 8J23FR005, and by the Internal Grant Agency of Faculty of AgriSciences at Mendel University in Brno, project number IGA24-AF-IP-021.

DOI: https://doi.org/10.15414/2024.9788055227443

The effect of bee pollen on some parameters of antioxidant status of rats

Marcela Capcarova[1], Anna Kalafova[1], Monika Schneidgenova[1], Patricia Simkova[1], Miroslav Juracek[2], Milan Simko[2], Branislav Galik[2]

[1]Institute of Applied Biology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra
[2]Institute of Nutrition and Genomics, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra

Abstract

Bee pollen is one of the rich sources of flavonoids. The aim of this study was to investigate the effect of bee pollen in two doses on some parameters of blood antioxidant status in rats. The experiment involved 30 adult Wistar rats. The rats were divided into 3 groups (n=10 in each): control group (C) and two experimental groups (E1, E2). The experimental rats were given bee pollen at different doses as follows: E1 300 $mg.kg^{-1}$ body weight and E2 500 $mg.kg^{-1}$ body weight. The experiment lasted 90 days. Animals serving as controls were fed diets without added bee pollen. The activity of superoxide dismutase (SOD) and total antioxidant status (TAS) were measured by spectrophotometer Genesys 10 using commercial kits (Randox, Bratislava). Consumption of bee pollen at the dose of 500 $mg.kg^{-1}$ led to a significant ($P<0.05$) increase in TAS compared to control. The addition of bee pollen increased SOD activity in both experimental groups compared to the control group. Bee pollen was able to improve antioxidant parameters of treated rats. Further studies are needed.

Keywords: bee pollen, antioxidant, rats, TAS, SOD

Acknowledgement

This study was supported by APVV grant no 19/0243, KEGA 007SPU-4/2022, KEGA 017SPU-4/2023 and VEGA grant 1/0304/23.

DOI: https://doi.org/10.15414/2024.9788055227443

Microsatellite panel optimisation for studying of diversity in bees

Eva Cvingráfová, Petra Bartoňová, Aleš Knoll

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

Abstract

Bees are an essential element of our ecosystem. Beekeeping is currently facing several challenges, which include not only the spread of disease, but also climate change and the associated loss of habitats. Bee colonies are particularly threatened by bacterial diseases (*Paenibacillus larvae*, *Streptococcus pluton*) or mites (e.g. *Varroa destructor*). All of this causes not only a loss in the number of bee colonies, but a decrease in genetic variability in colonies. The decline of bees has a particular impact on agricultural production (85% of agricultural crops need insects to fertilize).

The main objective of this pilot study is the selection of microsatellite (MS) markers and the optimisation of the methodology for determining the selected MS panel, which will enable reliable testing of genetic diversity of honeybee population (*Apis mellifera*), because the diversity of the population in the Czech Republic is unknown and may differ from others in the world. The results obtained will provide information not only to scientific communities, but also to nature conservation authorities, beekeepers, and farmers in the Czech Republic. This MS panel will allow to determine the current state of genetic variability of bee colonies in the Czech Republic and the results obtained will serve as a basis for corrective measures in the protection of bee health and the entire ecosystem.

In cooperation with beekeepers, samples of 77 bees were taken from various hives throughout the Czech Republic. DNA was isolated from the bee's head and chest. The total amount of 22 MS has been divided into 4 panels and only one panel was selected for this pilot study. The proposed panel of 8 microsatellite markers was analysed using multipex PCR and fluorescent capillary electrophoresis on ABI PRISM 3500 genetic analyser and evaluated using the Gene Mapper v6 software. According to data obtained by fragmentation analysis, microsatellites Ap223 and Ap226 were identified as the least polymorphic, with a low number of allelic variants. Conversely, locus HB-C16-01 exhibited the highest polymorphism indicating its significance for genetic diversity analysis. Microsatellites with a higher polymorphic information content (PIC), typically above 0.5, are considered markers high informativeness including A007, A014, A079, Ap068, Ap226, and HB-C16-01. The average genetic diversity for the entire set of samples reached a value of 0.5425 indicating moderate genetic diversity within the studied population.

For reliable testing of genetic diversity in bee populations microsatellite markers from this multiplex, specifically A007, A014, A079, Ap068, Ap226 and HB-C16-01. These microsatellite markers can be used for testing of genetic diversity of honeybee population in Czech Republic.

Keywords: *Apis mellifera*, genetic variability, microsatellite markers

Acknowledgements: This study was supported by project MZe ČR QK22020324.

DOI: https://doi.org/10.15414/2024.9788055227443

Evaluation of the Influence of Platinum Nanoparticles on the Motility and Viability of Rabbit Spermatozoa *In Vitro*

Lucia Dianová[1], Filip Tirpák[1,2], Marko Halo Jr.[1], Nikola Štefunková[1], Tomáš Slanina[1], Michal Lenický[1], Klaudia Jaszcza[3], Iveta Urban[1], Peter Massányi[1]

[1]*Institute of Applied Biology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976 Nitra, Slovak Republic*
[2]*Division of Animal Sciences, University of Missouri, 920 East Campus Drive Columbia, Missouri 65211-5300, USA*
[3]*Department of Animal Physiology and Endocrinology, Faculty of Animal Science, University of Agriculture in Krakow, al. Mickiewicza 24/28, 30-059 Krakow, Poland*

Abstract

Metal nanoparticles are currently improving numerous existing processes in medicine, the food industry, electronics, textiles, cosmetics, and others. The steadily increasing usage of nanoparticles in many consumer products raises concerns about its impact on living organisms. The sensitivity of spermatozoa to various xenobiotics detected in the environment become a well-known fact. This experiment aims to evaluate the basic quality parameters of spermatozoa motility and viability *in vitro*, affected by various concentrations of platinum nanoparticles (Pt NPs). Samples of rabbit ejaculate (n=3) were exposed to different concentrations of Pt NPs (62.5 – 1.95 µg/mL; 6h; 37°C). Progressive motility (PRO) was evaluated using CASA technology in time intervals of 0 and 6 hours. In terminal time interval mitochondrial toxicity test (MTT) was measured for viability assessment of spermatozoa. The results of the experiment revealed that all evaluated quality parameters of spermatozoa were notably affected by Pt NPs. In the initial time interval, PRO was significantly increased at concentrations 31.25 µg/mL, 15.63 µg/mL ($P<0.05$), and 3.91 µg/mL ($P<0,01$). After 6 hours PRO decreased rapidly mainly at the highest used concentration 62.5 – 15.63 µg/mL ($P<0,0001$) and also significantly at 7.81 µg/mL ($P<0,001$), 3,91 µg/mL ($P<0,01$) and 1.95 µg/mL ($P<0.05$). On the contrary, results of MTT showed rising mitochondrial activity after 6 hours of exposition of spermatozoa to 31.25 µg/mL Pt NPs. The current short in vitro study could offer partial conclusions about the effect of Pt NPs on rabbit spermatozoa, but for a better understanding of their effect, further analyses are needed.

Keywords: Platinum, nanoparticles, spermatozoa, motility, viability

Acknowledgments: The research was financially supported by projects VEGA 1/0698/22, APVV-16-0289, APVV-21-0168, KEGA 035SPU-4/2023, and by the Grant Agency of SUA in Nitra No. 20-GASPU-2021. This publication was also created with support within the Operational Program Research and Innovation for the project: Support of research activities in the field of animal production, 313011U414, co-financed from the resources of the European Regional Development Fund.

DOI: https://doi.org/10.15414/2024.9788055227443

Irisin affects the transcriptome of the porcine anterior pituitary cells during the mid-luteal phase of the oestrous cycle

Kamil Dobrzyń[1], Barbara Zarzecka[1], Marta Kieżun[1], Grzegorz Kopij[1], Mariia Himultidinova[1], Oghuzan Koker[1], Agnieszka Rak[2], Tadeusz Kamiński[1], Nina Smolińska[1]

[1]*Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland*
[2]*Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Poland*

Abstract

The connection between metabolic disorders and reproductive dysfunctions has been intensively studied for many years. Irisin, a member of exercise-induced adipomyokines, is involved in regulating metabolism and has been perceived as a potential mediator between metabolism and reproductive functions. The complete lack of data concerning the adipomyokine actions in the pituitary, a crucial element of the hypothalamic-pituitary-gonadal (HPG) regulatory axis, prompted us to investigate its influence on the global gene expression in the porcine anterior pituitary cells (APc) during the mid-luteal phase of the oestrous cycle. The APc were incubated with irisin (300 ng/mL) or without any treatment (control; n=5) for 24h. Total RNA isolated from the APc was subjected to Next Generation Sequencing (NGS) using the Illumina NovaSeq 6000 platform. The obtained results revealed 163 genes with the expression altered in the presence of irisin (DEGs; $\log2(FC) \geq |0.56|$; $p<0.05$). In this group, 64 DEGs were up-regulated, and 99 were down-regulated in the presence of the adipomyokine. The analysis of functional annotation (Gene Ontology - GO) assigned the revealed DEGs to 242 GO terms, grouped into three following categories: biological processes (BP), cellular components (CC) and molecular functions (MF). The most enriched GO terms in particular categories were: “defence response to virus” (GO:0051607, 8 DEGs) in BP category, “cilium” (GO:0005929, 6 DEGs) in CC, and “calcium ion binding” (GO:0005509, 10 DEGs) in MF category. KEGG enrichment analysis revealed 9 signalling pathways involving the revealed DEGs, such as “Oxytocin signalling pathway” (ko04921, 140 DEGs) or “AMPK signalling pathway” (ko04152; 113 DEGs). The obtained results indicate the modulatory effect of irisin on the transcriptome of the porcine APc what suggests that adipomyokine plays an important role in the regulation of reproductive functions through the modulation of pituitary functioning.

Keywords: irisin, NGS, anterior pituitary, oestrous cycle, pig, reproduction

Acknowledgement: This study was supported by the Polish National Science Centre (2020/39/B/NZ9/01061; 2020/39/D/NZ9/010092018/31/B/NZ9/00781).

DOI: https://doi.org/10.15414/2024.9788055227443

The effect of extremely low-frequency electromagnetic field (ELF-EMF) on the expression of epigenetic mechanism-related genes in the endometrium of pigs during the peri-implantation period

Anita Franczak[1], Pawel Wydorski[1], Agata Zmijewska[1]

[1]*University of Warmia and Mazury, Faculty of Biology and Biotechnology, Department of Animal Anatomy and Physiology, Olsztyn, Poland*

Abstract

Epigenetic patterns in the genome, including DNA methylation and histone acetylation, are crucial for proper transcriptional activity. Any alterations to the epigenetic mechanisms of gene regulation can impact physiological processes, including the regulation of early pregnancy events. Recent studies have shown that exposure to extremely low-frequency electromagnetic fields (ELF-EMF), can lead to changes in the epigenetic elements responsible for regulating gene expression in the endometrium during the peri-implantation period. Specifically, genes that encode the protein chromobox 4 (*CBX4*), lysine methyltransferase 2A (*KMT2A*), lysine demethylase 6A (*KDM6A*), and lysine demethylase 6B (*KDM6B*) are required to maintain the transcriptionally repressive state of many genes, controlling chromatin organization. This study determined whether the ELF-EMF exposure (2 hours, 50 Hz) could alter *CBX4, KMT2A, KDM6A*, and *KDM6B* mRNA transcript abundance in endometrial slices *in vitro* collected from pigs during the peri-implantation period (day 15-16 of early pregnancy, n = 4). The mRNA transcript abundance was examined using a Real-time PCR. The study showed that exposure of the endometrium to the ELF-EMF led to down-regulation of *CBX4* and *KMT2A* mRNA transcript abundance, while *KDM6A* mRNA transcript abundance was up-regulated ($P \leq 0.05$). The expression of *KDM6B* mRNA was not altered due to the ELF-EMF exposure. In conclusion, the ELF-EMF radiation may impact the expression of genes that encode the proteins involved in epigenetic-related changes in the endometrium of pigs during the peri-implantation period. Further studies are required to explore the consequences of epigenetic-related alterations.

Keywords: ELF-EMF, endometrium, peri-implantation period, pigs

Acknowledgment: This work was supported by the National Science Centre, Poland, grant PRELUDIUM no. 2021/41/N/NZ9/01638 and grant no. 12-610-005-110 from the University of Warmia and Mazury in Olsztyn, Poland

DOI: https://doi.org/10.15414/2024.9788055227443

Influence of *Trigonella foenum-graecum* L. microgreens on HUVEC cells

Zofia Goc[1], Grzegorz Formicki[1], Agnieszka Greń[1], Tomas Jambor[2], Norbert Lukac[1]

[1]University of the National Education Commission, Krakow, Faculty of Exact and Natural Sciences, Department of Animal Physiology, Cracow, Poland
[2]Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Nitra, Slovak Republic

Abstract

Microgreens are tiny salad greens that in the past, have gained popularity as culinary ingredients to improve the texture, colour, taste, aroma, and aesthetic appeal of various food dishes. More recently, they have been marketed as nutritious supplements to diets due to its low caloric value as well as their high micro nutrients and antioxidant content. One of these important microgreen is *Trigonella foenum-graecum* L., with the species name fenugreek. The main effective compounds of fenugreek are including saponins, mucilage, steroids, alkaloids, and unsaturated fatty acids. In the present *in vitro* study, we evaluated the potential effect of *Trigonella foenum-graecum* L. microgreens on human umbilical vein endothelial cells (HUVEC). These cells were cultured in the presence of different concentrations of experimental microgreen (from 10 µg/mL to 300 µg/mL) during 24 h exposure. Metabolic activity was evaluated by alamarBlue assay, while the cell membrane integrity was quantified through CFDA-AM assay. Results of alamarBlue assay demonstrated that higher experimental doses (100, 150, 200, 250 and 300 µg/mL) significantly ($p<0.01$; $p<0.001$; $p<0.0001$, $p<0.0001$, $p<0.001$) increased the metabolic activity of HUVECs cells. Additionally, the cell membrane integrity was significantly ($p<0.05$; $p<0.01$) increased at 200 and 250 µg/mL of *Trigonella* after 24 h exposure. In conclusion, further experimental research is necessary to determine the biological significance of microgreen and their protective effects on human health.

Keywords: *Trigonella foenum-graecum L.,* HUVEC, metabolic activity, membrane integrity

Acknowledgement: The research was financially supported by Slovak research and development agency APVV-SK-PL-23-0037, and by the grant of Slovak University of Agriculture in Nitra 07-GASPU-2021. This publication was also created with the support of the Research and Innovation operational program for the project: Support of research activities in VC ABT, 313011T465, co-financed from the resources of the European Regional Development Fund.

DOI: https://doi.org/10.15414/2024.9788055227443

Effect of different doses of *Hippophae rhamnoides* L. on the concentration of GSH and MDA in the brain of diabetic rats

Zofia Goc[1], Edyta Kapusta[1], Agnieszka Greń[1], Renata Muchacka[1], Grzegorz Formicki[1], Marcela Capcarová[2], Anna Kalafová[2], Monika Schneidgenová[2], Nikoleta Šimonová[2]

[1]*University of the National Education Commission, Krakow, Faculty of Exact and Natural Sciences, Department of Animal Physiology, Cracow, Poland*
[2]*Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Nitra, Slovak Republic*

Abstract

The long-term impact of diabetes on the brain are manifested at the structural and neurophysiological levels. A common theory of the pathogenesis of brain dysfunction in diabetes links cell death to oxidative stress mediated by free radicals. Sea buckthorn (Hippophae rhamnoides L.) is characterized by a number of beneficial properties because it contains a number of bioactive substances, especially polyphenols with antioxidant properties. As a strong antioxidant, it is increasingly used in medical and therapeutic applications. Therefore, aim of this *in vivo* research was to determine the effects of different doses of *Hippophae rhamnoides* L. on the concentration of of reduced glutathione (GSH) and malonyldialdehyde (MDA) in the brain of Zucker diabetic fatty rats (ZDF), which represent an animal model of type 2 *Diabetes mellitus*. Animals were provided with water and diet on ad libitum base. Rats were divided into groups as follows: lean untreated rats, diabetic rats without any additives given distilled water daily using gastric gavage, diabetic rats treated with sea buckthorn at various doses 250, 500, 1000 mg·kg^{-1} b.w. daily by gastric gavage. After three months of the experiment, brains were collected and the concentration of GSH and MDA was measured. The obtained results showed a significant (*$p<0.01$; $p<0.05$;$p<0.05$*) increase in GSH content at 200, 500 and 1000 mg·kg^{-1} b.w of sea buckthorn exposure. However, higher experimental doses (500 and 1000 mg·kg^{-1} b.w) significantly (*$p<0.05$*) decreased the concentration of lipid peroxidation marker. This results suggests that further studies are necessary to evaluate the effects of *Hippophae* on oxidative stress and to identify its utilization as a natural antioxidant and nutritional supplement.

Keywords: brain, diabetes, sea buckthorn, GSH, MDA

Acknowledgement: The research was financially supported by projects KEGA 007SPU-4/2022

DOI: https://doi.org/10.15414/2024.9788055227443

Improving livestock biosecurity trough trace mineral supplementation

Ľubomíra Grešáková[1], Andrea Lauková[1], Monika Pogány Simonová[1], Katarína Tokarčíkova[1,2]

[1]Institute of Animal Physiology Centre of Biosciences Slovak Academy of Sciences, Košice, Slovak Republic
[2]Department of Animal Morphology, Physiology and Genetics, Faculty of AgrSciences, Mendel University in Brno, Brno, Czech Republic

Abstract

Biosecurity refers to a complex of strategies and practices to prevent the introduction and spread of pathogens and diseases on and between farms to preserve good health status of farm animals. Trace elements (TEs) play an important role in animal immunity, health and performance, and therefore feed supplementation with trace minerals is one of husbandry strategies how to improve animal health and livestock productivity. Besides keeping all biosecurity rules, some feed mineral additives can support the intestinal health and gut microbiome diversity to better manage animal diseases and disorders. Although, the nutrient requirement of pigs for Zn and Cu range at concentrations of 50 to 110 ppm and 5 to 10 ppm, respectively; pharmacological levels of both minerals (2000-3000 ppm for Zn, 125-250 ppm for Cu) used to supplement nursery diets to reduced post-weaning diarrhoea and improve growth performance in in both nursery and grow-finish pigs. The mode of action of the great Zn and Cu concentrations in pig feed related to antimicrobial activity, development of gut morphology and maintenance of gut integrity. The negative effect of using pharmacological levels of both TEs in swine production consist in the increasing antimicrobial resistance and the environmental contamination by heavy metals. Therefore, the use of pharmacological dosage of any Zn forms as growth promoters in piglet diets is prohibited in EU since 2022. On the other hand, appropriate nutrient supply including supplementation with TEs mainly from their organic sources should be a key component in livestock health programs and provides the foundation for other health management strategies as well as biosecurity practices. The objective of our research is studying the role of mineral supplementation in livestock farming as a factor in the transmission of pathogens between farm animals from point of view biosecurity.

Keywords: biosecurity, trace minerals, feed supplementation, gut health, livestock, environment

Acknowledgments: This research is based upon work from COST Action BETTER, CA20103, supported by COST (European Cooperation in Science and Technology), VEGA 2/0008/21, and APVV-21-0301.

DOI: https://doi.org/10.15414/2024.9788055227443

Effect of pentoxifyline on stallion spermatozoa parameters

Marko Halo Jr[1], Monika Šmondrková[2], Lucia Dianová[1], Michal Lenický[1], Kateryna Vanivska[1], Martin Massányi[3], Tomáš Slanina[1], Jiřina Zemanová[1], Katarína Tokárová[1], Eva Mlyneková[2], Marko Halo[2], Peter Massányi[1]

[1]*Institute of Applied Biology, Slovak University of Agriculture in Nitra, Slovak Republic*
[2]*Institute of Animal Husbandry, Slovak University of Agriculture in Nitra, Slovak Republic*
[3]*AgroBioTech Research Centre, Slovak University of Agriculture in Nitra, Slovak Republic*

Abstract

Pentoxifylline, a methylxanthine derivative, has been shown to enhance motility of human, ram, and equine ejaculated spermatozoa *in vitro*. Semen extenders should maintain the fertility of chilled semen for 24–48hours. This study was aimed to determine the impact of different pentoxifyline concentrations on the motility and viability of chilled stallion spermatozoa. Each stallion semen sample was diluted in a ratio of 1:2, with various pentoxifyline concentrations (0.112-3.89 mg/mL) dissolved in a conventional extender. The control samples (CON) were prepared by diluting ejaculate only using the conventional extender. The motility was analysed using a CASA system at different time intervals (0, 6, 12, 24, and 30 h) and the viability was evaluated using a mitochondrial toxicity test (MTT) performed at the end of the incubation at 5 °C. The samples that were treated with a pentoxifyline improved motility and progressive motility of stallion spermatozoa, mainly after 12, 24, and 30 hours of incubation. After 30 hours of incubation, viability of stallion spermatozoa showed non-significant increased values in experimental samples compared to control sample. The results of this study describe the pentoxifyline as an optimal supplement for improving the quality of stallion semen during chilled storage. To conclude, our study demonstrated that different pentoxifylline concentrations are suitable additives to conventional stallion semen extenders.

Key words: pentoxifyline, spermatozoa, stallion, CASA, viability

Acknowledgments: The research was financially supported by projects VEGA 1/0698/22, VEGA 1/0437/24, KEGA 035SPU-4/2023, APVV-21-0168, and by the Grant Agency of SUA in Nitra No. 20-GASPU-2021. This publication was also created with support within the Operational Program Research and Innovation for the project: Support of research activities in the field of animal production, 313011U414, co-financed from the resources of the European Regional Development Fund.

DOI: https://doi.org/10.15414/2024.9788055227443

Impact of Bisphenols on the Secretory Activity of Testosterone in Human Carcinoma NCI-H295R Cells

Marek Helczman, Nikola Štefunková, Tomáš Jambor, Anton Kováčik, Peter Massányi, Norbert Lukáč

Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Applied Biology, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovak Republic

Abstract

Bisphenols, particularly Bisphenol A (BPA) and Bisphenol F (BPF), are widespread environmental contaminants known for their endocrine-disrupting effects. This study investigates the impact of BPA and BPF on the secretory activity of testosterone in the human adrenocortical carcinoma cell line NCI-H295R. We employed a comprehensive methodology, including the preparation of biological material through the cultivation and passaging of NCI-H295R cells, and their exposure to varying concentrations of BPA and BPF. The study assessed cell viability and mitochondrial activity after 24-hour cultivation. The cells were cultured in DMEM/F12 medium supplemented with antibiotics, antifungals, NaHCO3, ITS + Premix, and BD Nu-Serum. The cells were transferred to sterile 96-well or 6-well plates, incubated in a CO_2 incubator, and then replaced with a new medium containing bisphenols at concentrations ranging from 0.1 to 100 µM (0,1; 0,5; 1; 10; 25; 50; 100). Statistical analysis using GraphPad Prism 9.5.0 and Microsoft Excel confirmed the cytotoxic effects and inhibitory action on testosterone secretion. The lowest metabolic and mitochondrial activity was observed at 100 µM. A statistically significant decrease in testosterone production compared to the negative control occurred with exposure to BPA at concentrations of 1µM ($P < 0.05$) and higher ($P < 0.001$). There was also a significant decrease in BPF exposure at concentrations of 10 µM and higher. BPA exhibited higher toxic effects than BPF, and inhibited testosterone production to a greater extent, suggesting that BPF could be considered a better substitute for BPA. This research not only underscores the detrimental impact of bisphenols on cellular viability and steroidogenesis but also highlights the necessity for stringent assessments of environmental contaminants' safety thresholds.

Keywords: endocrine disruptors, testosterone, BPA, BPF, NCI-H295R Cells

Acknowledgment: This work was supported by the Slovak Research and Development Agency under the contracts No. APVV-16-0289; APVV-20-0218; APVV-21-0168; and by the VEGA 1/0571/23, and KEGA 023SPU-4/2022: Integration of connectivity using web platforms into the teaching strategy of the courses of the study programme "Applied Biology".

DOI: https://doi.org/10.15414/2024.9788055227443

Determination of selected biogenic amines in common carp (*Cyprinus carpio*)

Silvia Jakabová[1], Július Árvay[1], Lucia Benešová[2], Jozef Golian[1]

[1]*Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Institute of Food Sciences, Nitra, Slovak Republic*

[2]*Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Agrobiotech Research Centre, Nitra, Slovak Republic*

Abstract

Biogenic amines are organic compounds found in various food including fish. They are formed through the breakdown of amino acids during the process of fish spoilage. Biogenic amines significantly influence food quality and can pose health risks to consumers. High levels of biogenic amines can indicate poor quality of fish meat and potential health risks if consumed. Fillets from common carp (*Cyprinus carpio*) were heat-treated and freeze-stored in order to investigate biogenic amines regarding the processing method. Biogenic amines were determined after pre-column derivatization with the dansyl chloride by HPLC-DAD method. Totally four different biogenic amines occurred in the samples in the following order of abundance and quantity: spermidine > putrescine > spermine > tyramine. Biogenic amines putrescine and spermidine were quantified in the fresh carp fillets in the mean concentration 2.80±0.06 mg/kg DW and 15.23±0.10 mg/kg DW, respectively. One-month stored fish fillets had increased concentration of the both biogenic amines, moreover spermine was also present (16.63±0.36 mg/kg DW). Tyramine was determined in the baked (31.4±0.30 mg/kg DW) and 3-month stored fish (15.04±0.32 mg/kg DW). Statistical differences between the variants, differing in the processing, were tested ($P<0.05$) by Tukey pairwise test. Statistical significant differences were found between all variants. In conclusion, further experimental investigations are required to follow the trends in biogenic amines formation in the processed freshwater fish products.

Keywords: common carp, biogenic amines, food processing

Acknowledgement: This work was supported by the Slovak Research and Development Agency under the contract no. APVV-22-0402, APVV 17-0508, and by the VEGA 1/0239/21, and by the project “Fostering Internationalisation in Higher Education by BioFood Virtual Labs” no. 2021-1-SK01-KA220-HED-000032062.

DOI: https://doi.org/10.15414/2024.9788055227443

The potential impact of *Trigonella foenum-graecum L.* microgreens on interleukin-6 release in HUVEC cells *in vitro*

Tomas Jambor[1], Bazany Denis[1], Hana Greifova[1], Nikola Stefunkova[1], Zofia Goc[2], Norbert Lukac[1]

[1]*Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Applied Biology, Nitra, Slovak Republic*
[2]*University of the National Education Commission, Faculty of Exact and Natural Sciences, Institute of Biology and Earth Sciences, Cracow, Poland*

Abstract

Over the past ten years, interest in fresh, functional and nutraceutical foods have been on the rise, driven by society's growing interest in healthy eating. In this point of view, microgreens as a new class of specialty crops have begun to gain significant popularity. It is primarily caused by a rich profile of biologically active compounds included in different types of microgreen crops, herbs and wild species. Microgreens *Trigonella foenum-graecum L.* contains a high concentration of phytochemicals, which have antioxidant, antidiabetic, hepatoprotective and anti-inflammatory effects. The aim of the present in vitro study was to evaluate the effect of *Trigonella foenum-graecum L.* microgreens on interleukin 6 (IL-6) release in human umbilical vein endothelial cells (HUVEC). These cells were exposed to selected doses of experimental microgreens (10 µg/mL - 300 µg/mL) during 24 h. Subsequently, IL-6 as a pro-inflammatory cytokine was quantified by the ELISA method. The gained results revealed a slight decrease in IL-6 release at 10, 50 and 100 µg/mL of *Trigonella* after 24 h exposure. However, higher experimental doses (150, 200, 250 and 300 µg/mL) significantly ($p<0.01$; $p<0.05$; $p<0.001$) inhibited the release of this cytokine. This pilot study suggests that more detailed and systematic research is definitely required for a better understanding of the health benefits associated with microgreen consumption.

Keywords: *Trigonella foenum-graecum L.,* HUVEC, inflammation

Acknowledgments: The research was financially supported by Slovak research and development agency APVV-SK-PL-23-0037, by the Scientific Agency of the Slovak Republic VEGA No. 1/0083/21, and by the grant of Slovak University of Agriculture in Nitra 07-GASPU-2021. This publication was also created with the support of the Research and Innovation operational program for the project: Support of research activities in VC ABT, 313011T465, co-financed from the resources of the European Regional Development Fund.

DOI: https://doi.org/10.15414/2024.9788055227443

Effect of *Trigonella foenum-graecum L.* microgreens on bovine spermatozoa motility and cell membrane integrity *in vitro*

Tomas Jambor[1], Michal Ďuračka[2], Eva Tvrdá[3], Norbert Lukac[1]

[1]*Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Applied Biology, Nitra, Slovak Republic*
[2] *AgroBioTech Research Centre, Slovak University of Agriculture in Nitra, Slovak Republic*
[3]*Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Biotechnology, Nitra, Slovak Republic*

Abstract

Trigonella foenum-graecum L. microgreens exhibit antidiabetic, antineoplastic, hepatoprotective and immunomodulatory properties. Thanks to a varied profile of biologically active compounds, it is considered a medicinal plant widely used for its aphrodisiac effects. On the other hand, the impact of *Trigonella* on male reproduction and the possible mode of its action are not evaluated in detail. Herein, the aim of the study was to examine the effect of *Trigonella* microgreen extract on bovine spermatozoa *in vitro.* Concretely, the study evaluated the impact of experimental concentrations (1 – 300 µg/mL) on spermatozoa motility and cell membrane integrity after 2 h and 24 h exposure. Motility was assessed by computer-assisted sperm analysis (CASA), while the second parameters was monitored by CFDA-AM assay. The results clearly demonstrate that any applied concentration did not significantly affect these parameters after short-term (2 h) exposure. Lower experimental concentrations (1 – 50 µg/mL) significantly ($p<0.05$) stimulated spermatozoa motility, while the rest of the experimental doses did not affect this parameter significantly after 24 h exposure. A similar tendency was recorded in cell membrane integrity. A significant ($p<0.05$) effect was observed at 1, 10 and 50 µg/mL of the experimental microgreen while the higher doses did not affect this parameter significantly. This pilot study suggests that more detailed and systematic research is definitely required for a better understanding of the health benefits associated with microgreen consumption.

Keywords: *Trigonella foenum-graecum L.,* spermatozoa, reproduction,

Acknowledgments: The research was financially supported by Slovak research and development agency APVV-SK-PL-23-0037, by the grant of Slovak University of Agriculture in Nitra 07-GASPU-2021, and by the Scientific Agency of the Slovak Republic VEGA No. 1/0083/21. This publication was also created with the support of the Research and Innovation operational program for the project: Support of research activities in VC ABT, 313011T465, co-financed from the resources of the European Regional Development Fund.

DOI: https://doi.org/10.15414/2024.9788055227443

Meat colour evaluation after bee bread supplementation of female Japanese quails

Anna Kalafova[1], Nikoleta Simonova[1], Monika Schneidgenova[1], Patricia Simkova[1], Emilia Hanusova[2], Cyril Hrncar[3], Peter Hascik[4], Jan Brindza[5], Renata Mamrakova[1], Marcela Capcarova[1]

[1]*Institute of Applied Biology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 94976, Slovak Republic*
[2]*Institute of Small Farm Animals, Research Institute for Animal Production Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic*
[3]*Institute of Animal Husbandry, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 94976, Slovak Republic*
[4]*Institute of Food Sciences, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 94976, Slovak Republic*
[5]*Institute of Plant and Environmental Sciences, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 94976, Slovak Republic*

Abstract

The aim of this research was to determine whether bee bread affect the meat colour of the female Japanese quail's (*Coturnix japonica*) thigh and breast muscle. Meat colour was determined from samples of the *pectoralis major* and *biceps femoris* muscles using a CM-2600d spectrophotometer (Osaka, Japan) 24 hours *post-mortem*. For the assessment of colour, we established the colour space using the Commission Internationale de l'Eclairage (CIE) Lab system. Animals were divided into 4 groups (E1, E2, E3 and control) according to the doses of bee bread into feed mixture HYD 11 supplied *ad libitum* as follows: E1 (n=9) 2 $g.kg^{-1}$, E2 (n=9) 4 $g.kg^{-1}$, E3 (n=9) 6 $g.kg^{-1}$. The control group (n=9) was the group without additives (K). The experimental intervention lasted for 170 days. There were no statistically significant differences ($P > 0.05$) in the meat colour between the experimental groups and the control group. According to the findings of the study, bee bred in the feeding mixture of female Japanese quails did not affect meat colour in thigh and breast muscle.

Key words: Japanese quails; meat colour; bee bread

Acknowledgments: This work was financially supported by the KEGA 007SPU-4/2022, KEGA 017SPU-4/2023, VEGA 1/0304/23 and APVV-19-0243.

DOI: https://doi.org/10.15414/2024.9788055227443

Detection of antibiotic resistance in *E. coli* from calves

Lívia Karahutová[1], Dobroslava Bujňáková[1]

[1]*Institute of Animal Physiology Centre of Biosciences Slovak Academy of Sciences*

Abstract

Monitoring programs based on clinical studies provide the fastest system for monitoring the emergence of resistance to new antimicrobial agents, but they do not provide a true description of the occurrence of resistance. Indicator commensal bacteria isolated from healthy animals provide more reliable data on the occurrence of resistance in a more comprehensive population than pathogenic isolates. The aim of this study was to classify *E. coli* strains originating from eight farms with calves into phylogenetic groups (intestinal, extraintestinal) and select a group of indicator intestinal strains (belonged to phylogroups A, B1 and C – 43% of obtained isolates) to detect phenotypic antibiotic resistance (AR) via Slovakian automated diagnostic system Bel-MIDITECH (Bratislava, SVK). A third of the isolates (27%) were sensitive to 20 tested antibiotics (ATBs); 18% were resistant to one ATB (TET or COL); 4% to two (TET+COL) and resistance to three (AMP, TET, COT), four (AMP, SAM, GEN, TET), five (AMP, SAM, CXM, CTX, COT) and six combinations (AMP, GEN, TOB, CIP, TET, COT) of ATBs was occurred in similar values. A more worrying situation was resistance to 7 (21% of isolates; AMP, SAM, GEN, TOB, CIP, TET, COT), 8 (7% of isolates; AMP, SAM, GEN, TOB, CIP, TET, COL, COT) and even up to 11 (5% of isolates; AMP, CXM, CTX, CAZ, FEP, ETP, TOB, AMI, CIP, TET, COT) ATBs. We noticed considerable differences in the occurrence of AR on individual farms. While on the first five farms, AR was occurred only rarely (resistance to a maximum of 2 ATBs) it was worse on the remaining three farms. Since diarrhea is one of the most serious diseases of newborn calves, the obtained AR profiles were provided to owners and their veterinarians, which may help them to better treat *E. coli* diseases in their herds in the future. The next step will be the detection of virulence factors, which will help to better determine the pathotype of *E. coli* causing the disease on individual farms.

Keywords: *E. coli*, antibiotic resistance, antibiotic treat.

Acknowledgement: This work was supported by [VEGA] (Vedecká grantová agentúra Ministerstva školstva, vedy, výskumu a športu Slovenskej republiky a Slovenskej akadémie vied) grant number [2/0010/21].

DOI: https://doi.org/10.15414/2024.9788055227443

Association of chemerin levels with progression of colorectal cancer

Jacek Kiezun[1], Anna Koprowicz-Wielguszewska[1], Marlena Gudelska[1], Katarzyna Kisielewska[1], Janusz Godlewski[1], Bartlomiej E. Krazinski[1]

[1]*Department of Human Histology and Embryology, School of Medicine, University of Warmia and Mazury in Olsztyn, Poland*

Abstract

Chemerin is an adipokine, with pleiotropic biological activity. Chemerin was demonstrated to contribute to diverse regulations in large intestine both, under physiological and pathological conditions. However, its role in colorectal cancer (CRC) remains unclear. This study aimed to evaluate the content of chemerin in tissue homogenates of the tumor and unchanged large intestine, and blood sera collected from patients with CRC. Tissue samples of CRC tumor, peritumoral or distant mucosa with submucosa (M/SM) and peritumoral or distant muscularis externa (ME) were collected from postoperative material of 15 patients with CRC. Tumor fragments and large intestine compartments (M/SM and ME) were separated from resected material and snap-frozen in liquid nitrogen. The blood samples were collected from 55 patients with CRC and 25 healthy volunteers. The chemerin levels in tissue homogenates and blood sera were determined by ELISA and then correlated with the clinico-pathological data (primary tumor status T, cancer spread to regional lymph nodes N, distant metastases M, TNM stage) of the patients. Peritumoral M/SM was characterized by the highest chemerin content. In contrast, the lowest chemerin levels were determined in ME of both peritumoral and distant fragments of the large intestine and these differences (vs peritumoral M/SM) were statistically significant. Moreover, chemerin levels in peritumoral M/SM of patients with cancer spread to the regional lymph nodes (N1 and N2) were over 3-fold lower than chemerin concentration in peritumoral M/SM of N0 patients. Primary tumor status (T) and presence of distant metastases (M) did not correlate with chemerin levels. There were no significant differences in blood sera chemerin concentration in CRC patients compared to healthy volunteers. The expression of chemerin may play a role in the invasion of cancer cells to the regional lymph nodes. Our results suggest that higher levels of chemerin may be associated with protective, anti-cancerous action in CRC. The exact role of chemerin in CRC and its mechanism of action on cancerous and/or peritumoral stromal cells requires further studies.

Keywords: chemerin, colorectal cancer, clinico-pathological data.

Acknowledgement: This research was supported by the University of Warmia and Mazury Rector's Grant, Olsztyn, Poland.

DOI: https://doi.org/10.15414/2024.9788055227443

Omentin-1 regulates endometrial angiogenesis through the VEGF system expression

Marta Kieżun[1], Kamil Dobrzyń[1], Oguzhan Köker[1], Ewa Zaobidna[1], Grzegorz Kopij[1], Marlena Gudelska[2], Katarzyna Kisielewska[2], Barbara Zarzecka[1], Mariia Himultidinova[1], Nina Smolińska[1], Tadeusz Kamiński[1]

[1]*Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland*
[2]*School of Medicine, University of Warmia and Mazury in Olsztyn, Poland*

Abstract

Omentin-1, known for its pleiotropic properties is also perceived as a factor involved in the reproductive processes. We hypothesised that the hormone may also be involved in endometrial angiogenesis. The study aimed to evaluate the effect of omentin-1 on the secretion of vascular endothelial growth factors (VEGF) A-D and placental growth factor (PlGF) as well as VEGF receptors (VEGFR) 1-3 protein abundance in the *in vitro* cultured porcine endometrial explants. Experimental tissues were obtained during the mid-luteal phase of the oestrous cycle – the stage of the highest ovarian secretory activity. Tissues were *in vitro* cultured in the presence of omentin-1 (25, 50, 100 ng/mL) or with medium alone (control; n=5 in each group) The results were statistically analysed by one-way ANOVA and Duncan *post hoc* test, and significant differences were considered at $p<0.05$. The concentrations of VEGFs and PlGF in the culture media were measured by the ELISA method, whereas the protein abundance of VEGFRs was evaluated with the Western Blot method. We observed that omentin increased the secretion of VEGF-A (100 ng/mL), VAGFA-C (all doses) and VEGF-D (50 ng/mL), did not affect VEGF-B release, and decreased PlGF concentration in the culture media (all doses). Omentin increased the endometrial protein content of VEGRF1 and 2 (25, 100 ng/mL), and VEGFR3 (50 ng/mL). The presented results showed that omentin significantly affects the *in vitro* release of angiogenesis-related factors and the corresponding receptors' protein abundance and that the effects were dependent on the adipokine concentration. Cyclic blood and lymphatic vessel remodelling are some of the key processes underlying the proper preparation of the endometrium for embryo implantation. Therefore, it seems that omentin may be an important player in the regulation of endometrial angiogenesis.

Keywords: omentin, angiogenesis, VEGF, VEGFR, endometrium, oestrous cycle, pig

Acknowledgement: This study was supported by the Polish National Science Centre (projects No: 2020/39/D/NZ9/01009, 2020/39/B/NZ9/01061).

DOI: https://doi.org/10.15414/2024.9788055227443

Omentin-1 modulates prostaglandin synthesis in the porcine endometrium: *in vitro* insights from early pregnancy and mid-luteal phase of the oestrous cycle

Oguzhan Köker[1],Kamil Dobrzyń[1], Grzegorz Kopij[1], Marlena Gudelska[2], Barbara Zarzecka[1], Mariia Himultidinova[1], Nina Smolińska[1], Tadeusz Kamiński[1], Marta Kieżun[1]

[1]*Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland*
[2]*School of Medicine, University of Warmia and Mazury in Olsztyn, Poland*

Abstract

Our hypothesis is based on the assumption that omentin-1, a hormone belonging to the adipokines family, influences the endometrial synthesis of prostaglandins. The study aimed to detect the *in vitro* effects of omentin-1 at doses of 25, 50, and 100 ng/mL on the secretion of prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), as well as the protein abundance of microsomal prostaglandin E synthase-1 (mPGES-1) in the porcine endometrial tissue slices from days 10 to 12 of the oestrous cycle (mid-luteal phase of the cycle) and 15 to 16 of pregnancy (beginning of implantation). The concentrations of PGE_2 and $PGF_{2\alpha}$ in the culture media were evaluated by ELISA and protein abundance of mPGES-1 with the Western Blot method. For statistical analysis, one-way ANOVA and Duncan *post hoc* test were used. On days 15-16 of pregnancy, omentin-1 significantly increased PGE_2 (50 ng/mL) and decreased $PGF_{2\alpha}$ secretion (all doses), while there was no significant influence of the adipokine on days 10-12 of the oestrous cycle. In the case of mPGES-1, omentin-1 (all doses) increased the protein expression on days 10-12 of the oestrous cycle, whereas the adipokine (25, 50 ng/mL) decreased the protein content on days 15-16 of pregnancy. The obtained results confirm that omentin-1, at least *in vitro*, has a significant influence on the prostaglandin synthesis process in the porcine endometrium. This implies that the adipokine may be an effective factor in the regulation of key processes underlying the proper functioning of the endometrium, both during the oestrous cycle and early pregnancy. To fully explain the mechanisms of omentin-1 action in the endometrium, further research is essential.

Keywords: omentin, prostaglandin, PGE_2, $PGF_{2\alpha}$, mPGES-1, endometrium, oestrous cycle, pregnancy, pig

Acknowledgement: This study was supported by the Polish National Science Centre (projects No: 2020/39/D/NZ9/01009, 2020/39/B/NZ9/01061).

DOI: https://doi.org/10.15414/2024.9788055227443

Monitoring the effects of glycyrrhizin and shiitake extract on steroid hormone production by human ovarian tumor cells *in vitro*

Adriana Kolesarova[1,2], Olha Balytska[1], Michal Mihal[1], Katarina Majerik Behinska[1], Tibor Lukac[1,3], Tatiana Bojnanska[4]

[1]*Institute of Applied Biology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic*
[2]*AgroBioTech Research Centre, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic*
[3]*One Pharma, s.r.o., Janka Kráľa 5, 974 01 Banská Bystrica, Slovak Republic*
[4]*Institute of Food Sciences, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic*

Abstract

Steroid hormones, particularly estradiol and progesterone, play pivotal roles in regulating various physiological processes, including reproduction. Dysregulation of their production can lead to significant health consequences. Glycyrrhizin, has been reported to possess various pharmacological properties, including anti-inflammatory and anti-cancer effects. Previous studies have suggested its potential influence on steroid hormone synthesis pathways. Shiitake (*Lentinula edodes*) extract is renowned for its immunomodulatory and anti-cancer properties but its effects on steroid hormone production remain largely unexplored. The aim of this *in vitro* study was to investigate the effect of glycyrrhizin and shiitake extract separately at concentrations of 10, 50, 100, 250, 500 and 1000 µg/ml during 48-hour cultivation on the production steroid hormones: progesterone and estradiol by ovarian cancerous cells (human granulosa tumor cells COV434). To carry out this experiment, we applied the ELISA method. The results of our study revealed that while shiitake extract exhibited no significant effect on hormone production, glycyrrhizin notably increased the levels of progesterone at concentrations 250 µg/ml and higher and the same effect was noticed at production estradiol which was significantly ($P \leq 0.05$) higher at concentrations 500 and 1000 µg/ml. These findings suggest a potential role of glycyrrhizin in modulating steroid hormone synthesis, specifically changes in estradiol and progesterone production in cancer cells. However, further research is needed to confirm the hypothesis and underlying mechanisms and clinical implications of glycyrrhizin in hormone-related disorders.

Keywords: Glycyrrhizin, Shiitake extract, steroid hormones, estradiol, progesterone, COV434 cell line.

Acknowledgments: The work was supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic project APVV-21-0206, VEGA 1/0620/24, KEGA 035SPU-4/2023, and the Operational Program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, co-financed by the European Regional Development Fund.

DOI: https://doi.org/10.15414/2024.9788055227443

Virtual laboratories and digital learning environment for internationalization in tertiary education

Adriana Kolesárová[1], Silvia Jakabová[1], Tomáš Jambor[1], Ľubomír Belej[1], Eva Ďurčanská[1], Dominik Hollý[1], Petr Sláma[2], Grzegorz Formicki[3], Péter Sipos[4]

[1]*Slovak University of Agriculture, Nitra, Slovak Republic*
[2]*Mendel University in Brno, Brno, Czech Republic*
[3]*University of National Education Commission, Krakow, Poland*
[4]*University of Debrecen, Debrecén, Hungary*

Abstract

Tertiary education in the pandemic period strongly felt the need for digitization of educational materials, which was reflected in many educational projects, including project within the Cooperation partnerships in higher education no. 2021-1-SK01-KA220-HED-000032062 under the title Fostering Internationalisation in Higher Education by BioFood Virtual Labs. The BioFoodVirLabs international project is being solved in cooperation with Slovak Agricultural University in Nitra by three other partner universities: Mendel University in Brno in the Czech Republic, University of Debrecen in Hungary and University of National Education Commission in Krakow in Poland. The main goal of the project is to support digital skills in the field of biology, biotechnology and food sciences through the development and promotion of new forms of distance learning aimed at higher education. The innovative component is an approach based on the creation of the concept of virtual laboratories in three scientific areas – Biology, Biotechnology and Food Sciences. Two main outputs are solved within the project – virtual libraries and virtual laboratories that are logically linked together through the preparation of digital education materials for the creation of virtual laboratories. The model represents an interesting form of implementation of modern digital technologies that enable the transformation of education and partially remove obstacles to distance education in the teaching of practical professional subjects in the field of biological, biotechnological and food sciences disciplines. Creation of digital education materials, their collection, exchange, or mediation between partners on a common virtual platform represents the development of digital readiness for various forms of tertiary education, as the digital platform will provide continuity an access to materials for teachers and students and the digital resilience and capacity of the participating universities will be strengthened.

Keywords: virtual laboratories, virtual libraries, digital education materials, internationalisation in tertiary education

Acknowledgement: This work was supported by the Erasmus + KA220 Cooperation partnerships in higher education under the contract no. 2021-1-SK01-KA220-HED-000032062.

DOI: https://doi.org/10.15414/2024.9788055227443

Barefoot and shod horses, rehabilitation of defects in their hoof capsule

Anežka Kopečková[1], Kristína Žuffová[2], Terezie Zavadilová[1]

[1]Mendel University in Brno, Faculty of AgriSciences, Department of Morphology, Physiology and Animal Genetics, Czech Republic
[2]University of Veterinary Sciences Brno, Faculty of Veterinary Medicine, Section of Large Animals Diseases, Equine Clinic, Czech Republic

Abstract

Healthy hooves are essential for a horse's movement and overall well-being. Many breeders prioritize the health of their horses and the condition of their musculoskeletal system. Monitoring and caring for the hooves are critical in preventing various issues. The hoof, a derivative of the skin, encases the end of the third phalanx and facilitates movement by protecting the underlying tissues from mechanical damage and ensuring a pain-free gait. There is an ongoing debate within the breeding community regarding the efficacy of the barefoot approach versus traditional shoeing, particularly in terms of suitability for different conditions and environments. Advocates for the barefoot method argue that it is more natural and allows the hoof to adapt better to various surfaces.

This thesis introduces the study of barefoot versus shod horses, focusing on the rehabilitation of the hoof capsule. It compares the evolution of the hoof capsule shape in two horses, one barefoot and one shod, detailing the interventions employed in each hoof care strategy. Radiographic analysis of the distal parts of the thoracic limbs before and after treatment provides empirical evidence of the outcomes. The thesis concludes with a summary of the findings, elucidating the reasons for the preferred treatment method and proposing future research directions on this topic.

Keywords: barefoot, horseshoe, hoof, hoof capsule

DOI: https://doi.org/10.15414/2024.9788055227443

Persistent Organic Pollutants and Oxidative Stress in Freshwater Fish

Anton Kovacik[1], Marek Helczman[1], Tomas Jambor[1], Marian Tomka[2], Martin Fik[3], Eva Kovacikova[4], Peter Massanyi[1]

[1]*Institute of Applied Biology, Slovak University of Agriculture in Nitra, Slovakia*
[2]*Institute of Biotechnology, Slovak University of Agriculture in Nitra, Slovakia*
[3]*Institute of Animal Husbandry, Slovak University of Agriculture in Nitra, Slovakia*
[4]*AgroBioTech research centre, Slovak University of Agriculture in Nitra, Slovakia*

Abstract

Persistent organic pollutants (POPs), synthetic chemicals infamous for their persistence, bioaccumulation, and toxicity, cast a long shadow over the health of freshwater ecosystems. Among their arsenal of detrimental effects, POPs inflict oxidative stress, an imbalance between free radical generation and antioxidant defences within fish cells. Some POPs, like polychlorinated biphenyls (PCBs), can directly act as pro-oxidants, producing ROS directly within cells. Other POPs, like dioxins, can deplete antioxidant enzymes and molecules, weakening the body's natural defences against ROS. Certain POPs, including organochlorine pesticides, can impair mitochondrial function, leading to increased ROS production and decreased energy production. POPs can induce the activity of enzymes responsible for their detoxification, but these processes can also generate ROS as byproducts. Some POPs can interfere with cellular signalling pathways, leading to altered gene expression and increased production of pro-inflammatory molecules, further exacerbating oxidative stress. Consequences of oxidative stress are several. Lipid peroxidation: Damage to cell membranes and other vital cellular structures. DNA damage: Increased risk of mutations and cancer. Protein modification: Disruption of protein function and enzyme activity. Immune dysfunction: Weakened resistance to pathogens and parasites. Reproductive impairments: Reduced fertility and hatching success. Altered growth and development: Reduced growth rates and developmental malformations. Ripple effects on ecosystem health is followed by reduced biodiversity, altered ecosystem function, and increased vulnerability to other stressors. While significant progress has been made in understanding the link between POPs and oxidative stress in fish, several key questions remain: Chronic and combined effects; Inter-species variability; Sublethal effects. By addressing these knowledge gaps and developing effective mitigation strategies, we can work towards safeguarding freshwater ecosystems and the fish populations that underpin their health and biodiversity.

Keywords: POPs, Reactive Oxygen Species, Freshwater Ecosystem, Biomonitoring

Acknowledgement: This work was supported by the SRDA under the contracts No. APVV 16-0289 and No. APVV-21–0168, by The Ministry of Education, Research, Development and Youth of the Slovak Republic under the project VEGA 1/0571/23, and by the Grant Agency of SUA in Nitra No. 20-GASPU-2021. This publication was also created with support within the Operational Program Research and Innovation for the project: Support of research activities in the field of animal production, 313011U414, co-financed from the resources of the European Regional Development Fund.

DOI: https://doi.org/10.15414/2024.9788055227443

Cemtirestat supplementation does not influence macroscopical and mechanical properties of femoral bone in ZDF rats

Veronika Kovacova[1], Radoslav Omelka[1], Vladimira Mondockova[1], Roman Biro[1], Noemi Penzes[1], Piotr Londzin[2], Joanna Folwarczna[2], Karol Svik[3], Marta Soltesova Prnova[3], Monika Martiniakova[1]

[1]Faculty of Natural Sciences and Informatics, Constantine the Philosopher University in Nitra, Slovak Republic
[2]Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Poland
[3]Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Slovak Republic

Abstract

Aldose reductase (AR), the first enzyme of the polyol pathway, plays a significant role in the cascade of metabolic imbalances responsible for detrimental effects of hyperglycemia. Cemtirestat, a highly selective and efficient AR inhibitor with antioxidant ability, is considered a promising drug in the treatment of several diabetic complications, especially peripheral neuropathy. Our study investigated the impact of prolonged cemtirestat supplementation on macroscopical and mechanical properties of femoral bone in Zucker diabetic fatty (ZDF) rats, as diabetic bone damage is an important microvascular complication of diabetes mellitus. Adult ZDF rats (n=18) were assigned to three groups: untreated diabetic rats (D group) and diabetic rats orally treated with cemtirestat at doses of 2.5 mg/kg/day (E1 group) and 7.5 mg/kg/day (E2 group) for 2 months. According to our results, no significant differences in total body weight of rats, femoral weight, and femoral length were found between cemtirestat-treated and untreated groups. The three-point bending test revealed no effect of cemtirestat treatment on femoral bone mechanical properties, specifically on yield point load (YPL), displacement, energy and stress for the YPL; maximum load (ML), displacement, energy and stress for the ML; fracture load (FL), displacement, energy and stress for the FL. All aforementioned findings do not support a protective impact of cemtirestat against diabetic bone damage in ZDF rats, which serve as a suitable animal model of type 2 diabetes mellitus.

Keywords: cemtirestat, aldose reductase, bone macroscopical properties, bone mechanical properties, ZDF rats, type 2 diabetes mellitus

Acknowledgement: This work was supported by the projects VEGA 1/0416/22 and VEGA 1/0328/24.

DOI: https://doi.org/10.15414/2024.9788055227443

Stabilised form of thymol in rabbit diet, its absorption from gastrointestinal tract and effect on intestinal morphology.

Radoslava Kristofova[1], Karin Zitterl-Eglseer[2], Maria Teresa Capucchio[3,4], Dorotea Ippolito[3], Francesco Gai[4], Fardad Firooznia[5], Ales Franc[6], Iveta Placha[1]

[1]*Centre of Biosciences-Slovak Academy of Sciences-Institute of Animal Physiology, Kosice, Slovak Republic*
[2]*Centre of Animal Nutrition and Welfare, University of Veterinary Medicine Vienna, Austria*
[3]*Department of Veterinary Sciences, University of Turin, Italy*
[4]*National Research Council of Italy-Institute of Sciences of Food Production, Italy*
[5]*Department of Biology, City College of New York, USA*
[6]*Department of Pharmaceutical Technology, Faculty of Pharmacy, Masaryk University Brno, Czech Republic*

Abstract

Thymol, a major compound of *Thymus vulgaris* L., exhibits strong beneficial properties related to its phenolic structure. One of the crucial aspects of its beneficial effect is the amount present in the gut as a result of its release from feed, and consequent ability to pass through the intestinal wall in each part of the gastrointestinal tract.

To improve the stability of thymol against enzymatic degradation, ensure its penetration into the intestinal mucosa, we incorporated coated self-emulsifying pellets of thymol into rabbit diets for 21 days and than was withdrawn for 7 days. The level of thymol in plasma, duodenal and jejunal wall was analysed by means of solid phase microextraction and GC/MS; while histomorphometry of different intestinal segments was investigated.

Based on thymol analyses and statistical evaluation we assume its intensive biotransformation processes with consequent absorption in duodenal wall during its application (plasma, P=220.1±81.5 ng/mL; duodenal wall, DW=752.7±208.3 ng/g dry matter-DM, jejunal wall JW=541.1 ng/g DM; Spearman´s correlation P vs DW, r_s*=0.9000*, *P=0.0833*). During the withdrawal period, thymol in P was significantly lower than in DW and JW (9.1±2.4 ng/mL vs 76.9±11.1 ng/g DM, 71.8±10.8 ng/g DM, *P<0.05*) and pointed on ongoing processes of biotransformation. Histomorphological analyses of the duodenal and jejunal wall structure showed, that they were not damaged by thymol biotransformation processes. We suppose, that intensive absorption and biotransformation of thymol in DW and JW in our experiment is a consequence of thymol application in stabilised form, which ensure its release in gastrointestinal tract with the most intensive absorption rate.

Keywords: thymol pellets, bioavailability, histomorphology, rabbit

Acknowledgement: This work was supported by VEGA 2/0009/20, 2/0005/24; CNR-SAS-2022-02, Open-Mob-2022-01, COST CA22109, Action Austria-Slovakia MPC-2023-01046

DOI: https://doi.org/10.15414/2024.9788055227443

Protective effect of mitochondria-targeted antioxidant Mito-TEMPO on rabbit sperm function during cryopreservation

Lenka Kuzelova[1,2], Andrea Svoradova[3,4], Jakub Vozaf[1], Andrej Balazi[3], Jaromir Vasicek[1,3], Peter Chrenek[1,3]

[1]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Biotechnology, Nitra, Slovak Republic
[2]Slovak University of Agriculture in Nitra, AgroBioTech Research Centre, Nitra, Slovak Republic
[3]NPPC - Research Institute for Animal Production Nitra, Lužianky, Slovak Republic
[4]Mendel University in Brno, Faculty of AgriSciences, Department of Morphology, Physiology and Animal Genetics, Brno, Czech Republic

Abstract

The freezing and thawing processes generate and accumulate high concentrations of reactive oxygen species (ROS), which negatively impact spermatozoal function. Therefore, supplementation with an exogenous source of antioxidants in the freezing diluent is crucial. The objective of this study was to examine the effectiveness of the mitochondria-targeted antioxidant Mito-TEMPO on the quality of rabbit sperm following the freezing-thawing cycles. Semen samples from six New Zealand White rabbits were pooled and cryopreserved in an extender enhanced with various concentrations of Mito-TEMPO (0, 0.5, 5, and 50 µM) across five separate repetitions. Sperm motility parameters after thawing were assessed using Computer-Assisted Sperm Analysis. Additional sperm quality parameters, including viability, apoptosis, acrosome integrity, intracellular ROS, and mitochondrial activity, were evaluated via flow cytometry. Adding 5 and 50 µM Mito-TEMPO to the extender significantly enhanced ($P \leq 0.05$) total sperm motility, progressive motility, mitochondrial activity, acrosome integrity, and viability compared with semen samples from other groups. The inclusion of the mitochondria-targeted antioxidant Mito-TEMPO in the semen extender prior to cryopreservation could improve the quality of rabbit semen after the thawing of cryopreserved samples.

Keywords: rabbit; semen; cryopreservation; Mito-TEMPO

Acknowledgments: This research was funded by the Slovak Research and Development Agency (grant no. APVV-20-0006), VEGA 1/0002/23 and INTERREG, HUSK/2302/1.2/018.

DOI: https://doi.org/10.15414/2024.9788055227443

Is Porcine Granulosa Cells' Hormone production influenced by DHA and EPA?

Martina Lecová[1], Diana Babjáková[1], Kristína Rodáková[1], Maria Romerowicz-Misielak[2], Katarzyna Kozioł[2], Drahomíra Sopková[1], Radoslava Vlčková[1]

[1]Department of Biology and Physiology, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak Republic
[2]Biotechnology Institute, Rzeszów University, Rejtana st. 16c, 35-959 Rzeszów, Poland

Abstract

Docosahexaenoic acid (DHA; 22:6) and eicosapentaenoic acid (EPA; 20:5) are ω-3 polyunsaturated fatty acids (PUFAs) that derive from α-linolenic acid (ALA; 18:3). Alpha-linolenic acid is an essential FA that cannot be synthesised by the body and must come from a diet. Long-chain FAs have been documented to have a positive effect on an animal body since they modulate several cell functions. This study aimed to determine the effect of different concentrations of DHA and EPA on the secretion of progesterone (P4), testosterone (T), oestradiol-17ß (E2), and insulin-like growth factor I (IGF-I) by porcine granulosa cells. This study was performed on isolated porcine granulosa cells that were cultivated for the first 24 hours without any additives. In the next step, the media were collected and new ones without (control group) or with the addition of DHA1 (10 µg/ml), DHA2 (1 µg/ml), DHA3 (0.1 µg/ml), EPA1 (10 µg/ml), EPA2 (1 µg/ml), and EPA3 (0.1 µg/ml) additives were used to cultivate the cells for another 24 hours. After this period, the media were collected for the analysis of the hormones using immunoassays. The concentrations of P4 increased *($P < 0.05$)* only after EPA1 addition and the concentration of E2 increased *($P < 0.01$)* only after DHA3 addition to the culture media compared to the controls. The most prominent increase was observed in the secretion of T after the addition of DHA1, DHA2, and EPA1 *(all $P < 0.01$)*. On the other hand, the secretion of IGF-I decreased after adding EPA1 and EPA2 *(both $P < 0.05$)* compared to the control group. The other concentrations of FAs revealed no significant changes in hormone secretion. These preliminary results proved that different concentrations of DHA and EPA stimulated steroidogenesis in the porcine granulosa cells, mostly androgen production while suppressing the secretion of IGF-I (the effect of EPA). It may be assumed that the long-chain FAs support androgen production and suppress IGF-I production in antral follicles that may lead to apoptosis of porcine granulosa cells.

Keywords: porcine, granulosa cells, DHA, EPA, hormones

Acknowledgments: This work was supported by the projects VEGA 1/0414/23 and APVV-22-0071.

DOI: https://doi.org/10.15414/2024.9788055227443

Determination of beehive by-product on motility characteristics of stallion spermatozoa *in vitro*.

Michal Lenický[1], Ewlina Sidor[2], Lucia Dianová[1], Marko Halo[1], Tomáš Slanina[1], Denis Bažány[1], Nikola Štefunková[1], Małgorzata Dżugan[2], Peter Massányi[1]

[1]Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Institute of Applied Biology, Nitra, Slovak Republic
[2]Department of Chemistry and Food Toxicology, Institute of Food Technology and Nutrition, University of Rzeszów, Ćwiklińskiej 1a St., 35-601 Rzeszów, Poland

Abstract

The beneficial effects of beehive products have been known for thousands of years. In addition to honey, propolis or royal jelly, the beehive also contains drone brood. The homogenised drone brood (DB) are rich in proteins, vitamins, bioelements, hormones and they have antioxidant activity. The aim of our study was to determine the effect of drone brood (DB) on motility characteristics of stallion spermatozoa under *in vitro* conditions. From drone brood homogenates were prepared solutions (0.9% NaCl) with DB concentrations of 0.25, 0.5, 1.0, 2.0 and 4.0 mg.mL^{-1}. Ejaculate was diluted 1:3 into the solutions. Pure physiological solution served as a control. Computer-assisted sperm analysis (CASA system) was used to determine total motility (MOT, %), progressive motility (PRO; %), velocity curved line (VCL, µm/s) and distance curved line (DCL, µm) at 0h, 1h and 3h of incubation at 37°C. A concentration of 4 mg.mL^{-1} caused an increase in MOT *(P<0.05)* compared to control after 1h. After 3h, a significantly higher *(P<0.0001)* MOT was observed in all groups compared with control. Significantly higher PRO compared to control was observed after 1h at concentrations of 0.5, 1.0, 2.0 *(P < 0.05)* and 4.0 mg.mL^{-1} *(P<0.001)*. Similarly, after 3h, all groups showed significantly higher *(P<0.0001)* PRO compared to control. The DCL parameters showed a similar trend when we observed significantly higher values in all concentrations after 1h and 3h, compared to the control. Significantly higher VCL compared to control was observed across all experimental groups at 0h, 1h and 3h. Our results suggest that DB homogenate has a beneficial effect on percentage of total and progressive motility especially at 4.0 mg.mL^{-1}. The spermatozoa moved faster and overcame a longer distance.

Keywords: drone brood, motility, spermatozoa, stallion

Acknowledgement: The research was financially supported by projects VEGA 1/0698/22, 1/0083/21, APVV-16-0289, APVV-21-0168 and KEGA 035SPU-4/2023.

DOI: https://doi.org/10.15414/2024.9788055227443

Dose-dependent effect of *Trigonella foenum-graecum L*. microgreens on angiogenesis in HUVEC cells *in vitro*

Norbert Lukac[1], Bazany Denis[1], Hana Greifova[1], Nikola Stefunkova[1], Agnieszka Gren[2], Tomas Jambor[1]

[1]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Applied Biology, Nitra, Slovak Republic
[2]University of the National Education Commission, Faculty of Exact and Natural Sciences, Institute of Biology and Earth Sciences, Cracow, Poland

Abstract

Microgreens are a new class of speciality crop, defined as tender immature greens produced from the seeds of vegetables, herbs, or grains including wild species. They are harvested at the soil/substrate level upon the appearance of the first pair of true leaves, when cotyledons are fully expanded and still turgid, usually within 7 – 21 days from seed germination. Their current popularity stems from their vivid colours, delicate texture, unique flavour, and fortified phytonutrient content followed by a significant bioactive potential. *Trigonella foenum-graecum L.* is a leguminous annual plant extensively used in Ayurvedic medicines for anti-diabetic, antiseptic and anti-inflammatory benefits. At the same time, there is strong evidence that *Trigonella* microgreens cultivation may increase the content of biologically active compounds and promote their health benefits effects. Our *in vitro* study examined the effect of *Trigonella foenum-graecum L*. microgreens on angiogenesis in human umbilical vein endothelial cells (HUVEC). The experimental cell line was cultured in the presence of different concentrations of *Trigonella* microgreens (from 10 µg/mL to 300 µg/mL) for 24 h. Afterwards, interleukin 8 (IL-8) as a potent promoter of angiogenesis was evaluated by the ELISA method. The results of the present study confirmed that lower concentrations of experimental microgreens (10, 50 and 100 µg/mL) significantly ($p<0.01$) stimulate the release of proangiogenic factor IL-8. Overleaf, higher applied concentrations including 150, 200, 250 and 300 µg/mL significantly ($p<0.001$; $p<0.05$) inhibited the presented parameter.

Keywords: *Trigonella foenum-graecum L.,* HUVEC, angiogenesis

Acknowledgments: The research was financially supported by Slovak research and development agency APVV-SK-PL-23-0037, by the grant of Slovak University of Agriculture in Nitra 07-GASPU-2021, and by the Scientific Agency of the Slovak Republic VEGA No. 1/0083/21. This publication was also created with the support of the Research and Innovation operational program for the project: Support of research activities in VC ABT, 313011T465, co-financed from the resources of the European Regional Development Fund.

DOI: https://doi.org/10.15414/2024.9788055227443

Determination of potential impact of *Trigonella foenum graecum L.* on progressive motility and DNA fragmentation in bovine spermatozoa *in vitro*

Norbert Lukac[1], Michal Ďuračka[2], Eva Tvrdá[3], Tomas Jambor[1]

[1]*Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Applied Biology, Nitra, Slovak Republic*
[2]*AgroBioTech Research Centre, Slovak University of Agriculture in Nitra, Slovak Republic*
[3]*Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Biotechnology, Nitra, Slovak Republic*

Abstract

Trigonella foenum graecum L. is frequently used in traditional medicine and as a natural additive food thanks to its significant anti-atherogenic, antidiabetic, antianorexic, antioxidant, anticarcinogenic, antihyperlipidemic, and anti-inflammatory effects, as confirmed by several human and animal studies. The modern cultivation of *Trigonella,* as a microgreen, has enhanced its exceptional properties and stimulated the content of biologically active substances that can positively influence the reproductive system of males. The present *in vitro* study evaluates the potential impact of *Trigonella* microgreens extract on bovine spermatozoa motility and DNA fragmentation after 2 h and 24 h cultivation. The progressive motility of bovine spermatozoa was quantified by computer-assisted sperm analysis (CASA), followed by chromatin-dispersion test to evaluate for DNA fragmentation. The obtained results suggest that progressive motility was not significantly affected after 2 h exposure. Overleaf, prolonged time of cultivation significantly ($p<0.05$) affects the presented parameters at 1 µg/mL and 10 µg/mL. In the case of DNA fragmentation, prolonged time of cultivation did not cause significant changes in this parameter.

Keywords: *Trigonella foenum-graecum L.,* spermatozoa, progressive motility, DNA integrity

Acknowledgments: The research was financially supported by Slovak research and development agency APVV-SK-PL-23-0037, by the grant of Slovak University of Agriculture in Nitra 07-GASPU-2021, and by the Scientific Agency of the Slovak Republic VEGA No. 1/0083/21. This publication was also created with the support of the Research and Innovation operational program for the project: Support of research activities in VC ABT, 313011T465, co-financed from the resources of the European Regional Development Fund.

DOI: https://doi.org/10.15414/2024.9788055227443

Impact of *Aronia melanocarpa* on the female hormonal profile

Katarina Majerik Behinska[1], Olha Balytska[1], Jana Kopcekova[2], Jana Mrazova[2], Adriana Kolesarova[1,3]

[1]*Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Institute of Applied Biology, Nitra, Slovak Republic*

[2]*Slovak University of Agriculture, Faculty of Agrobiology and Food Resources, Institute of Nutrition and Genomics*

[3]*Slovak University of Agriculture, AgroBioTech Research Centre, Nitra, Slovak Republic*

Abstract

Environmental burdens and risk factors within the food chain heighten the risk of reproductive disorders. Natural compounds in fruits like *Aronia melanocarpa* can modulate critical aspects of steroidogenesis in the female reproductive system and mitigate adverse environmental influences. This study aimed to investigate the impact of chokeberry for 4 and 8 weeks on the hormonal profiles of 9 postmenopausal women. We focused on assessing adenohypophyseal hormones (FSH, PRL, LH, TSH), steroid hormones (progesterone, 17ß-estradiol, and testosterone), and thyroid hormone (T4) levels using the ELISA method. Our findings revealed no statistically significant differences in the secretion of adenohypophyseal hormones, specifically FSH, TSH, and LH, between the aronia-treated groups and the control group after both 4 and 8 weeks of consumption. However, a notable decrease in the secretion of 17ß-estradiol ($P \leq 0.001$) and testosterone ($P \leq 0.05$) was observed after 8 weeks of chokeberry exposure. Furthermore, prolactin levels decreased significantly after both 4 and 8 weeks of treatment ($P \leq 0.05$). Progesterone and thyroid hormone T4 levels remained unaffected throughout the study period. These results highlight chokeberry's potential to modulate female hormone levels, underscoring the need for further research to elucidate its mechanisms and broader health impacts.

Keywords: Aronia, polyphenols, steroidogenesis, reproduction, hormones

Acknowledgement: This work was supported by the Slovak Research and Development Agency under the contract no. APVV-18-0312, VEGA 1/0620/24, amd KEGA 035SPU-4/2023.

DOI: https://doi.org/10.15414/2024.9788055227443

The effect of culture system on the *in vitro* production of embryos from fresh and vitrified cattle oocytes

Alexander Makarevich[1], Linda Dujíčková[1], Lucia Olexiková[1]

[1]National Agricultural and Food Centre (NPPC), Research Institute for Animal Production Nitra, 95141 Lužianky, Slovak Republic

Abstract

Successful protocols of *in vitro* embryo production (IVP) presuppose sufficient yield of preimplantation embryos at the blastocyst stage. Usually, two culture systems are used for IVP of bovine embryos: culture at atmospheric oxygen content (20% O_2; AOC) or culture at reduced oxygen content (5% O_2; ROC). AOC system requires conventional CO_2 incubator and co-culture with a feeder cells (embryonal fibroblasts, oviductal cells, Vero cells, BRL cells or others), what involves additional procedure of passaging and maintaining these cells in continuous culture at monolayer. ROC system requires a special incubator providing reduced oxygen conditions and an additional nitrogen bomb for pushing out excess oxygen from the gas mixture. The aim of this study was to examine, which of these two culture systems can provide higher yield of bovine blastocysts after in vitro fertilization of fresh or vitrified oocytes. Oocytes (n= 1040), aspirated from slaughterhouse cow ovaries, were matured *in vitro* (IVM; M199, 10% FBS, 0.25 mmol·L^{-1} sodium pyruvate, 50 µg/mL gentamicin, 1 I.U FSH/LH (Pluset)) at 38.5 °C and 5% CO_2. Part of them were vitrified in M199 medium with 30 % ethylene glycol, 1 M sucrose and 10% FBS in minimum volume on the electron microscopy grids by ultra-rapid vitrification technique. Afterwards, both fresh (n= 558) and vitrified (n= 482) oocytes were fertilized *in vitro* (IVF) using frozen bull semen. After IVF presumptive zygotes were incubated either in a B2 Menezo medium with 10% FBS, 10 mg/mL BSA, 50 µg/mL gentamicin on a monolayer of BRL-1 (Rat epithelial cells; ECACC, UK) cells in a conventional CO_2 incubator at 38.5 °C, 5% CO_2 and 20% O_2 (**AOC; n= 568)**, or in IVC medium (ivf bioscience) in a ESCO MIRI incubator at 38.5 °C, 6.1% CO_2 and 5% O_2 (**ROC; n= 472**) until the blastocyst stage (8 days). Experiments were performed in 5 replicates. Zygotes, obtained from fresh oocytes, reached higher cleavage (76.72%) and blastocyst (39.34%) rates $p < 0.05$), when cultured in ROC, compared to AOC (65.6% and 29.25%, resp.). Similar trend was also observed in vitrified oocytes, where cleavage (71.9%) and blastocyst (22.8%) rates were significantly higher ($p < 0.05$) under reduced oxygen conditions than in atmospheric oxygen content (56.2% and 14.0%, resp.). In conclusion, the low O_2 culture system in ESCO incubator appeared to be more efficient in blastocyst production than the system of co-culture with feeder cells in a conventional CO_2 incubator. This observation is important mainly for IVP of embryos from cryopreserved oocytes, which are particularly sensitive to culture conditions.

Keywords: bovine, oocyte, embryo, culture, vitrification, reduced oxygen

Grant support: *Slovak Research and Development Agency, grant no. APVV-19-0111.*

DOI: https://doi.org/10.15414/2024.9788055227443

Is obesity beneficial for bone health?

Monika Martiniakova[1], Roman Biro[1], Noemi Penzes[1], Anna Sarocka[1], Veronika Kovacova[1], Vladimira Mondockova[1], Radoslav Omelka[1]

[1]*Faculty of Natural Sciences and Informatics, Constantine the Philosopher University in Nitra, Slovak Republic*

Abstract

Current studies suggest that adipose tissue can negatively affect bone health, challenging the traditional paradigm that increased fat mass is beneficial for the skeleton. Various molecular pathways by which adipose tissue communicates with bone tissue have been proposed. This interplay is active and dynamic and involves multiple factors such as hormones (e.g. leptin, adiponectin, resistin), pro-inflammatory cytokines (e.g. TNF-α, IL-6, CRP), vitamin D. In addition, bone tissue influences metabolic parameters, including body weight control through bone-derived factors (e.g. osteocalcin, osteopontin). In general, biochemical markers of bone turnover are lower in obese individuals compared to lean subjects. This difference appears to be more relevant for markers of bone resorption than those of bone formation. Despite the fact that obese adults have higher BMD and a lower risk of some fractures (e.g. hip, pelvis, wrist), several site-dependent fractures (e.g. humerus, ankle, upper leg, elbow, vertebrae, rib) are common in obesity. Moreover, the prevalence of low-trauma fractures is similar in obese and non-obese individuals. The pathophysiology of fractures in obese adults is not fully understood, but an elevated risk of falls, various patterns of falling, and unfavorable impacts of adipose tissue on bone tissue are likely contributing factors. Cytokines from visceral fat are known to be pro-resorptive and high intramuscular fat is associated with poorer muscle function, moderates the effect of loading, and increases the risk of falling. In an increasingly obese and aging population, understanding the interactions between obesity and bone fractures becomes an urgent need to reduce health care expenditures.

Keywords: obesity, adipose tissue, bone tissue, bone health, fractures

Acknowledgement: This work was supported by the Ministry of Education, Research, Development and Youth of the Slovak Republic, grant numbers KEGA 034UKF-4/2022, KEGA 012UKF-4/2023.

DOI: https://doi.org/10.15414/2024.9788055227443

The effect of zinc nanoparticles on the spermatozoa parameters in vitro

Peter Massányi, Barbora Remeňová, Marko Halo, Tomáš Slanina, Lucia Dianová, Michal Lenický, Filip Tirpák, Jaroslav Kováčik

Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Institute of Applied Biology, Nitra, Slovak Republic

Abstract

Zinc is an essential element that is important for the proper functioning of the body. In addition to being part of various biological processes, it plays an irreplaceable role in the proper course of spermatogenesis, testicular development, spermatozoa capacity during fertilization, and affects spermatozoa motility. In recent decades, nanoparticles have become very popular in various fields of industry or medicine, mainly due to their size (1 – 100 nm) and physico-chemical properties. In this study, we investigated the effect of eight different concentrations of zinc nanoparticles on stallion spermatozoa parameters in vitro, using CASA analysis. Also, the overall viability of the spermatozoa using a mitochondrial toxicity test was determined. All measurements were performed in time intervals 0, 30, 60 and 90 minutes from the collection. All experimental samples were compared with a control sample containing saline only. The experimental samples contained semen diluted with saline, while each sample also contained an addition of zinc nanoparticles at various concentrations ranging from 0.01171875 mg/ml to 24 mg/ml. As part of the evaluation of the MTT result, any significant decrease or increase in viability compared to the control sample was found. Evaluation of spermatozoa motility and progressive motility recorded a significant decrease in samples with the highest concentration (12 and 24 mg/ml). A similar result with a negative effect in the samples with the highest concentration for both distance and velocity parameters were recorded. The results show that high concentrations of zinc nanoparticles (12 and 24 mg/ml) have a negative effect on spermatozoa parameters, but lower concentrations have a positive, although not statistically significant effect.

Keywords: spermatozoa, stallion, zinc, nanoparticles

Acknowledgement: This work was supported by the Slovak Research and Development Agency under the contract No. APVV-16-0289, APVV-21-0168, and projects VEGA 1/0698/22, KEGA 035SPU-4/2023, and by the Grant Agency of SUA in Nitra project No. 20-GASPU-2021. This publication was also created with support within the Operational Program Research and Innovation for the project: Support of research activities in the field of animal production, 313011U414, co-financed from the resources of the European Regional Development Fund.

DOI: https://doi.org/10.15414/2024.9788055227443

Isorhamnetin and its effect on human ovarian *stratum granulosum* tumor cells *in vitro*

Michal Mihal[1], Katarina Majerik Behinska[1], Olha Balytska[1], Adriana Kolesarova[1,2]

[1]*Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Institute of Applied Biology, Nitra, Slovak Republic*

[2]*AgroBioTech Research Centre, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic*

Abstract

Isorhamnetin is a flavonoid, which is present in a wide variety of fruits and vegetables for example onions, gingko, tea and sea buckthorn. It is well known that isorhamnetin can stimulate cell proliferation and increase the total antioxidant capacity of the cells and also can decrease cell survival and viability of tumor cells. The aim of this study was to determine the effect of isorhamnetin at concentrations 5; 10; 20; 40; 80 µmol/mL (24 h) on the viability of human ovarian granulosa tumor cell line (COV434), as well the secretion of transforming growth factor beta-2 (TGF-β2) and the presence of its receptor (TGF-β2R), markers of proliferation and apoptosis (Bcl-2, Bax) and apoptosis-inducing factor (AIF). Cell viability was evaluated by AlamarBlue™ cell viability assay, the growth factor production, and the presence of their receptors was assayed by ELISA methods, and Bax and Bcl-2 expression was tested by western blot. Our results confirmed the effect of isorhamnetin on cell viability. Isorhamnetin significantly (P≤0.001) decreased the viability of COV434 (20; 40; 80 µmol/mL). Isorhamnetin had no significant effect on TGF-β2, TGF-β2R, Bax, Bcl-2 and AIF in COV434 cells but confirmed our hypothesis also in comparison with other authors of the available literature, when it showed a tendency to increase Bax and decrease anti-apoptotic protein Bcl-2 at the highest concentration (80 µg.ml^{-1}) of isorhamnetin. Based on these findings, we can suggest a potential positive effect of flavonoids such as isorhamnetin, on reproductive health. However, further research is needed for a better understanding of the therapeutic potential of isorhamnetin.

Keywords: isorhamnetin, ovarian cells, steroidogenesis, growth factors, tumor

Acknowledgement: This work was supported by the projects of the Ministry of Education, Science, Research and Sport of the Slovak Republic APVV-18-0312, APVV-21-0206, VEGA 1/0620/24, KEGA 035SPU-4/2023 and the Operational Programme Integrated Infrastructure for the project: demand-oriented research for sustainable and innovative food, Drive4SIFood 313011V336, co-financed by the European Regional Development Fund.

DOI: https://doi.org/10.15414/2024.9788055227443

The inhibitory effect of alfacalcidol on mineralisation in primary rat osteoblast cell culture

Vladimira Mondockova[1], Veronika Kovacova[1], Roman Biro[1], Noemi Penzes[1], Monika Martiniakova[1], Radoslav Omelka[1]

[1]*Constantine the Philosopher University in Nitra, Faculty of Natural Sciences and Informatics; Slovakia*

Abstract

The active metabolite of vitamin D (1α,25$(OH)_2D_3$) is an important regulator of bone cell activity with a significant role in osteoblast differentiation and mineralisation. The synthetic vitamin D analogue alfacalcidol (1α$(OH)D_3$) has been used for treatment of different diseases. However, previous researches pointed to a different behaviour of osteoblasts in regulating bone mineralisation in response to vitamin D in vitro compared to in vivo conditions. Moreover, pharmacokinetic studies suggest that 1α$(OH)D_3$ may have an effect independent of the plasma-1,25$(OH)_2D_3$ levels. This study aims to evaluate the impact of vitamin D on the production of calcium deposits in rat osteoblasts, which indicates successful bone formation in vitro. Primary rat osteoblastic cells were seeded in 6-well plates and after reaching 80% of confluence, they were treated every 3rd day with different concentrations (10, 50, 100 nM) of alfacalcidol. Alfacalcidol was dissolved in osteogenic medium (growth medium supplemented with 5 mM β-glycerophosphate, 10 nM dexamethasone, and 50 µg/mL L-ascorbic acid). After 21 days, cells were fixed with 10% formalin solution and stained by bright orange-red Alizarin Red S (ARS, 2%). For calcium quantification, ARS staining quantification assay was used. Mineralisation was assessed by extraction of calcified mineral at low pH (10% acetic acid) and subsequently neutralised with ammonium hydroxide. The colorimetric detection was carried out spectrophotometrically at 405 nm in a 96-well format. Based on the results, the mineralisation process was significantly reduced by all concentrations of alfacalcidol, suggesting that this synthetic analogue could have an inhibitory effect on bone formation, similar to the known effect of active metabolite of vitamin D in vitro. Further studies are needed to reveal possible mechanisms of independent action of alfacalcidol in target cells, including bone cells.

Keywords: alfacalcidol, osteoblasts, mineralisation

Acknowledgement: This study was supported by the grants KEGA 034UKF-4/2022 and KEGA 012UKF-4/2023.

DOI: https://doi.org/10.15414/2024.9788055227443

Analysis of estrogen receptors expression in the gastrointestinal tract of the domestic chicken at various stages of development

Zuzanna Mucha[1], Agnieszka Greń[1], Marcin Lis[2], Jacek Kieżun[3], Grzegorz Formicki[1]

[1]*University of the National Education Commission, Institute of Biology and Earth Sciences, Department of Animal Physiology, Kraków, Poland*
[2]*University of Agriculture, Faculty of Animal Science, Department of Zoology and Animal Welfare, Kraków, Poland*
[3]*University of Warmia and Mazury, Medical Faculty, Department of Human Histology and Embryology, Olsztyn, Poland*

Abstract

The effect of estrogen on the motor activity of the mammalian gastrointestinal tract is currently the subject of scientific discussion. So far, their occurrence has been confirmed in the muscles that build the esophagus, stomach and intestines of these animals. The action of these hormones occurs through their interaction with estrogen receptors present in the smooth muscles of the gastrointestinal tract. However, little is known about the presence of estrogen receptors in the gastrointestinal tract of birds. Moreover, there is no mention in the literature of the expression of these receptors in the gastrointestinal tract of animals during their embryonic development. The aim of the study was to investigate the expression of estrogen receptor alpha and estrogen receptor beta in the muscles of the bird's digestive tract. According to the hypothesis, the expression of estrogen receptors may begin during or after the embryonic development of the bird. Therefore, embryos of domestic hens (*Gallus gallus domesticus* L.) on the 7th, 10th, 14th and 18th days of development and one-day-old chicks were used as a research model. An attempt was made to determine estrogen receptors in histological material depicting sections of the gastrointestinal tract at selected stages of development. Immunohistochemical staining methods were used for this purpose, using antibodies specific for each type of estrogen receptor. The method used allowed for precise imaging of the location of estrogen receptors in the tissues studied. Consideration of the different stages of development of the chicken embryo allowed us to analyze changes in the expression of estrogen receptors in the gastrointestinal muscles as development progressed.

Keywords: estrogen receptor, embryonic development, *Gallus gallus domesticus*, immunohistochemistry

DOI: https://doi.org/10.15414/2024.9788055227443

Evaluation of the antioxidant potential of cannabidiol in reducing oxidative stress in chicken embryonic liver tissue

Renata Muchacka[1], Edyta Kapusta[1], Agnieszka Greń[1], Zofia Goc[1], Grzegorz Formicki[1]

[1]*University of the National Education Commission, Department of Animal Physiology, Krakow, Poland*

Abstract

Today, many factors can cause an imbalance between the production and neutralisation of free radicals, leading to oxidative stress in organisms. For this reason, there is growing interest in researching substances with antioxidant potential to provide additional protection against RFTs. One of these is cannabidiol, a compound belonging to the phytocannabinoids, extracted from hemp seed (*Cannabis sativa* L.). The aim of the present study was to evaluate the antioxidant properties of CBD in chicken embryo liver tissues. The *in ovo* experiment was carried out on eggs of broiler chickens of the Ross 308 line, previously incubated. The experimental material was divided into 5 experimental groups: group I - control without pricking and injection, group II - into which the carrier alone was injected, and groups III, IV, V subjected to injection of cannabidiol together with the carrier at concentrations of 2 ppm, 10 ppm and 20 ppm, respectively. On day 14 of incubation after embryos were sacrificed, livers were collected for assays. Reduced glutathione and malondialdehyde levels were determined as oxidative-antioxidant indices. The analysis showed an increase in GSH values in liver tissue in each of the designated study groups compared to the control group, but the differences obtained were not statistically significant. In contrast, the results obtained for MDA showed a reduction in malondialdehyde levels in all study groups compared to the control. The administration of cannabidiol concentrations of 10 ppm and 20 ppm produced statistically significant differences. These results confirm that cannabidiol causes a reduction in lipid peroxidation and that it has an antioxidant-supporting effect on the antioxidant system. The interesting biological properties of cannabidiol offer prospects for exploiting its potential against oxidative stress-related diseases. However, further studies are needed to confirm the therapeutic properties on humans.

Keywords: CBD, chicken embryo, liver, GSH, MDA

DOI: https://doi.org/10.15414/2024.9788055227443

Dose-dependent effect of vitamin K2 on biochemical and microstructural parameters of bone in ovariectomized rats

Radoslav Omelka[1], Martina Babikova[1], Veronika Kovacova[1], Vladimira Mondockova[1], Roman Biro[1], Noemi Penzes[1], Karol Svik[2], Marta Soltesova-Prnova[2], Juraj Vozar[3], Monika Martiniakova[1]

[1] Faculty of Natural Sciences and Informatics, Constantine the Philosopher University in Nitra, Slovakia
[2] Institute of Experimental Pharmacology and Toxicology, Centre of Experimental Medicine of the Slovak Academy of Sciences, Bratislava, Slovakia
[3] Pharmaceutical company BIOMIN, a.s., Cífer, Slovakia

Abstract

Vitamin K2 (VK2) is used in some countries in the prevention of osteoporosis and bone loss, especially with vitamin D3 (VD3). However, routine VK2 supplementation is not commonly recommended in postmenopausal osteoporosis, as many studies have provided contrasting findings. Our study investigated the impact of VK2 (menaquinone-7) individually at different doses and in combination with VD3 (alfacalcidol) on ovariectomy-induced bone loss in rats. Adult female rats were divided into 6 groups: sham-operated; ovariectomized (OVX) untreated; OVX treated with VK2 in doses of 4 µg/rat (K2-4) and 8 µg/rat (K2-8); OVX simultaneously receiving VD3 (0.2 µg/kg bw) and VK2 in doses of 4 µg/rat (D3K2-4) and 8 µg/rat (D3K2-8) during 8 weeks. Plasma calcium and phosphate were increased in groups D3K2-4 and D3K2-8 versus OVX; higher plasma calcium was detected also in K2-4 and K2-8 groups. In K2-4 and D3K2-4 groups, reduced bone turnover was observed versus OVX group. Higher doses of VK2 (K2-8 and D3K2-8 groups) decreased only bone resorption. Increased trabecular relative bone volume (accompanied by an increase in trabecular number) and bone surface were determined in groups D3K2-4 and D3K2-8 versus OVX. In group D3K2-4, elevated trabecular thickness and trabecular bone mineral density were reported. In the cortical bone, an enhanced periosteal bone apposition was identified in all VK2 (including VD3) groups. An increased area of secondary osteons was found in K2-4 and D3K2-4 groups versus OVX. Presented results indicate the beneficial effect of a lower dose of VK2, especially in combination with VD3, on biochemical and microstructural parameters of bone in OVX rats. This study was supported by the grants KEGA 034UKF-4/2022 and KEGA 012UKF-4/2023.

Keywords: vitamin K2, vitamin D3, osteoporosis, bone microstructure, ovariectomized rats

DOI: https://doi.org/10.15414/2024.9788055227443

Breed-specific blood parameters reference interval variations in cattle

Ales Pavlik[1], Andrej Batik[1], Petr Vevera[1], Petr Slama[1]

[1]Mendel University in Brno, Faculty of AgriSciences, Department of Animal Morphology, Physiology and Genetics, Zemedelska 1, 613 00 Brno, Czech Republic

Abstract

Internal environment of cattle is crucial for their health and performance, with blood parameters serving as key indicators. Factors such as breed, sex, and environmental conditions influence these parameters. Breed-specific differences may arise due to genetic predispositions and selection for specific traits. Sex hormones also impact blood parameters differently in cows and bulls. Environmental factors like feed quality and stress levels further affect cattle's internal environment. Considering these factors is crucial for accurate interpretation of blood tests and effective cattle care, as normal ranges vary accordingly. Researchers revealed significant variations in blood parameters among different cattle breeds, including hematocrit, hemoglobin, glucose, cholesterol, urea, creatinine, proteins, and minerals. Understanding breed-specific considerations is essential for interpreting blood test results and monitoring cattle health effectively. Reference ranges (intervals) play a critical role in assessing cattle health, established through statistical analysis of healthy individuals under standardized conditions. Regular monitoring within these ranges enables early detection of health issues and effective management practices in cattle production, though interpretation should consider clinical signs and diagnostic findings. Ongoing research is necessary to further understand breed-related variations and improve cattle health management.

In the assessment of the health status of Aberdeen Angus and Gasconne cattle, which was conducted as part of this experiment, and monitored from birth to pregnancy in heifers and to slaughter in bulls, it's observed that certain blood parameters deviate from the reference intervals established for cattle. These deviations necessitate a nuanced approach when evaluating the health of these breeds.

Keywords: beef cattle, internal environment, reference range

Acknowledgement: Present study was supported by the project of National Agency for Agricultural Research no. QH 71156.

DOI: https://doi.org/10.15414/2024.9788055227443

The impact of metformin and sea buckthorn on biochemical and macroscopical parameters of femoral bone in ZDF rats

Noemi Penzes[1], Roman Biro[2], Veronika Kovacova[2], Vladimira Mondockova[1], Radoslav Omelka[1], Anna Kalafova[3], Marcela Capcarova[3], Monika Martiniakova[2]

[1]*Constantine the Philosopher University, Faculty of Natural Sciences and Informatics, Department of Botany and Genetics, Nitra, Slovak Republic*
[2]*Constantine the Philosopher University, Faculty of Natural Sciences and Informatics, Department of Zoology and Anthropology, Nitra, Slovak Republic*
[3]*Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Nitra, Slovak Republic*

Abstract

Type 2 diabetes mellitus (T2DM) is associated with serious secondary complications, including diabetic bone damage. Metformin is an oral anti-diabetic drug for the treatment of T2DM, but some reports indicate its adverse effects. Sea buckthorn is a rich source of bioactive substances with increased antioxidant activity; therefore, it could alleviate the progression of T2DM. In the present study, the impact of metformin and sea buckthorn applied individually and simultaneously on biochemical and macroscopical parameters of femoral bone was investigated in ZDF rats, which were divided into 4 groups: C (diabetic control), E1 (metformin application, 150 mg/kg bw/day), E2 (sea buckthorn application, 500 mg/kg bw/day), E12 (simultaneous application, 150 mg/kg bw/day of metformin + 500 mg/kg bw/day of sea buckthorn) for 3 months. Significantly decreased levels of triglycerides were observed in the E12 group compared to the C and E1 groups. Conversely, the levels of glucose, insulin, alkaline phosphatase, and cholesterol were not affected by metformin and sea buckthorn treatment. Supplementation with sea buckthorn leads to increased body weight in ZDF rats. No significant differences were recorded for femoral weight and length between the analysed groups. In conclusion, simultaneous treatment with metformin and sea buckthorn alleviated hypertriglyceridemia. Sea buckthorn supplementation increased the body weight of ZDF rats. Therefore, sea buckthorn could be used as a food supplement to alleviate T2DM-related complications.

Keywords: metformin, sea buckthorn, biochemical parameters, macroscopical parameters, diabetic bone damage, ZDF rats

Acknowledgement: Supported by the Ministry of Education, Research, Development and Youth of the Slovak Republic, grant numbers: VEGA 1/0416/22 and VEGA 1/0328/24.

DOI: https://doi.org/10.15414/2024.9788055227443

Effect of meadow grassland and chicory on growth performance and rumen microbiota in grazing lambs with endoparasites

Daniel Petrič[1], Klára Mikulová[1], Dominika Batťányi[1], Michaela Komáromyová[2], Marián Várady[2], Zora Váradyová[1]

[1]*Institute of Animal Physiology of Centre of Biosciences, Slovak Academy of Sciences, Šoltésovej 4-6, SK-040 01 Košice, Slovakia*
[2]*Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice, Slovakia*

Abstract

Most natural areas represent a plant species diversity that are nutritional centers and pharmacies with a huge amount of primary (nutrients) and secondary (pharmaceutical) compounds vital to the nutrition and health of ruminants. Bioactive substances of plant origin (polyphenols) can be beneficial for ruminants by fighting against gastrointestinal parasites and bacteria, and at the same time can also improve nutrition. Grazing ruminants are at constant risk of getting infected by larvae of parasitic gastrointestinal nematodes (GIN). In small ruminants, GIN diseases are mainly caused by the parasite *Haemonchus contortus*, whose adult individuals live attached to the wall of the abomasum and feed on the host's blood. The ruminal microbiome is responsible for the dietary and metabolic needs of ruminants that can be influenced by several host factors such as age, breed, disease, infection, and feed. Changes in the composition of nutrients alter the composition of the ruminal microbiota and its enzymatic activity. Our objective was to determine the growth performance and ruminal microbiota and fermentation in GIN-infected lambs during 145 days of grazing on two meadow pastures containing a mixed plant species diversity (CON) and experimentally sown chicory (CHIC). Sixteen Improved Valachian lambs aged 3–4 months with an average weight of 13.6 ± 0.52 kg were divided into two groups (n = 8/group): control animals grazing on the CON plot and experimental animals grazing on the CHIC plot. Lambs were infected orally with 5000 third-stage larvae of *H. contortus*. Mean body weight and cumulative gain in live weight were higher in the CHIC group as compared to the CON group by day (D) D90 ($P < 0.001$), D132 ($P < 0.05$), and D145 ($P < 0.05$). Total bacterial populations and relative abundance of *Streptococcus bovis* were significantly higher ($P < 0.05$) for the CHIC group than the CON group. The specific microbial enzymatic activity of amylase and xylanase was significantly higher for CHIC than CON. In the CHIC group, the microbiota was likely more associated with amylase in the particulate fraction. Similarly, the xylanase activities accelerated xylan biodegradation to volatile fatty acids and gases during the rumen processing of lignocellulosic biomass. It can be concluded that meadow grasslands with mixed plant species diversity and chicory improved growth performance and can affect ruminal fermentation and microbiota of lambs with endoparasitosis.

Keywords: ruminal fermentation, enzymatic activity, *Haemonchus contortus,* bioactive compounds

Acknowledgment: This study was supported by funds from the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences (VEGA 2/0008/21).

DOI: https://doi.org/10.15414/2024.9788055227443

Effect of fipronil maternal exposure on further development of mouse offspring

Jozef Pisko[1], Kamila Fabianová[2], Zuzana Šefčíková[1], Dušan Fabian[1]

[1]Centre of Biosciences of the Slovak Academy of Sciences, Institute of Animal Physiology, Šoltésovej 4-6, 04001 Košice, Slovakia

[2]Biomedical Research Center, Institute of Neurobiology, Slovak Academy of Sciences, Šoltésovej 4-6, 040 01 Košice, Slovakia

Abstract

Fipronil is a highly effective broad-spectrum insecticide, widely used as a veterinary pest control agent. It belongs to the phenyl-pyrazole class of chemicals and is a potent disruptor of the insect central nervous system through interference with the gamma-aminobutyric acid (GABA) regulated chloride channels. It came to broad public attention in 2017, when fipronil-contaminated eggs were found in several European countries, despite its prohibited use in food-producing animals.

The aim of the current study was to evaluate the effect of fipronil on mice offspring after the 4-day maternal exposure. Fipronil was orally administered to naturally fertilized female mice during preimplantation period in two concentrations: **0.9 mg/kg b.w. (F1)** and **0.009 mg/kg b.w. (F2)** , which is the acute reference dose according to the WHO and the dose with a previously proven negative effect on the quality of preimplantation embryos.

From a metabolic point of view, we noted significantly increased body weight in 2-day-old offspring, and body fat and lean body mass in 45-day-old offspring in the F2 experimental group. In offspring exposed to higher concentrations of fipronil (F1 group), we noticed a significant increase in body weight on days 21 and 45, as well as in body fat on day 45. Moreover, we observed delayed eye opening and an earlier onset of first estrus in the F1 experimental group in comparison with controls.

In order to explore the locomotor activity and the anxiety-related behavior in mouse offspring obtained from fipronil treated dams, we used the open field test. The locomotion was reduced in both F1 and F2 experimental animals in comparison with controls, but this decrease was significant only in F1 experimental group. For assessment of depressive-like behavior in fipronil exposed mice, the forced swimming test was employed. Significant increase in level of depression was evident in both experimental (F1 and F2) groups in comparison with controls.

We can conclude that maternal exposure to fipronil during the preimplantation period has various negative effects on their offspring even at the acute reference dose.

Keywords: mouse, offspring, *in vivo,* fipronil

Acknowledgement: The research was financially supported by the Slovak Academy of Sciences project VEGA 2/0041/23 and by the Slovak Research and Development Agency project APVV-22-0071.

DOI: https://doi.org/10.15414/2024.9788055227443

Potential role of plants used in traditional medicine in ovarian steroidogenesis

Shubhadeep Roychoudhury[1], Petr Slama[2], Adriana Kolesarova[3,4]

[1]*Department of Life Science and Bioinformatics, Assam University, Silchar, India*
[2]*Laboratory of Animal Immunology and Biotechnology, Department of Animal Morphology, Physiology and Genetics, Faculty of AgriSciences, Mendel University in Brno, Brno, Czech Republic*
[3]*Institute of Applied Biology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic*
[4]*AgroBioTech Research Centre, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic*

Abstract

The primary objective of the current investigation was to evaluate the effects of plants used in traditional medicine on the secretion of steroid hormones progesterone and 17-β estradiol by porcine ovarian granulosa cells *in vitro*. As detected by ELISA, there was no significant influence of *Terminalia bellerica* extract on the secretion of the steroid hormones. When tested for *Glycyrrhiza glabra*, a substantial decrease ($p<0.05$) in the release of progesterone was noted although there was no notable fluctuation in 17-β estradiol release in comparison to control. On the other hand, *Butea monosperma* application registered an upliftment ($p<0.05$) in release of progesterone whereas 17-β estradiol did not change significantly as compared to control. Obtained results indicate *G. glabra* and/or its chemical constituents as a potential regulator of ovarian steroidogenesis. *B. monosperma* and/or its chemical constituents might also play some role in the regulation of ovarian steroidogenesis. However, further confirmatory studies at the level of receptors are necessary to validate the claims.

Keywords: *Terminalia bellerica*, *Glycyrrhiza glabra*, *Butea monosperma*, ovary, progesterone, estradiol.

Acknowledgements: The authors are thankful to the Ministry of Education, Science, Research and Sport of the Slovak Republic for support through projects VEGA 1/0620/24, KEGA 035SPU-4/2023, and Erasmus+ KA220-HED Cooperation Partnerships in higher education “Fostering Internationalisation in Higher Education by BioFood Virtual Labs” (2021-1-SK01-KA220-HED-000032062).

DOI: https://doi.org/10.15414/2024.9788055227443

The effect of fipronil on the expression of glutathione metobalism transcripts

Laura Rušinová, Veronika Kovaříková, Zuzana Šefčíková, Štefan Čikoš, Alexandra Špirková, Jozef Pisko, Dušan Fabian

Institute of Animal Physiology, Centre of Biosciences of the Slovak Academy of Sciences, Šoltésovej 4-6, 04001 Košice, Slovakia

Abstract

Fipronil as insecticide belongs in the phenylpyrazoles class. Fipronilis a widespread active compound, which is commonly used to control pests in agriculture, household and health care. Accumulating evidence indicates that fipronil is involved in the induction of oxidative stress. We analysed transcripts of genes involved in the glutathione metabolism and action. We isolated total RNA from several mouse blastocyst samples and prepared complementary cDNA. To determine relative quantity of selected mRNAs we used real time PCR with specific oligonucleotide primers. We found that fipronil significantly decreased quantity of gene transcripts connected with glutathione metabolism: glutamate-cysteine ligase, glutathione synthetase, glutathione reductase (Gclc, Gsr and Gss). We found no significant effect of fipronil on glutathione peroxidase 4 and glutathione peroxidase 8 (Gpx4, Gpx8) transcripts. These results showed that fipronil acting on early embryonic cells reduces expression (transcription, mRNA stability, or both) of genes critical for synthesis of glutathione molecule (Gclc, Gss) and for formation of reduced glutatione (Gsr) that is necessary for hydrogen peroxide elimination. This could attenuate the ability of fipronil-treated blastocyst to cope with oxidative stress.

Keywords: fipronil, glutathione, embryo

Acknowledgments: This work was supported by the Slovak Academy of Sciences project VEGA 2/0041/23and the Slovak Research and Development Agency project APVV-22-0071.

DOI: https://doi.org/10.15414/2024.9788055227443

Alterations in the chemical composition of meat following bee bread supplementation in male Japanese quails

Monika Schneidgenova[1], Anna Kalafova[1], Nikoleta Simonova[1], Patricia Simkova[1], Emilia Hanusova[2], Cyril Hrncar[3], Peter Hascik[4], Jan Brindza[5], Renata Mamrakova[1], Marcela Capcarova[1]

[1]*Institute of Applied Biology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 94976, Slovak Republic*
[2]*Institute of Small Farm Animals, Research Institute for Animal Production Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic*
[3]*Institute of Animal Husbandry, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 94976, Slovak Republic*
[4]*Institute of Food Sciences, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 94976, Slovak Republic*
[5]*Institute of Plant and Environmental Sciences, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 94976, Slovak Republic*

Abstract

The objective of this research was to determine whether bee bread supplementation affect the chemical composition of the thigh and breast muscles of male Japanese quails (Coturnix japonica). We determined the chemical composition of meat from the samples collected from the *pectoralis major* muscle and the *biceps femoris* muscle. The content of water, proteins, fats was analyzed using the FT IR (Fourier Transform Infrared Spectroscopy) method with the use of a Nicolet 6700 (Pragolab Ltd.). Animals were divided into 4 groups (E1, E2, E3 and control) according to the doses of bee bread incorporated into feeding mixture HYD 11 offered *ad libitum* following: E1 (n=8) 2 $g.kg^{-1}$, E2 (n=8) 4 $g.kg^{-1}$, E3 (n=8) 6 $g.kg^{-1}$. The control group (n=8) was the group without additives (C). The duration of the experimental treatment period was 180 days. There were no statistically significant differences ($P > 0.05$) in the chemical composition in selected parameters between the experimental groups and the control group. Based on the findings of the study, bee bread in the feeding mixture of male Japanese quails did not affect chemical composition of meat in thigh and breast muscle.

Keywords: Japanese quails; meat; bee bread

Acknowledgments: This work was financially supported by the KEGA 007SPU-4/2022, KEGA 017SPU-4/2023, VEGA 1/0304/23 and APVV-19-0243.

DOI: https://doi.org/10.15414/2024.9788055227443

Effects of anabolic steroids on the reproductive system

Kristýna Skoupá[1], Andrej Bátik[1], Kamil Šťastný[2], Zbyšek Sládek[1]

[1]*Department of Animal Morphology, Physiology and Genetics, Faculty of AgrSciences, Mendel University in Brno, Brno, Czech Republic*
[2]*Department of Infectious Diseases and Preventive Medicine, Veterinary Research Institute, Brno, Czech Republic*

Abstract

Anabolic-androgenic steroids (AAS), synthetic derivatives derived from testosterone, are generally known for their muscle growth-promoting effects. However, their effect on the reproductive system is exactly the opposite. They do not cause the growth of reproductive organs, but on the contrary have a strong toxic and degenerative effect. In our study, we used 18 pigs, hybrids of Large White × Landrace (sow) × Duroc (boar) and divided them into three groups of six: control (without AAS), testosterone (treated with an i.m. injection of 17β-testosterone propionate) and nandrolone (treated with an i.m. injection of 19nor-17β-testosterone propionate). After slaughter, testes, heart, kidney and liver were sampled and proteomic, morfological and histological analysis was performed.

Testicular size was significantly reduced in subjects administered AAS. In the testosterone group by 30%, in the nandrolone group by up to 40%. At the histological level, there were massive degenerative changes. In the groups treated with AAS, there was a reduction of the germinal epithelium in the seminiferous convoluted tubules, in the nandrolone group, complete destruction of the epithelium down to a layer of few cells occurred in most cases. Deformation of the membranes of seminiferous tubules and degeneration of Sertoli cells occurred. Further to the degeneration and loss of Leydig cells and thus to the reduction of natural testosterone production. There was a partial loss of germ cells, mainly a decrease in mature spermatids and sperm. It is therefore possible to predict a decrease in the fertility of individuals, in the case of the nandrolone group, where the effects were stronger, even complete infertility. Changes related to the function of the reproductive system were also observed at the level of proteomics in the heart, kidney and liver. In the heart, up-regulation of N-cadherin (expressed normally by somatic cells of gonadal precursors) was observed in both groups with AAS, most likely as a response to large testicular atrophy. On the contrary, a down-regulation of the adipokine chemerin was observed, which is assumed to have inhibitory effects on testosterone production and on Leydig cell steroidogenesis. Major seminal plasma glycoprotein PSP-I, which is a biomarker of sperm quality, was down-regulated in the liver and kidneys.

We can therefore state that AAS have a negative effect on the reproductive organs of pigs and their use in large doses leads to infertility.

Keywords: anabolic steroids, reproductive systém, pigs, histology, proteomics

Acknowledgement: The study was funded by Ministry of Agriculture of the Czech Republic (project QL24010272) and by Mendelu University in Brno (project AF-IGA2023-IP-021).

DOI: https://doi.org/10.15414/2024.9788055227443

Effect of punicalagin on the viability of bovine mononuclear cells

Lucie Polakova[1], Tina Babackova[1], Eva Venusova[1], Adriana Kolesarova[2], Shubhadeep Roychoudhury[3], Ales Pavlik[1], Petr Slama[1]

[1]Mendel University in Brno, Faculty of AgriSciences, Department of Animal Morphology, Physiology and Genetics, Brno, Czech Republic
[2]Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Applied Biology, Nitra, Slovak Republic
[3]Assam University, Department of Life Science and Bioinformatics, Silchar, India

Abstract

The aim of this study was to investigate the effect of punicalagin, specifically ellagic acid, derivative of punicalagin, on the viability and oxidative stress of bovine mononuclear cells. Those cells were isolated from blood and incubated with ellagic acid or without ellagic acid (control) for 2 hours and 20 hours. There were used these concentrations: 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml, 300 µg/ml. After the mentioned incubation, apoptosis and necrosis were analysed using flow cytometry. After 2 hours of incubation, there was a significant increase in the number of live cells compared to the control at all concentrations. After 20 hours of incubation, the number of necrotic cells decreased the most at a concentration of 50 µg/ml, along with an increase in the number of live cells. Conversely, the concentration of 300 µg/ml was cytotoxic to the cells. The results of this experiment correlate with evaluation of superoxide production.

Keywords: phytonutrients, punicalagin, ellagic acid, lymphocyte, monocyte

Acknowledgement: This work was supported by the Ministry of Education, Youth and Sport of the Czech Republic, project number 8J23FR005.

DOI: https://doi.org/10.15414/2024.9788055227443

Bee bread as obesity prevention

Patricia Simkova[1], Anna Kalafova[1], Monika Schneidgenova[1], Jan Brindza[2], Marcela Capcarova[1]

[1]Slovak University of Agriculture in Nitra, Institute of Applied Biology, Faculty of Biotechnology and Food Sciences, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic
[2]Slovak University of Agriculture in Nitra, Institute of Plant and Environmental Sciences, Faculty of Agrobiology and Food Resources, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic

Abstract

Obesity in the population is growing at a rapid rate. By 2030, more than half of the EU population is projected to be obese. The combination of a decline in occupational physical activity and accessibility of low-cost, tasty meals high in calories has produced an atmosphere conducive to obesity. However, a sizable fraction of the world's population is still slender, indicating that there are intrinsic genetic factors that affect an individual's propensity to become obese. Multiple risk genes, each with relatively minor influence, combine to determine this hereditary susceptibility to obesity and type 2 *diabetes*. Determining how nature and nutrition interact is essential to creating plans to stop or lessen the negative impacts of obesity and type 2 *diabetes*. The most common preclinical animal model for studying metabolic diseases is a small rodent, such as a mouse or rat. Compared to non-mammalian animals, the physiology of mice and rats is more similar to that of humans since they are mammals. Nowadays, mice and rats are used in around 60% of all preclinical animal research. In our experiment, we introduced a gastric probe directly into the stomach of ZDF (Zucker diabetic fatty) rats with bee bread and investigated its effect on weight and food intake, which are precursors not only to obesity but also to *diabetes mellitus*. Body weight in ZDF rats was monitored and recorded weekly throughout the experiment (12 weeks). The mean weight of rats in the diabetic groups (D, DP) was significantly higher ($P<0.001$) than that in the healthy group (Z). The significantly higher ($P<0.001$) weight in the D and DP group compared to the Z group could be due to the manifestation of *diabetes mellitus*. The significantly higher ($P<0.05$) weight of DP rats compared to D rats was due to the positive effects of bee bread. Mean feed consumption was significantly ($P<0.001$) lower in the Z group compared to the D and DP groups. Feed intake was also lower in the DP group compared to the D group, but this difference was not significant ($P>0.05$). From our results, it can be deduced that bee bread, although having a positive effect on delaying the onset of *diabetes mellitus*, had no effect on weight loss in rats. Also, bee bread had no significantly demonstrable effect on the reduction of food consumption in rats treated with it. However, bee bread could be a good nutritional supplement that could delay the onset of *diabetes mellitus* and give patients more time to correct their lifestyle and control their weight before the onset of serious health problems.

Keywords: ZDF rats, obesity, bee bread

Acknowledgement: This study was supported by APVV grant no 19/0243, VEGA grant 1/0304/23 and KEGA 007SPU-4/2022 and 017SPU-4/2023.

DOI: https://doi.org/10.15414/2024.9788055227443

In vitro, the influence of rose hip on selected motility parameters of turkey spermatozoa

Tomáš Slanina, Radovan Floriančič, Peter Massányi

Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of applied biology

Abstract

Artificial insemination is nowadays the preferred method of breeding poultry. Collected turkey ejaculate is standardly always diluted, while the type and the composition of used semen diluent (semen extender) significantly affect the quality of storage spermatozoa. In terms of the quality of semen extenders and their economic effectiveness, a constant requirement exists to improve these media. Many studies confirmed that the rose hips exhibit a wide range of bioactivities, such as anti-inflammatory, antioxidant, antiproliferative etc. For this reason, this study aimed to study the effect of rosa hips extracts as a potentially suitable component of semen extenders on selected motility parameters of turkey spermatozoa cultivated at 5 °C under *in vitro* conditions. Experimental samples were prepared by diluting semen in 6 different concentrations of rose hips water extract solution, without seeds, in the saline solution: 2, 1, 0.5, 0,25, 0.125, 0.0625 $\mu l.ml^{-1}$. Spermatozoa motility parameters were measured after 0, 1, 2, 3, 4 and 5 hours. The analysis of Motility (MOT), Velocity Curved Line (VCL) and Beat Cross Frequency (BCF) of spermatozoa was assessed by the CASA system (Computer Assisted Semen Analyzer) – program Andro Vision®. In all monitored parameters, a negative effect of water rosehip extract was recorded from 1 hour of cultivation at the highest concentrations in samples M2, M1 and or M0.5. Contrariwise, we detected a significantly positive effect on spermatozoa motility at lower concentrations, from the third hour of cultivation in samples M0.25, M0.125 and M0.0625. The values of VCL and BCF of spermatozoa were also higher than the control sample but without a statistically significant difference. In conclusion, we can conclude that the water extract of rosa hips can benefit the motility parameters of storing turkey spermatozoa in vitro conditions. However, this effect does not appear immediately, but only after several hours of cultivation, which points to a protective effect rather than a stimulating one.

Key words: semen extender, *Rosa canina*, *Meleagris gallopavo*, motility

Acknowledgements: This work was supported by projects APVV-19-0243 a KEGA 035SPU-4/2023.

DOI: https://doi.org/10.15414/2024.9788055227443

Effect of irisin on LH and FSH secretion by the porcine anterior pituitary cells through NFκβ signalling pathway

Nina Smolińska[1], Barbara Zarzecka[1], Kamil Dobrzyń[1], Marta Kieżun[1], Grzegorz Kopij[1], Mariia Himultidinova[1], Oghuzan Koker[1], Cecilia Dall'Aglio[2], Agnieszka Rak[3], Tadeusz Kamiński[1]

[1]*Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland*
[2]*Department of Veterinary Medicine, University of Perugia, Italy*
[3]*Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Poland*

Abstract

We aimed to investigate the expression of integrins αV (*ITGAV*) and β5 (*ITGB5*) genes (qPCR) as well as localisation of αVβ5 integrin heterodimer (F-IHC) in the anterior pituitary (AP) of gilts on days 2-3, 10-12, 14-16 and 17-19 of the oestrous cycle. We also aimed to determine the impact of irisin (100, 200 and 300 ng/mL) on basal and GnRH and/or insulin (INS)-stimulated LH and FSH release by the AP cells on days 10-12 and 17-19 of the cycle (ELISA), as well as to determine the impact of irisin on the activation of MAPK and NFκβ signalling pathways (Western blot). The highest expression of *ITGAV* was observed on days 14-19 and 17-19, lower on days 10-12 and the lowest on days 2-3. There were no significant differences in the expression of *ITGB5* across the cycle. ELISA analysis revealed that on days 10-12 of the cycle, irisin alone or combined with integrin antagonist, cycloRGD, had no effect on the basal and GnRH- and/or INS- stimulated LH and FSH secretion. On days 17-19 of the cycle, irisin (300 ng/mL) combined with cycloRGD enhanced basal FSH secretion. The adipomyokine generally diminished GnRH- and/or INS- stimulated FSH secretion. The hormone had no effect on the basal, as well as GnRH- and INS-stimulated LH secretion, however, combined with cycloRGD generally enhanced GnRH- or INS-stimulated LH secretion on those days. The results of Western blot analysis revealed the stimulatory effect of irisin on the activation of the NFκβ signalling pathway and no effect on the activation of the MAPK pathway. The confirmed expression of integrins genes, as well as heterodimer protein in the porcine AP, the middle branch of the hypothalamic-pituitary-gonadal axis, as well as irisin influence on the secretion of hormones responsible for the control of reproduction, seems to confirm the potential role of irisin in the regulation of reproductive functions.

Keywords: irisin, anterior pituitary, integrins, cycloRGD, LH, FSH, oestrous cycle

Acknowledgement: Acknowledgement: This study was supported by the Polish National Science Centre (2020/39/B/NZ9/01061; 2020/39/D/NZ9/010092018/31/B/NZ9/00781).

DOI: https://doi.org/10.15414/2024.9788055227443

The impact of maternal obesity on expression of genes connected with insulin signaling in mouse blastocysts

Alexandra Špirková[1], Martina Kšiňanová[2], Štefan Čikoš[1], Veronika Kovaříková[1], Zuzana Šefčíková[1], Jozef Pisko[1], Janka Babeľová[1], Dušan Fabian[1]

[1]Institute of Animal Physiology, Centre of Biosciences of the Slovak Academy of Sciences, Šoltésovej 4-6, 04001 Košice, Slovak Republic
[2]Institute of Molecular Physiology and Genetics, Centre of Biosciences of the Slovak Academy of Sciences, Dúbravská cesta 9, 840 05 Bratislava, Slovak Republic

Abstract

Obesity isn't just a cosmetic concern. It can lead to other health problems, including diabetes, heart disease, and some cancers. It has been shown that maternal obesity negatively affect development of early embryo. There are data indicating that disturbation of insuling signaling can be beyond the impaired early embryo development in obese mothers. Therefore we investigated whether expression of genes connected with insulin signaling will be altered in mouse blastocysts derived from obese mouse females. We examined mRNA expression of selected cell receptors and protein kinases. We isolated embryos at the stage of blastocyst from control and obese females. Then we isolated total RNA and prepared complementary DNA. Using PCR with specific oligonucleotide primers we analyzed presence of particular transcripts in several independent cDNA samples of blastocysts. To confirm the identity of the amplified sequences we used electrophoresis agarosse gels stained with GelGreen. Our results showed a significant increase in the amount of insulin receptor transcripts in blastocysts recovered from obese mice. We found no significant changes in the expression of examined mitogen-activated proteinkinases. Neither we found no changes in the abundance of transcripts coding a kinase enzyme involved in the activation of protein kinase B/Akt kinase. Yet, further studies will be necessary to elucidate the impact of maternal obesity on insulin signaling in embryonic cells.

Keywords: maternal obesity, insulin receptor, preimplantation embryo

Acknowledgement: This work was supported by the Slovak Academy of Sciences project VEGA 2/0041/23 and the Slovak Research and Development Agency project APVV-18-0389.

DOI: https://doi.org/10.15414/2024.9788055227443

Evaluation of the effect of ZnO nanoparticles on oxidative balance and viability in H295R cells

Nikola Štefunková[1], Hana Greifová[1], Anton Kováčik[1], Tomáš Jambor[1], Lucia Zuščíková[1], Denis Bažány[1], Norbert Lukáč[1], Klaudia Jaszcza[2], Iveta Urban[1], Peter Massányi[1]

[1]Institute of Applied Biology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic
[2]Department of Animal Physiology and Endocrinology, Faculty of Animal Science, University of Agriculture in Krakow, al. Mickiewicza 24/28, 30-059 Krakow, Poland

Abstract

Nowadays, a wide range of industries, including the food, pharmaceutical, and packaging sectors, use nanoproducts extensively. Out of all the nanomaterials, zinc oxide nanoparticles (ZnO NPs) are the third most produced worldwide, after nano-silica and nano-titanium dioxide. ZnO NPs have been applied to pharmaceuticals, food additives, dental hygiene products, and food packaging. As the use of zinc oxide ZnO NPs in everyday products grows, so does concern about health risks. Nevertheless, little is known about the toxicities and mechanisms underlying the modifications in physiological functions and metabolism caused by ZnO NP. As a result, the purpose of this study was to determine the effect of ZnO nanoparticles on oxidative balance in the human adrenocortical carcinoma cell line H295R, which is often employed as a cellular model. Moreover, we evaluated mitochondrial activity. H295R cells were cultivated for 24 hours with different concentrations of ZnO nanoparticles (0.1 - 10 mg/ml) and compared to untreated control. A mitochondrial toxicity assay was used to assess mitochondrial activity, a NBT assay was used to determine superoxide radicals' production. RX Monaco was used to determine superoxide dismutase and glutathione peroxidase concentration in cell lysates. After 24-hour cultivation with 0.1, 0.5, 1, 5, and 10 ml/ml ZnO NPs, a significant decrease in mitochondrial activity was observed. After 24-hour cultivation with 5, and 10 mg/ml ZnO NPs, a significant decrease in SOD and GpX concentration was observed. A significant increase in GpX concentration was observed after cultivation with 0.1 mg/ml ZnO NPs. We didn't observe significant modifications in the superoxide radical generation. Further investigations are required to elucidate molecular mechanisms of action of ZnO nanoparticles on steroidogenesis and viability.

Keywords: GpX, H295R, MTT, nanoparticles, SOD, viability

Acknowledgments: The research was financially supported by the Scientific Agency of the Slovak Republic VEGA No. 1/0083/21, VEGA No. 1/0698/22, VEGA No. 1/0571/23, KEGA 023SPU-4/2022, and by the Slovak Research and Development Agency Grant APVV-21-0168, APVV-20-0218, APVV-19-0243.

DOI: https://doi.org/10.15414/2024.9788055227443

Bacterial species composition on the skin of healthy reptiles

Lucia Štempelová, Viola Strompfová

Institute of Animal Physiology, Centre of Biosciences of the Slovak Academy of Sciences, Šoltésovej 4-6, Košice, Slovakia

Despite the increasing amount of studies dealing with the skin bacteria of vertebrates, reptiles have received less attention. The aim of this study was bacterial species identification of strains isolated from healthy skin of reptiles. Superficial swab samples (n = 40) from the skin of 40 captive bred reptiles (17 species) were collected from the Zoological garden in Košice (Slovakia). The skin swabs were taken from the back part of the body of animal, except the turtle due to the shell (skin on the legs was sampled). The swabs were inoculated on selective (Mannitol Salt agar, Pseudomonas agar, MacConkey agar) and non-selective growth media (Brain Heart Infusion) within 24 h and incubated at 37 °C for 24–48 h. One or two morphologically different colonies from each sample were selected and used for species identification. The isolated bacteria were identified based on MALDI-TOF mass spectrometry after previous sample preparation using standard methodology with ethanol, formic acid and acetonitrile. The most common bacterial genera on healthy skin of reptiles were *Staphylocccus* sp. (*S. xylosus* (n = 22), *S. sciuri* (n = 16), *S. klosii* (n = 6), *S. cohnii* (n = 3), *S. arlettae* (n = 2), *S. haemolyticus* (n = 1) and *S. warneri*), *Bacillus* sp. (*B. cereus* (n = 11), *B. megaterium* (n = 6) and *B. oceanisediminis* (n = 2)) and *Enterococcus* sp. (*E. faecalis* (n = 5)*, E. casseliflavus*). No coagulase-positive staphylococcal species were identified. Gram-negative species such as *E. coli* or *Acinetobacter* sp. were also detected. Interestingly, *Salmonella* sp. was also identified in two healthy reptiles (*Elaphe schrenckii*)

Keywords: skin, reptiles, microbiota

Acknowledgments: The study was supported by the project VEGA 2/0004/24.

DOI: https://doi.org/10.15414/2024.9788055227443

A modified method for the isolation/preparation of primary small intestinal epithelial cells as *in vitro* intestinal models

Katarína Tokarčíkova[1,2], Ľubomíra Grešáková[1], Petr Sláma[2], Andrej Bátik[2], Petr Vevera[2], Aleš Pavlík[2]

[1]*Institute of Animal Physiology, Centre of Biosciences Slovak Academy of Sciences, Šoltésovej 4, 040 01 Košice, Slovak Republic*
[2]*Department of Animal Morphology, Physiology and Genetics, Faculty of AgrSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic*

Abstract

In vitro models of intestinal absorption could contribute to the understanding transport mechanisms of various substances across the intestinal barrier and they are useful in monitoring biological processes in the gut. Moreover, *in vitro* models could significantly reduce the ethical issues and costs associated with the use of animals in research, thus fulfilling the 3Rs philosophy. Advantage of *in vitro* intestinal models is a long-term cultivation of intestinal epithelial cells compared to *ex vivo* culture condition. Therefore, there is an effort to find and optimise condition for a long-term cultivation of *in vitro* models that the most accurately mimic the physiology and morphology *in vivo*. Long-term *in vitro* cultivated intestinal epithelial cells under certain conditions, so-called „mini-guts“ or three-dimensional (3D) organoids, surpass conventional *in vitro* intestinal models and are considered the next generation of *in vitro* tools capable of mimicking the architecture and microenvironment of the intestine *in vivo*. The aim of our experiment was to find and modify a suitable method for the isolation of mouse intestinal crypts, as a source of intestinal stem cells, for a long-term cultivation of intestinal 3D epithelial organoids. Our preliminary results have shown higher intestinal crypt yield using a PBS based chelating buffer with 2 mM EDTA, and repeated centrifugations at 250-300 x g for 5 min at 4°C. The highest yield of intestinal crypt cells was obtained in the 2nd and 3rd fractions. Optimizing the conditions for long-term cultivation of 3D intestinal organoids/enteroids is subject to our further investigation.

Keywords: 3D models, organ-on-a-chip, cell culture

Acknowledgement: The research was financially supported by projects VEGA 2/0008/21, and National Agency for Agricultural Research no. QL24010214.

DOI: https://doi.org/10.15414/2024.9788055227443

Toxic aspect of nanoparticles at the cellular level

Kateryna Vanivska[1], Lucia Dianová[1], Michal Lenický[1], Marko Halo[1], Peter Ivanič[2], Iveta Urban[1], Nikola Štefunková[1], Peter Massanyi[1]

[1]*Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Nitra, Slovak Republic*
[2]*Slovak Biological Services, Banská Bystrica, Slovak Republic*

Nanoparticles are structures ranging in size from 1 to 100 nanometers that have unique physical and chemical properties that make them distinctive for important applications. The aim of this work is to provide an overview of the characteristics of nanoparticles, their mechanisms of interaction with cells, and an assessment of their potential toxicity at the cellular level. The study includes the analysis of physical properties of nanoparticles such as high surface to volume ratio, quantum effects and specific optical properties and chemical properties such as enhanced reactivity, surface modification capabilities and stability. The work focuses on the mechanisms of nanoparticle interaction with cells, including endocytosis, adhesion and biointeraction with biomolecules. These mechanisms can lead to oxidative stress, inflammatory responses and genetic changes that cause cell death or mutations. The effect of nanoparticles on various organ systems, including the respiratory, cardiovascular, gastrointestinal and nervous systems, is being analyzed. Silver nanoparticles (AgNPs) are particles with a significant toxicological risk. AgNPs induce oxidative stress by generating reactive oxygen species (ROS), leading to damage of lipids, proteins and DNA in cells. Exposure to AgNPs induces inflammatory responses with activation of immune cells and release of pro-inflammatory cytokines, which can cause chronic inflammation and related diseases. AgNPs exhibit genotoxicity, causing genetic mutations and chromosomal aberrations through direct interaction with DNA or through mediation of ROS, which raises the risk of cancer and inherited genetic diseases. This study provides a comprehensive overview of the characteristics and toxic effects of nanoparticles, contributing to a better understanding of their interactions with cells and potential health risks.

Keywords: cells, nanoparticles, toxicity, silver nanoparticles.

Acknowledgement: This work was supported by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V02-00043. This work was also supported by the Research and Development Promotion Agency under the contract No. APVV-21–0168 and APVV-19-0243.

DOI: https://doi.org/10.15414/2024.9788055227443

Laminin enrichment of rabbit of DBA$^+$ primitive spermatogonial cells: a preliminary study

Jaromir Vasicek[1,2], Andrea Svoradova[2,3], Andrej Balazi[2], Jakub Vozaf[1]

[1]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Biotechnology, Nitra, Slovak Republic; jaromir.vasicek@gmail.com
[2]NPPC - Research Institute for Animal Production Nitra, Lužianky, Slovak Republic
[3]Mendel University in Brno, Faculty of AgriSciences, Department of Morphology, Physiology and Animal Genetics, Brno, Czech Republic

Abstract

Spermatogonial stem cells (SSCs) represent a small and unique cell population within the testis, which are capable of self-renewal and can differentiate into functional germ cells. Recently, it was observed that bull, ram or boar SSCs can be enriched using differentiation plating technique with the use of extracellular matrix components such as laminin in combination with bovine serum albumin (BSA). In this preliminary study, we focused on the enrichment of rabbit SSCs using laminin coated culture flasks. Briefly, culture flasks were coated firstly with BSA (0.5 mg/ml) by incubating for 2 h with constant shaking in orbital shaker at 37 °C, and then coated with laminin (20 µg/ml) under same conditions as for BSA coating prior seeding of testicular cells (TCs). TCs were isolated from testes of five humanly sacrificed rabbits. Testis tissue was gently minced with scissors into small parts and then enzymatically digested in two steps with collagenase II and trypsin-EDTA. TCs were diluted in 5% DMEM-high glucose medium and seeded in pre-coated culture flasks at the concentration of 0.54 x 10^6/cm^2 and incubated for 2 h or overnight at 37 °C and 5% CO_2. After that, medium with nonadherent cells was discarded, adherent cells were gently washed with PBS twice and revealed from the laminin coating by trypsinization and washed. One million of cells from fresh and both cultured TCs samples were used for DBA-FITC staining. At least 10,000 of stained cells were analysed in each sample using a FACSCalibur flow cytometer. According to analysis, laminin coating significantly ($P<0.05$) increased proportion of DBA$^+$ cells only in TCs samples cultured overnight. In conclusion, further experiments are needed to optimize the enrichment of rabbit SSCs.

Keywords: rabbit testes, spermatogonial cells, laminin,

Acknowledgement: This work was supported by the grant: VEGA 1/0011/23.

DOI: https://doi.org/10.15414/2024.9788055227443

Effect of biologically active compounds in drone brood on bone tissue cells metabolism

Petr Vevera[1], Michala Steinerova[1], Katarina Tokarcikova[1,2], Andrej Batik[1], Petr Slama[1], Ales Pavlik[1]

[1]Department of Animal morphology, Physiology and Genetics, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic
[2]Institute of Animal Physiology, Centre of Biosciences Slovak Academy of Sciences, Šoltésovej 4, 040 01, Košice, Slovak Republic

Abstract

Drone brood, which is sometimes also called drone milk, is one of materials that can be gotten from honeybees. Today its usage is not highly spread, even though it contains many potentially beneficial compounds. In addition to compounds such as protein or fat, it also contains biologically active substances like antioxidants, vitamins and both male and female hormones. Mixture of all these substances can have potential for people and animal health. One of the problems from which animals and people suffer is osteoporosis. Osteoporosis can be characterized as non-coordinated resorption of bones intracellular matter without recovery. This can be caused either by hormonal imbalance or an increased need of mineral compounds which make up the inorganic part of intracellular matter. In chicken breeding, the second cause is non-negligible both in breeding of meat and egg laying chicken. Meat hybrids of chicken are specific by their speed of growth, which can cause problems with bone consolidation. In case of egg laying chicken, there can be problems with increased need of minerals for eggshell formation. In this research we will test the effect of drone brood on bone cells of chicken. Bone is formed and constantly rebuild by two populations of cells. Osteoblasts are cells which build the bone matrix and enable its mineralization. Osteoclasts on the other hand disassemble the bone matrix and make the minerals saved in the bone resorbable. Progenitor cells of both osteoblasts and osteoclasts were isolated from femur of juvenile chicken. Mesenchymal stem cells which are progenitor cells of osteoblasts were isolated from epiphysis of femur and hematopoietic cells of monocyte lineage which are progenitors of osteoclasts will be isolated from bone marrow of the same bone. These cells were then cultivated in six-well culture plates in DMEM high glucose with addition of fetal bovine serum and antibiotics in governed environment of 37 °C and with 5 % of CO2 in atmosphere. Later, the cells were passaged into twenty-four-well culture plates. In those the studied cells were exposed to various concentrations of the solution of drone brood. The effect of the solutions will be evaluated.

Keywords: osteoblasts, osteoclasts, drone brood

Acknowledgment: This research is financed by the Internal Grant Agency of the Faculty of AgriSciences, Mendel University in Brno (IGA24-AF-IP-028).

DOI: https://doi.org/10.15414/2024.9788055227443

Variability of mercury concentrations within the digestive tract of muskrats.

Izabela Wiśniowska[1], Łukasz J. Binkowski[1], Katarzyna Tomczyk[1], Robert Stawarz[1]

[1]*Institute of Biology and Earth Sciences, University of the National Education Commission, Krakow Podchorążych 2, 31-054 Krakow, Poland*

Abstract

Mercury (Hg) is introduced into the environment through natural processes (e.g. volcanic eruptions and evaporation from the surface of the seas and oceans) and human activities, particularly industry. The most toxic form of Hg, methylmercury (MeHg), is mainly associated with the aquatic environment, where methylation takes place, and makes aquatic organisms first to be exposed. As a result, MeHg enters their body with food, bioaccumulates in their tissues, and causes harmful effects.

Eleven individuals of muskrats (*Ondatra zibethicus* L.) from the Zator fishponds area (southern Poland) were shot in April 2012. After that, they were weighed, skinned, sexed (five females and six males), and dissected. Samples of the digestive system were resected and collected: liver, stomach, large intestine, cecum and their content. Age was determined based on the wear of molars, which indicated that the examined individuals were 24 ± 3 months old. Concentrations of Hg were measured in the wet weight of samples using the method CV-AAS (spectrometer MA-2, Nippon, Japan).

The primary objective of the study was to investigate the levels of Hg concentrations in the digestive tract, with a specific focus on identifying any statistically significant differences between sample types and sexes. The concentrations in the content of the digestive tract ranged from 0.0012 µg/g (in stomach content) to 0.0055 µg/g (in large intestine content). We found that the differences between sample types and sexes were not significant, as well as their interaction. However, when we combined the results of both sexes the highest median of mercury concentrations in tissues was noted in the caecum (0.0033 µg/g) and the lowest in the stomach content (0.0019 µg/g).

These results confirmed the presence of Hg in the studied ecosystem, but its accumulation does not vary in various parts of the digestive tract by muskrats.

Keywords: muskrat, Hg, bioaccumulation, digestive system, AAS

DOI: https://doi.org/10.15414/2024.9788055227443

The effect of exposure to an extremely low-frequency electromagnetic field on the abundance of endothelial nitric oxide synthase in the endometrium

Pawel Jozef Wydorski[1], Agata Zmijewska[1], Anita Franczak[1]

[1]*University of Warmia and Mazury, Faculty of Biology and Biotechnology, Department of Animal Anatomy and Physiology, Olsztyn, Poland*

Abstract

The endothelial nitric oxide synthase (eNOS) is one of the isoforms of nitric oxide synthases responsible for the production of nitric oxide (NO). Increased expression of eNOS in the glandular epithelium and luminal endometrium has been found in women with recurrent miscarriages and unexplained infertility. It has been found that the extremely low-frequency electromagnetic field (ELF-EMF) can increase the amount of eNOS in mammalian cells. This phenomenon can lead to the development of oxidative stress and may subsequently impair physiological functions. The overproduction of NO leads to the rise in oxidative stress, therefore the aim of the study was to determine whether the ELF-EMF (2 h, 50 Hz) alters NOS3 mRNA transcript and protein abundance in the endometrial slices in vitro obtained from pigs during the peri-implantation period (day 15–16 of early pregnancy, n = 4). The mRNA and protein NOS3 expression were examined using Real-time PCR assay and ELISA, respectively. *NOS3* mRNA transcript abundance ($p = 0.004641$) and protein concentration of eNOS ($p = 0.000269$) were significantly increased in the endometrium exposed to ELF-EMF compared to the non-treated control. In conclusion, ELF-EMF can generate oxidative stress in the endometrium via increased eNOS concentration. As a result, ELF-EMF-related disruption of the process of implantation may occur. Future studies confirming the occurrence of oxidative stress and its consequences in the endometrium exposed to ELF-EMF are needed.

Keywords: endothelial nitric oxide synthase, extremely low-frequency electromagnetic field, endometrium

Acknowledgement: This work was supported by PRELUDIUM grant no. 2021/41/N/NZ9/01638 from the National Science Center, Poland, and partially co-supported by grant no. 12-610-005-110 from the University of Warmia and Mazury in Olsztyn, Poland.

DOI: https://doi.org/10.15414/2024.9788055227443

Effect of irisin on the transcriptomic profile of the porcine pituitary cells during implantation

Barbara Zarzecka[1], Kamil Dobrzyń[1], Marta Kieżun[1], Grzegorz Kopij[1], Mariia Himultidinova[1], Oguzhan Köker[1], Agnieszka Rak[2], Tadeusz Kamiński[1], Nina Smolińska[1]

[1]*Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn*
[2]*Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Poland*

Abstract

It is well known that reproductive success is closely related to metabolic status. Irisin is a biologically active protein secreted mostly by the skeletal muscle and adipose tissue, involved in the regulation of the energy homeostasis of animals. The pituitary gland is an important organ regulating reproductive processes and proper functioning of the hypothalamic-pituitary-ovarian (HPO) axis. The main aim of this study was to determine the effect of irisin on differentially expressed genes (DEGs), differentially expressed lncRNAs (DELs), and alternatively spliced (AS) transcripts in the porcine anterior pituitary cells, collected during the implantation period (days 15-16 of pregnancy) and exposed to irisin (300 ng/mL). High-throughput sequencing of transcriptomes was performed on the Illumina NovaSeq 6000 platform. The obtained results revealed 500 DEGs ($\log2(FC) \geq |0.56|$; $p<0.05$), from which 350 were upregulated and 150 were downregulated in the irisin-treated group. DEGs were assigned to 257 functional annotations, grouped in categories: biological processes (172), cellular components (17) and molecular functions (67). KEGG analysis showed that the genes with expression changed by irisin participate in 12 signalling pathways, such as ovarian steroidogenesis, phospholipase D signalling pathway and pantothenate and CoA biosynthesis. We detected 747 DELs, of which 349 were known and 398 were described for the first time. Moreover, we identified 220 AS events. Validation of selected genes using the real-time PCR (RT-qPCR) method confirmed RNA-Seq results. Products of revealed DEGs take part in several processes important for the function of the anterior pituitary, such as enzyme activity, steroid hormone, regulation of glucose metabolic process, angiogenesis as well as intracellular signalling pathways and factors which regulate reproductive functions. These findings confirmed a significant effect of irisin on the transcriptome of the pituitary cells. It may be assumed that irisin takes part in the control of the proper course of pregnancy *via* the regulation of pituitary transcriptomic profile.

Keywords: adipomyokine, pituitary cells, RNA-Seq, differentially expressed genes, pregnancy

Acknowledgement: This study was supported by the Polish National Science Centre (2020/39/B/NZ9/01061; 2020/39/D/NZ9/01009; 2018/31/B/NZ9/00781).

DOI: https://doi.org/10.15414/2024.9788055227443

The effect of sage essential oil on rabbit spermatozoa motility *in vitro*

Jiřina Zemanová, Anton Kováčik, Michal Lenický, Eva Kováčiková, Peter Massányi

Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Institute of Applied Biology, Nitra, Slovak Republic

Abstract

The aim of our study was to observe the effect of Salvia officinalis essential oil on sperm motility. We collected semen from 7 rabbits, then mixed them to create a heterospermic sample. Subsequently, we created 2 control groups (K1, K2) without the addition of essential oil of sage and eight experimental groups with different concentrations of essential oil (A - 0.47 µl, B - 0.94 µl, C - 1.88 µl, D - 3.75 µl, E - 7.5 µl, F - 15 µl, G - 30 µl, H - 60 µl). We applied 100 µl of semen to each sample. We used the CASA method to measure sperm motility parameters in different time intervals (0, 120 and 180 min) at a temperature of 37°C. At time 0, we recorded the highest motility in experimental group A, as the addition of essential oil increased, the motility decreased in direct proportion, while the lowest motility was in experimental group F, which there was a significant difference compared to the K2 group. At time 1, sperm motility increased in groups without or with a lower addition of essential oil compared to time 0. We recorded the highest motility in experimental group B. A decrease in motility occurred after the addition of higher doses of essential oil, while we noted a significant difference in group E and F compared to groups K1 and K2. At time 2, there was a slight decrease in group K2. We recorded the highest motility in group K1 and a decrease in motility was again noted in groups with a higher addition of essential oil, while the significant difference was in group C and in group D and the difference in group E and in experimental group F compared to K1. From our observations, it follows that the addition of essential oil of sage in a lower concentration to the ejaculate has a positive effect on sperm motility, but by increasing the concentration and incubation time, the effect on motility is rather negative.

Keywords: rabbit, spermatozoa, CASA, Salvia officinalis, essential oil

Acknowledgement: This work was supported by the Slovak Research and Development Agency under the contract No. APVV-16-0289, and No. APVV-21–0168. This publication was also supported by The Ministry of Education, Science, Research and Sport of the Slovak Republic under the project VEGA 1/0698/22, and by the Grant Agency of SUA in Nitra project No. 20-GASPU-2021. This publication was also created with support within the Operational Program Research and Innovation for the project: Support of research activities in the field of animal production, 313011U414, co-financed from the resources of the European Regional Development Fund.

DOI: https://doi.org/10.15414/2024.9788055227443

The effect of kisspeptin on the secretion of progesterone and 17β-estradiol by endometrium of pigs during the peri-implantation period

Agata Zmijewska[1], Pawel Wydorski[1], Anita Franczak[1]

[1]*University of Warmia and Mazury, Faculty of Biology and Biotechnology, Department of Animal Anatomy and Physiology, Olsztyn, Poland*

Abstract

Kisspeptin (KISS) is a crucial regulator of the female reproductive system. The KISS acts via the kisspeptin receptor (KISSR) and possesses a stimulating effect on the secretion of gonadotropin-releasing hormone in pigs. However, the role of KISS in the porcine uterus and conceptuses is not well understood. Therefore, the aim of this study was to determine the expression of KISSR and the effect of KISS on the secretion of progesterone (P_4) and 17β-estradiol (E_2) in porcine endometrium *in vitro*.

To analyze the *KISSR* expression, endometrial slices were isolated from gilts during the peri-implantation period (days 15-16 of pregnancy) and on days 15-16 of the estrous cycle. The *KISSR* mRNA transcript abundance was examined using the Real-Time PCR method. To analyze the effect of KISS, endometrial explants were isolated from pigs during the peri-implantation period and *in vitro* pre-cultured for 2 hours. Then, they were incubated for the next 12 hours with KISS (1 µM, 100 nM, 10 nM, and 0.1 nM). Control slices were not exposed to KISS. The secretion of P_4 and E_2 was measured using a radioimmunology assay. The results demonstrated the presence of *KISSR* in the porcine endometrium, and a tendency ($p = 0.06$) to increase *KISSR* mRNA transcript abundance in the endometrium collected from pigs during the peri-implantation period compared to the respective days of the estrous cycle. The KISS increases P_4 and E_2 secretion by the porcine endometrium in a dose-dependent manner.

In conclusion, the porcine endometrium possesses the potential to respond to KISS, and the KISS affects the secretion of steroid hormones in the endometrium. However, further molecular studies are needed to understand the role of KISS in the uterus.

Keywords: Kisspeptin, endometrium, per-implantation period, pigs

Acknowledgment: This work was supported by the National Science Centre, Grant No. 2012/07/D/NZ4/04177 and grant no. 12-610-005-110 from the University of Warmia and Mazury in Olsztyn, Poland.

DOI: https://doi.org/10.15414/2024.9788055227443

The effect of acetamiprid TM3 Leydig cells morphology in a 3D system *in vitro*

Lucia Zuscikova[1], Hana Greifova[1], Nikola Stefunkova[1], Norbert Lukac[1], Iva Sovadinova[2], Eliska Sychrova[2], Denis Bazany[1], Tomas Jambor[1]

[1]*Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Nitra, Slovak Republic*
[2]*RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic*

Abstract

Acetamiprid, a new-generation chloronicotinyl insecticide, is commonly used to manage insect pests as an alternative to organophosphates and carbamates. However, despite its primary target being nicotinic acetylcholine receptors in insects, its extensive and widespread application has led to adverse effects in non-targeted organisms, including mammals. Many studies have demonstrated the negative impact of acetamiprid on the male reproductive system, leading to reduced serum testosterone levels, altered metabolic activity, and morphological changes in the testes. In the present study, we investigated the impact of acetamiprid on murine TM3 Leydig cells cultured in a 3D environment *in vitro*. The spheroids formed by TM3 Leydig cells were repeatedly exposed to varying concentrations of acetamiprid (ranging from 4 to 500 µM) for up to 7 days. Subsequently, we continuously monitored and evaluated the size and shape of the exposed spheroids. We observed no statistically significant changes (*P>0.05*) in the spheroid area (µm^2; size parameter) over both time horizons. The shape parameter (roundness, dimensionless parameter) of spheroids also remained unaffected (*P>0.05*) within the specified concentration range of 4 to 500 µM. While our findings suggest no immediate morphological impact on TM3 Leydig cells, further research is necessary to fully understand the potential long-term effects of acetamiprid exposure on Leydig cell morphology *in vitro*.

Keywords: acetamiprid, morphology, Leydig cells, 3D culture.

Acknowledgement: This work was financially supported by the Scientific Grant Agency of the of the Slovak Republic – VEGA 1/0083/21, the Cultural and Educational Grant Agency of the Slovak Republic - KEGA 054SPU-4/2024, the Slovak Research and Development Agency Grant APVV-20-0218, APVV-21-0168, APVV-19-0243 and the CEEPUS Network SK-1913-01-2425. The work was also supported by the RECETOX research infrastructure (ID LM2023069, MŠMT, 2023-2026) and the Czech Science Foundation project no. GA22-30004S.

DOI: https://doi.org/10.15414/2024.9788055227443

ANIMAL PHYSIOLOGY 2024 - book of abstracts from a scientific conference

This book compiles the abstracts from the 19th international scientific conference Animal Physiology 2024, conducted from 11–13 June 2024 in Liptovský Ján, Slovak Republic. The abstracts are presented as submitted by the author and accepted by the reviewers and scientific committee members. Supported by the Slovak Research and Development Agency of the Slovak Republic APVV-19-0243, APVV-20-0218, and APVV-21-0168.

Reviewers

Prof. DVM. Eva Petrovová, Ph.D. - University of Veterinary Medicine and Pharmacy in Košice, Slovak Republic

DVM. Miroslav Uroševic, Ph.D. - University of Novi Sad, Serbia

Editors

Anton Kováčik, Ph.D. - Slovak University of Agriculture in Nitra, Slovak Republic

Tomáš Jambor, Ph.D. - Slovak University of Agriculture in Nitra, Slovak Republic

Graphical design (front page and last page)

Tomáš Jambor, Ph.D. - Slovak University of Agriculture in Nitra, Slovak Republic

Published by

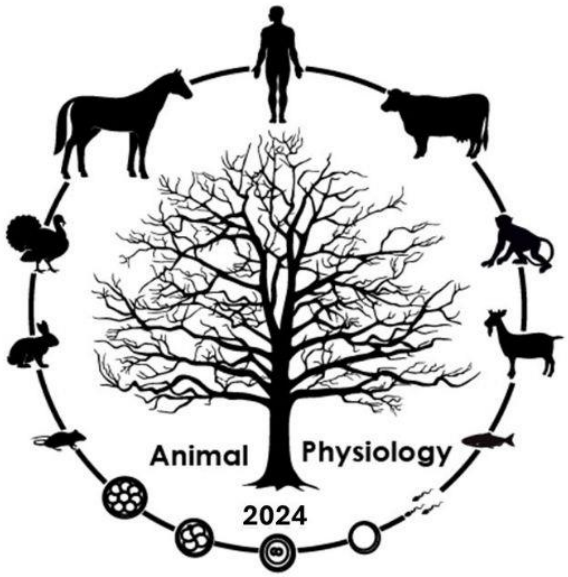
Slovak University of Agriculture in Nitra, volume 1, 2024, Slovak Republic.

Not edited at the Publishing House of SUA in Nitra.

ISBN 978-80-552-2744-3

DOI: https://doi.org/10.15414/2024.9788055227443

DOI: https://doi.org/10.15414/2024.9788055227443



inabi

SUA
Slovak University
of Agriculture
in Nitra

SUA·FBFS
Faculty
of Biotechnology
and Food Science