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INFLUENCE OF ELECTROMAGNETIC RADIATION ON TESTICULAR STRUCTURE AND STEROIDOGENESIS IN RATS

Viera Almášiová¹, Katarína Holovská¹, Viera Cigánková¹, Enikö Račeková²

Address(es):

¹Department of anatomy, histology and physiology, University of Veterinary Medicine and Pharmacy in Košice ²Neurobiology SAS in Kosice

Corresponding author: almasiova@uvm.sk

ABSTRACT

In the new era of intensive usage of modern electronic devices everybody is influenced by an accumulating effect of electromagnetic radiation (EMR) more widely than ever in the past. EMR is a self-propagating wave in space with electric and magnetic components and is physically classed as non-ionising radiation. The immediate whole body electromagnetic radiation -EMR was used to investigate testicular structure and testosterone synthesis of the Wistar rats. Sexually mature (48 days old) rats were subjected to pulsed electromagnetic fields at frequency of 2.45 GHz and mean power density of 2.8 mW/cm² for 3 h per 3 weeks. Histological structure of the testicular parenchyma and plasma testosterone concentrations were evaluated in 21 days post irradiation. The light microscopy revealed moderate degenerative changes in testicular parenchyma as well as decreased plasma testosterone levels in experimental animals compared to the control counterpart which indicate an adverse effect of EMR on spermatogenesis and steroidogenesis in rats. These findings confirmed the evidence, that the testes are amongst the most susceptible organs to the EMR. Next studies are required for elucidation of relation between the EMR and widely debated disorders of fertility and fecundity not only in the human population.

Keywords: electromagnetic radiation, testes, structure, testosterone, rats

ALA-D AS AN INDICATOR OF LEAD POISONING AMONG HUNTED BIRDS

Łukasz Jakub Binkowski, Bartłomiej Zyśk, Marek Guzik, Robert Stawarz, Tomasz Łaciak, Włodzimierz Wojtaś, Grzegorz Formicki, Agnieszka Greń

Address(es):

Institute of Biology, Pedagogical University of Cracow, Poland, Podbrzezie 3, 31-054 Cracow, Poland

Corresponding author: ljbinkowski@gmail.com

ABSTRACT

The major causes of lead (Pb) poisoning in waterbirds (mostly Anseriformes) are lead pellets from ammunition sources and fishing sinkers which may be ingested by the mistake by birds during foraging. They are being dissolved and got into the bloodstream causing a poisoning. To evaluate this exposition of particular specimens, biomarkers as the activity of δ aminolevulinic acid dehydratase in blood can be used. These measurements may be done in blood samples taken from birds during the ringing or the trapping. However, the use of this biomarker for hunted birds is still scarcely evaluated which is the significant lack of knowledge in respect of world-wide hunting activity and easiness of material collection. Such measurements are faster and cheaper in comparison to Pb determination in blood with Atomic Absorption Spectrometry. They are not as precise as AAS method but they better show the real impact of Pb on the physiology of the organism. In this study we present the results of the measurements of ALA-d activity in blood of hunted birds (Mallards and Coots). The samples were taken immediately after the shot from the heart to K₂EDTA vials. The activity was measured according to the standard European method. Lead was positively determined in the majority of blood sample. The relationship between Pb level and ALA-d activity was found.

Keywords: dehydratase, biomarker, waterfowl, lead poisoning, Pb

EFFECT OF SINGLE DOSE OF T-2 TOXIN ON HAEMATOLOGICAL PARAMETERS OF RABBITS

Marcela Capcarová^{1*}, Peter Petruška¹, Anna Kalafová¹, Adriana Kolesárová¹, Ľubomír Ondruška², Rastislav Jurčík², Ľubica Chrastinová², Anton Kováčik¹, Monika Schneidgenová¹, Eva Tušimová¹

Address(es):

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

²Animal Production Research Centre Nitra, Hlohovecka 2, 949 01 Nitra, Slovak Republic

Corresponding author: marcela.capcarova@uniag.sk

ABSTRACT

The aim of the present study was to determine effect of single dose of T-2 toxin on selected haematological parameters of rabbit's blood. Animals were randomly divided into two groups (control C and experimental E). Experimental group of rabbits received intramuscularly T-2 toxin (Romer Labs Division Holding GmbH, Tulln, Austria) at dose 0.08 mg per kg of body weight 72 hours before slaughter. Whole experiment lasted 90 days. In whole blood selected haematological parameters (WBC total white blood cell count, MID medium size cells count, GRA granulocytes count, RBC red blood cell count, HGB haemoglobin, HCT haematocrit, PLT platelet count, MPV mean platelet volume, and PDWc platelet distribution width) were measured using haematology analyser Abacus junior VET (Diatron[®], Austria). Single dose of T-2 toxin caused decrease in WBC, MID, GRA, PLT, and PDWc, however without significant differences (P>0.05). Insignificant increase (P>0.05) of RBC, HGB, HCT, and MPV was observed after T-2 toxin treatment. In conclusion, T-2 toxin in single dose 0.08 mg had no harmful effect on rabbits.

Keywords: T-2 toxin, haematological parameters, rabbits

Acknowlegments: This work was financially supported by VEGA scientific grant 1/0790/11 and Vega 1/0084/12.

LEVELS OF SELECTED INDICATORS IN THE BLOOD SERUM OF HORSES AND THEIR CORRELATION DEPENDENCY

Peter Čupka¹, Marek Halo², Peter Massányi¹, Anton Kováčik¹, Robert Stawarz³, Formicki Grzegorz³

Address(es):

¹Department of Animal Physiology, Slovak Universyity of Agriculture in Nitra ²Department of Animal Husbandry, Slovak Universyity of Agriculture in Nitra ³Pedagogical University in Krakow

Corresponding author: peter.cupka@uniag.sk

ABSTRACT

In this study 26 sport horses (14 mares and 12 stallions) were included in the trial. The content of urea, total proteins (TP),

glucose (GLU), cholesterol (CHOL), triglycerides (TG), bilirubin (BILI), AST, ALT, GGT and ALP was determined in the blood serum. Levels of monitoring indicators in stallions and mares were balanced. Significant difference (P<0.05) was recorded only in total proteins and AST content between stallions and mares. Other differences were statistically non significant. Medium correlation was found between UREA:AST (stallions), UREA:BILI (stallions), UREA:TG (mares), UREA:GLU (stallions), UREA:TP, TP:ALP (stallions, mares), TP:GTT (stallions, mares), TP:GLU (stallions), GLU:GGT (mares), CHOL:ALP (stallions), CHOL:GGT (stallions), TG:ALP (stallions), TG:GGT (stallions), TG:BILI (mares), BILI:GGT (stallions), AST:GGT (stallions), ALT:GGT (stallions), GGT:ALP (stallions). A high correlation was found between UREA:ALP (stallions), TG:BILI (stallions), BILI:ALP (stallions), AST:ALT (stallions).

Keywords: mares, stallions, blood serum, serum chemistry, correlations

Acknowlegments: This work was supported by the Scientific Agency of the Slovak Republic VEGA 1/0532/11.

THE COMPARISON OF SUSCEPTIBILITY OF CHICKEN AND DUCK EMBRYOS TO CADMIUM DURING *IN OVO* EXPOSURE

Dżugan M.¹, Lis M.², Głodek K.², Droba M.¹, Niedziółka J.W.²

Address(es):

¹Department of Food Chemistry and Toxicology, University of Rzeszów, Ćwiklińskiej 2, 35-601 Rzeszów

²Department of Poultry and Fur Animals Breeding and Animal Hygiene, University of Agriculture in Kraków

Corresponding author: mdzugan@univ.rzeszow.pl

ABSTRACT

Cadmium gives a real threat for living organisms due to its enhancing environmental contamination, a unique ability to bioaccumulation and multidirectional toxic effects. All birds are exposed to cadmium in their natural habitats, but in the case of water fowl the exposure seems to be much higher as a result of the contact with polluted bottom sediments. It is confirmed that reproductive disturbances of wild waterfowl have this etiology and avian eggs are considered to be sensitive indicators of heavy metal contamination in environmental monitoring. The aim of this study was to compare the effect of cadmium injected in ovo to chicken and Muscovy duck eggs for hatching results and lysosomal hydrolases activity in blood plasma of one-day hatchlings. Cadmium was injected on day 4 of incubation to hens egg in the increasing dose ranging from 0 to 24 μ g per egg. For ducks eggs, the injection was done on day 6 of incubation with doses 0-30 µg per egg (recalculated into higher egg weight). The results obtained indicate that cadmium ions applied in ovo at the dose 3 µg per egg were embryotoxic for hens embryos and markedly reduced hatchability (20 %) as compared to the control group (45 %). In ducks, the significant adverse effect of cadmium was observed for the dose 7.5 μ g per egg which reduced hatchability from 52.3 % observed for control group to 31.9 % (P<0.05). In both birds species cadmium induced significant changes in lysosomal hydrolases activity in blood plasma and the greatest differences for N-acetylglucosaminidase,

 β -mannosidase and arylsulphatase were observed. Compared to the control group, in the blood serum of chicks from the group receiving 3 µg Cd per egg N-acetylglucosaminidase activity increased by 79 % (P \leq 0.05) whereas for ducklings a weaker non-significant increase (by 22 %) for the same dose was observed. The results seem to indicate a species-specific differences in susceptibility of birds embryos to cadmium.

Acknowlegments: The study was financed by research project NN 304 291 140

SELENIUM BALANCE IN LAYER CHICKENS FED DIETS SUPPLEMENTED WITH SELENIUM FROM INORGANIC OR ORGANIC SOURCES

Ľubomíra Grešáková¹, Klaudia Čobanová¹, Štefan Faix¹, Katarína Venglovská²

Address(es):

¹Institute of Animal Physiology, Slovak Academy of Sciences, Šoltesovej 4-6, 040 01, Košice, Slovak Republic

²Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafarik University, Moyzesova 11, 040 01, Košice, Slovak Republic

Corresponding author: gresakl@saske.sk

ABSTRACT

The aim of this study was to compare the effects of feed supplementation with comparable Se doses from sodium selenite (SS) and selenised yeast (Se-yeast) on Se retention, balance and tissue deposition in layer chickens by the balance technique. One-day-old female chickens of the laying strain Isa Brown were randomly allocated to four dietary treatments consisting of basal diet (BD) containing only background Se (0.12 mg/kg dry matter (DM)) and two treatments based on identical BD supplemented with 0.2 mg Se/kg DM either from SS or from Se-yeast. The fourth group of layers fed BD enriched with 0.7 mg Se/kg DM from Se-yeast. After 4 weeks of experiment, no differences in blood Se levels were observed between chickens fed diets supplemented with equivalent doses of SS or Se-yeast, while the 8-week intake of Se-yeast resulted in significantly higher blood Se levels of layers than from SS (0.17 vs. 0.21 mg/L, P<0.01). The layers given BD showed significantly the lowest Se level. The daily Se balance was positive in all birds with the highest values in chickens fed larger amount of Se-yeast. The balance measurements carried out in week 4 and 8 showed no differences between groups supplemented with equivalent Se amounts in the selenium balance and Se retention (% of Se ingested), both differing from the control group in week 8 only. The 8-week intake of Se-yeast resulted in significantly higher Se deposition in muscles, heart, gizzard, pancreas, lungs, kidney, Bursa Fabricii and feathers of chickens than that from SS. The highest tissue Se concentrations in all layers were found in the kidney. The layers given diet with larger amount of Se-yeast showed the highest values in the majority tissues. The results demonstrate the better bioavailability of Se-yeast in young layers due to the greater body retention of this essential microelement than from sodium selenite, however the higher Se absorption from digestive tract of birds was as effective as inorganic Se source.

Keywords: sodium selenite; selenised yeast; Se retention; Se deposition

Acknowlegments: This study was supported by the Research and Development Support Agency, Slovak Republic, Grant No. APVV-0399-07

EFFECT OF VEGETABLE FATS ON STEROID HORMONAL RELEASE OF PORCINE OVARIAN GRANULOSA CELLS

Adriana Kolesárová^{*1}, Nora Maruniaková, Marek Halenár, Marína Medveďová and Shubhadeep Roychoudhury²

Address(es):

¹Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic; ²Department of Life Science & Bioinformatics, Assam University, Silchar 788011, Assam, India

Corresponding author: Adriana.Kolesarova@uniag.sk

ABSTRACT

Vegetable oils that are solid at room temperature are called vegetable fats. A study was conducted in western Slovakia to determine possible effect of used vegetable fats (100 µl/ml) and palm fats (100 µl/ml) after cooking in restaurants on secretion activity of steroid hormone progesterone and 17 β-estradiol on porcine ovarian granulosa cells in vitro. Progesterone and estradiol are essential for normal ovarian cycles and contribute to regulation of ovarian follicular development and remodeling. Release of these hormones by ovarian granulosa cells of Slovakian White gilts was detected by Enzyme Linked Immunosorbent Assay. Experimental results indicated that administration of used vegetable fats and palm fats significantly $(P \le 0.05)$ lowered the release of progesterone by porcine ovarian granulosa cells in comparison to control although no such difference was noted in case of estradiol production. Obtained data suggest that used vegetable fats and palm fats may influence steroidogenesis in animals.

Keywords: Steroidogenesis, progesterone, estradiol, ovarian granulosa cells, used vegetable fats, palm fats

Acknowlegments: The authors are thankful to ecol Trade, s.r.o., Nitra, Slovak Republic, http://www.ecoltrade.sk/ for provided used vegetable fats. This work was financially supported by the Ministry of Education of the Slovak Republic projects no. 1/0790/11 and 1/0022/13.

IN VITRO STUDY ON THE STEROIDOGENIC EFFECT OF USED VEGETABLE OILS IN PORCINE MODEL

Adriana Kolesárová^{*1}, Nora Maruniaková, Marek Halenár, Marína Medveďová and Shubhadeep Roychoudhury²

Address(es):

¹Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic; ²Department of Life Science & Bioinformatics, Assam University, Silchar 788011, Assam, India

Corresponding author: Adriana.Kolesarova@uniag.sk

ABSTRACT

Vegetable oils are extracted from seeds like rapeseed, soybean, corn, sunflower, safflower and are found in majority of processed foods. A study was conducted in western Slovakia to determine possible effect of used vegetable oils (100 µl/ml) after cooking in households, school canteens and fast food restaurants on secretion activity of steroid hormones progesterone and 17 βestradiol on porcine ovarian granulosa cells in vitro. Progesterone and estradiol are essential for normal ovarian cycles and contribute to regulation of ovarian follicular development and remodeling. Release of these hormones by ovarian granulosa cells of Slovakian White gilts was detected by Enzyme Linked Immunosorbent Assay. Experimental results indicated that administration of used vegetable oils after cooking in households, school canteens and fast food restaurants significantly ($P \le 0.05$) lowered the release of progesterone by porcine ovarian granulosa cells in comparison to control although no such difference was noted in case of estradiol secretion. Obtained data suggest that used vegetable oils after cooking may influence steroidogenesis in animals.

Keywords: Steroidogenesis, progesterone, estradiol, ovarian granulosa cells, used vegetable oil

Acknowlegments: The authors are thankful to ecol Trade, s.r.o., Nitra, Slovak Republic, http://www.ecoltrade.sk/ for provided used vegetable oils. This work was financially supported by the Ministry of Education of the Slovak Republic projects no. 1/0790/11 and 1/0022/13.

DIFFERENCES IN Na⁺, K⁺, Cl⁻IONS LEVEL AFTER INTRAPERITONEAL STREPTOZOTOCIN INJECTION IN CHOSEN BRAIN STRUCTURES

Marta Kopańska, Grzegorz Formicki, Kinga Kraska

Address(es):

Department of Animal Physiology and Toxicology, Institute of Biology,

Pedagogical University of Cracow

Corresponding author: martakopanska@poczta.onet.pl

ABSTRACT

 Na^+ , K^+ , Cl^- play fundamental role of transport in maintaining the necessary ionic gradients for cell metabolism and excitability in the nervous system. The aim of our work was to estimate the level of Na^+ , K^+ , Cl^- ions in selected brain structures after streptozotocin in: the: right hemisphere, the left hemisphere, cerebellum and trunk of the brain. The experiment was carried out on 24 male mice of Swiss strain. The measurements were performed after 48, 72 hours, 8 days and 16 days after streptozotocin injection in single dose - 65 mg/kg b.w. The level of ions was measured in the mixture of supernatant on the EasyLyte Na/K/Cl analyzer. Statistical analysis was performed using analysis of variances ANOVA. Homogoneity of variances was estimates using Dunnett test. We noticed influence of streptozotocin on ions level in selected brain structures. Taking into account Na^+ level there were significant changes in the right hemisphere after 16 days, in the left hemisphere after 16 days, in cerebellum after 48 hours and in trunk of the brain after 16 days. Taking into account K^+ level there were significant

changes in the right hemisphere after 16 days, in the left hemisphere after 48 hours, 8 and 16 days, in cerebellum after 8 and 16 days and in trunk of the brain after 16 days. Taking into account Cl-level there were significant changes in the right hemisphere after 48 hours, 8 and 16 days, in cerebellum after 48 and 72 hours and in trunk of the brain after 72 hours and 16 days. We can notice many fluctuations in ions level. It was probably the result of the body's attempt to adapt to the inject dose of streptozotocin. Although no high significant changes after each time, we noticed increased tendency in Na^+ , K^+ , $Cl^$ ions level.Significantly increased level of Na^+ , K^+ , Cl^- ions occures in diabetic patients.

THE VALUES OF SELECTED BIOCHEMICAL PARAMETERS IN THE BLOOD SERUM OF PIGLETS AFTER USE OF PHYTOADDITIVES

Janka Kotosová^{1*}, Janka Poráčová², Marta Mydlárová Blaščáková², Vincent Sedlák¹, Terézia Pošiváková¹

Address(es):

¹Department of Ecology, Faculty of Humanities and Natural Sciences, Presov University in Presov ²Department of Biology, Faculty of Humanities and Natural Sciences, Presov University in Presov

Corresponding author: jankakotosova@hotmail.com

ABSTRACT

In our work, we focused on monitoring the enzymatic activities of LDH, AST, ALT and GLU in the blood serum of pigs (piglets) from the age of 21 days old to 42 days old. Subscriptions were made on each week in the morning on an empty stomach, from the eye veny (vena ophtalmica). All experimental animals were fed with compound feed COS1, COS2, without any feed additives. Piglets were divided into two groups. The first group was the control group (n = 7 piglets) and the second was the experimental group (n = 7 piglets), which was givens age (Salvia officinalis L.) and oregano (Origanum vulgare L.) in the form of essential oils in concentrations of 0.05%. The average results of observed enzymatic activities of the 21day old piglets were as follows: control group of piglets: LDH - 10.3 μ kat/l, AST - 0.84 μ kat/l a ALT - 1.75 μ kat/l a GLU - 5.33 mmol/l and in the experimental group the results of observed enzymatic activities were: LDH - 9.09 µkat/l, AST - 0.71 µkat/l, ALT – 0.96 µkat/l a GLU – 5.97mmol/l. The sampling was repeated when the piglets reached 42 days old and in both groups we found out that the results of enzymatic activities in LDH and AST were higher and in ALT and GLU they were lower.

Keywords: piglets, sage (*Salvia officinalis* L.), common oregano (*Origanum vulgare* L.) enzymatic activity, feed additives

Acknowledgement: This research was supported by the project KEGA016PU-4/2012

EFFECT OF GREEN TEA EXTRACT ON MOTILITY PARAMETERS OF RABBIT SPERM

Jiřina Kročková, Anton Kováčik

Address(es):

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

Corresponding author: cipko26@yaho.com

ABSTRACT

Reproduction is a fundamental expression of living matter. Semen quality is the guarantee of successful insemination in breeding rabbits. The basic criterion in assessing of semen quality is sperm motility. There are many endogenous and exogenous factors affecting reproduction. Green tea has recently become a subject of investigation in connection with the prevention of various diseases and also its effects on reproduction. The aim of our study was to investigate the effects of different concentrations of green tea extract on the motility parameters of rabbit sperm in vitro at different time intervals (0, 60, 120 minutes) at 37°C. Sperm was collected from adult male rabbits (n=5). Samples were diluted with saline with addition of green tea extract-group A (0.75 mg/L), group B (1.5 mg/L), group C (2.25 mg/L), group D (3 mg/L) and group K without the addition of green tea extract. The measurements were performed using CASA analysis. We observed in creased motility parameters at all time intervals in the group with the lowest concentration of green tea extract compared to the control group. After the addition of higher concentrations of green tea extract (≥1.5 mg/L), we observed decrease of all motility parameters at all time intervals compared to the control group. The results of our work indicate that low doses of green tea extract (≤0.75 mg/L) positive affect sperm motility. However, higher doses decrease rabbit sperm motility.

Keywords: rabbit, sperm, CASA, green tee extract

EFFECT OF RESVERATROL ON MOTILITY PARAMETERS OF RABBIT SPERM

Jiřina Kročková, Anton Kováčik, Peter Čupka

Address(es):

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

Corresponding author: cipko26@yaho.com

ABSTRACT

The aim of our study was to evaluate motility parameters of rabbit sperm due to different concentrations of resveratrol at different time periods (0 and 120 minutes). Motility parameters were evaluated at room temperature (22-25 °C) by detailed analysis of CASA. The samples were collected from adult rabbits (n=5) and diluted with saline with addition of resveratrol - experimental group A (0.5 mg/mL), group B (0.75 mg/mL), group C (0.875 mg/mL), group D (1 mg/mL) and control group K witout resveratrol addition. The highest motility (85.36 %) was observed after addition of the lowest resveratrol concentration (group B) at time 0. The significantly (p<0.001)

lower values were measured in group A (71.40 %) and C (74.14 %) in comparison to the control group (89.32 %). After 2 hours incubation was lowest motility in the group B (53.63 %), while the highest in the group A (62.08 %). Progressive motility of the sperm had the same tendency. In the group with the highest concentration of resveratrol was the lowest progressive motility (65.84 %) and the highest in the group with the lowest concentration of resveratrol (81.95 %). After 2 hours incubation there were significant differences of pregressive motility in comparison to control group. The highest value was in the group D (57.24 %) and the lowest in the group A (50.32 %). In measurement of distance parameters we observed the lowest values after addition of the highest concentrations of resveratrol. Similar trend was also observed in the evaluation of speed parameters. In conclusion we can state that the addition of any amount of resveratrol to sperm decreased motility parameters immediately after addition and also after longer time of incubation. Motility was decreased directly proportionally with increasing of resveratrol concentrations and time of exposure. The obtained results show the negative impact of resveratrol on rabbit sperm motility.

Keywords: rabbit, sperm, CASA, resveratrol

HEMATOLOGICAL ALTERATIONS IN COMMON CARP (*CYPRINUS CARPIO* L.) EXPOSED TO PROCHLORAZ

A. Ludwikowska, H. Lutnicka, B. Bojarski

Address(es):

Faculty of Animal Science, University of Agriculture in Cracow

Corresponding author: aga.ludwikowska@onet.eu

ABSTRACT

In this study the influence of the fungicide prochloraz on common carp hematological parameters was investigated. The study was conducted on fish weighing 50 (\pm 10) g in aquaria under controlled environmental conditions. Animals were exposed to the fungicide for 14 days at concentration of 1 mg/L. Next, individuals were transported to clean water for 30 days recovery period. It was determined that prochloraz caused a slight increase in packed cell volume (PCV), mean corpuscular volume (MCV) levels and white blood cell (WBC) number after 3 and 14 days of exposure. Decrease in red blood cell (RBC) number was observed after 24 hour of exposure and it was more pronounced after 3 and 14 days of exposure. No differences were observed in levels of hemoglobin (Hb), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Hematological evaluation of fish can be useful in detection of environmental stressors, like fungicides.

Keywords: hematological parameters, fungicide, common carp

BREED DIFFERENCES IN SEVERAL CHARACTERISTICS OF BULL FROZEN-THAWED SPERM

Alexander V. Makarevich¹, Eliška Špaleková¹, Luděk Stádník², Elena Kubovičová¹, František Louda³

Address(es):

¹Animal Production Research Centre Nitra, Lužianky, Slovak Republic

²Czech Agricultural University in Prague, Czech Republic

³*Research Institute for Cattle Breeding Ltd. Rapotin, Vikýřovice, Czech Republic*

Corresponding author: makarevic@cvzv.sk

ABSTRACT

A number of factors influencing bull fertility are identified including scrotal circumference, sperm morphology, motility, mating ability, age, body condition and the breed of the bull. This study was aimed at comparison of some sperm characteristics in Czech Fleckvieh (n= 8) and Holstein (n= 8) bulls at the age of 1 to 6 years. Samples of ejaculate were obtained from each bull using an artificial vagina. After determination of pathologic sperm (staining with Congo red, Bromothymol blue and Janus green) and sperm motility, the samples of semen with progressive movement \geq 70% were diluted in AndroMed® extender containing soybean lecithin extract, frozen in a programmable freezing device and stored in liquid nitrogen until analyses. Sperm motility was analyzed by CASA. Sperm plasma membrane integrity and apoptosis incidence were detected under a Leica fluorescent microscope after staining with PNA-Alexa Fluor and Yo-Pro-1 in combination with propidium iodide (PI), using specific wavelength filters. Average age of bulls and incidence of pathological sperm were not different between CF and H breeds, whilst average body condition score (BCS) was significantly higher (p< 0.05) in CF (2.84 \pm 0.17) than in H (2.19 \pm 0.13) bulls. Total sperm motility and progressive movement measured by CASA immediately after thawing $(38.6\pm1.8 \text{ and } 34.19\pm1.91, \text{ resp})$ and following 0.5 h (46.44±1.79 and 44.14±1.8) were higher in Holstein bulls compared to CF bulls (34.15±2 and 30.27±2,13; 39.17 ± 2 and 36.12 ± 2.07 , resp.), but following 2 h these traits were not different. More sperm with plasma membrane damages were recorded in CF bulls (36.41±1.81%) compared to H bulls (26.33±1.63%). Similarly, higher incidence of apoptotic sperm was noted in CF bulls (19.12±1.71%) compared to H bulls (12.58±1.51%). We observed differences in sperm motility and viability between two tested breeds, which may be a result of interbreed difference in average body condition. These results should be taken into account at preparation of insemination doses.

Keywords: Holstein, Czech Fleckvieh, bull, sperm, motility, plasma membrane, apoptosis.

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THE EFFECT OF AGE OF PARENT FLOCK ON HATCHABILITY

Anna Musilová¹, Martina Lichovníková¹, David Hampel²

Address(es):

¹Department of Animal Breeding, Mendel University, Faculty of Agronomy, Zemědělská 1, 613 00, Brno, Czech Republic ²Department of Statistics and Operation Analysis, Faculty of Business and Economics, Zemědělská 1, 613 00, Brno, Czech Republic

Corresponding author: <u>anulka.ms@seznam.cz</u>

ABSTRACT

This study evaluates the effect of age of parent flock on embryonic mortality and hatchability. The embryonic mortality and hatchability play a major role in economy of hatcheries and they are one of the most important indicators of reproduction.

Parent flock of ISA Brown (IB) at 34, 56 and 58 weeks of age was used in the evaluation. Freshly laid eggs were incubated and analyzed for fertilization, hatchability and embryonic mortality. The flock was vaccinated against infectious bronchitis during the rearing period. Significantly higher hatchability (P<0.05) was found at 34 weeks of age of parent flock. Decrease of hatchability was statistically significant (P<0.05) between 56 and 58 weeks of age. Unusually high embryonic mortality was found in the second third of incubation. Eggshell quality was also evaluated. Significantly lower eggshell quality – strength (P<0.01), thickness (P<0.01) and weight (P<0.05) were found in ISA Brown.

Key words: hatchability, parent flock, embryonic mortality, fertilization

THE USE OF ELECTROPHORESIS IN LABORATORY DIAGNOSTIC OF SERUM PROTEINS IN VETERINARY MEDICINE

Veronika Nagyová¹*, Csila Tóthová¹, Helena Šoltésová¹, Mária Vargová¹, Oskar Nagy¹

Address(es):

¹Clinic of Ruminants, University of Veterinary Medicine and Pharmacy in Košice

Corresponding author: <u>nagyova.vero@gmail.com</u>

ABSTRACT

In this study, we explain the need for more detailed laboratory diagnostic of serum proteins in veterinary medicine. The application of proteomic approaches over the last decade has provided new tools for clarifying the molecular aspects of physiological states, and for understanding the etiology and pathogenesis of many diseases. Proteomics performs large-scale protein analysis, describes the entire protein complement of a cell, tissue, biological fluid or organism and provides information on protein expression, localization, functions and interactions. Majority of plasmatic proteins is synthesized in hepatocytes, with albumin representing their largest quantitative part. The second major contributor is the imune system. Plasma proteins perform a nutritive function, exert colloidal osmotic pressure, and aid in the maintenance of acid-base balance. Individual proteins serve as enzymes, antibodies, coagulation factors, hormones, and transport substances. Fresh serum contains all of the plasma proteins except fibrinogen, factor V, and factor VIII. These are consumed during clot formation. The electrophoretic technique is the current standard of reference for the fractionation of the serum proteins in clinical biochemistry. Serum proteins determined by electrophoresis involves albumin, $\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$ and γ -globulins. Individual blood serum proteins have different functions and their identification is used also as a diagnostic tool. Many of these proteins are the so-called acute phase proteins. For example, a1-acid glycoprotein concentration increases during inflammation or infection, as well as concentrations of other acute phase proteins (CRP, serum amyloid A protein, α 1-antitrypsin, haptoglobin, ceruloplasmin and fibrinogen) based on increased synthesis of hepatocytes with subsequent release of these proteins in blood. Alfa1-antitrypsin, member of the superfamily of proteinase inhibitors, has a crucial effect on inactivation of neutrophil elastase and other proteases, which maintains protease-antiprotease balance. Ceruloplasmin takes part in copper metabolism and its decreased plasmatic concentration is associated with copper deficiency. Concentrations of serum proteins are influenced by many physiological (age, pregnancy, lactacion etc.) and pathological (malnutrition, renal and hepatal diseases etc.) factors. Current widespread use of electrophoresis is commensurate with its reflection of a variety of changes in serum protein patterns in disease in different species of animals.

Keywords: serum proteins, electrophoresis, albumin, globulin, animals

INTERNAL ENVIRONMENT INDICATORS OF CZECH PIED BULLS DEPENDING ON LEPTIN GENE SNP

Aleš Pavlík¹, Petr Sláma¹, Aleš Knoll¹, Aleš Dufek³, Jan Šubrt²

Address(es):

¹Mendel University in Brno, Department of Animal Morphology, Physiology and Genetics, Brno, Czech Republic ²Mendel University in Brno, Department of Animal Breeding, Brno, Czech Republic ³Agriresearch Rapotín Ltd., Vikýřovice, Czech Republic

Corresponding author: pavlik@mendelu.cz

ABSTRACT

In this study the effect of leptin gene single nucleotide polymorphism (SNP) on cattle internal environment indicators were evaluated. In experiment 58 Czech Pied bulls divided in three groups (TT, CT and CC) according to leptin SNP were investigated. Serum leptin, insulin, thyroxine, triiodothyronine, triacylglycerols and non-esterified fatty acids concentrations were measured. No significant differences in serum T3, T4, TAG and NEFA concentrations between experimental groups, were found. Significantly higher concentration of serum insulin was recorded in group of TT bulls compared to CT a CC genotype. No effect of SNP of leptin gene on leptin serum concentration was recorded. No significant correlations between monitored hormones and metabolites in serum of Czech Pied bulls were found. Some differences in comparison with results found in other studies could be given by differences in fattening capacities particular breeds.

Keywords: cattle, hormones, blood, adipose tissue

ACTIVE CASPASE-3 DETECTION IN CARBAMATE BENDIOCARB TREATED RABBIT LIVER

Eva Petrovová^{1*}, Dávid Maženský¹, Lenka Luptáková², Ľubica Šťavová²

Address(es):

¹Department of Anatomy, Histology and Physiology, University of Veterinary Medicine and Pharmacy, Kosice ²Department of Biology and Genetics, University of Veterinary Medicine and Pharmacy, Kosice

Corresponding author: petrovova@uvlf.sk

ABSTRACT

Increasing use of pesticides all over the world makes it necessary to reveal the toxic risk in populations of non-targeted organisms. Bendiocarb is a carbamate pesticide which is used against a variety of insects, and the liver has an important role in its process of detoxication and excretion. In our experiment were used 56 adult rabbits which were divided into four groups (control, days 10, 20, 30 of administration) and all experimental rabbits received bendiocarb per os in a dose 5 mg/kg b.w. Almost no caspase positive cells were detected in the untreated samples. After 10 days of bendiocarb treatment, numerous immunoreactive cells were present throughout the organ, most commonly around the portal tract (PT). Positive cells were still abundant at 20 days close to the central vein, with a decline at 30 days, at which point vacuoles were observed in some cells together with replacement fibrosis. We concluded that bendiocarb has moderate effect on increase of programmed cell death, particularly after short-term administration.

Keywords: bendiocarb, caspase-3 activity, liver, rabbit, toxicity

EFFECT OF QUERCETIN EXPOSURE ON SOD ACTIVITY OF RABBITS: GENDER COMPARISON

Peter Petruška, Katarína Zbyňovská, Anna Kalafová, Adriana Kolesárová, Marcela Capcarová

Address(es):

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

Corresponding author: petruska.peter85@gmail.com

ABSTRACT

The aim of the present work was to analyze the activity of the chronic application of quercetin in various doses on level of activity superoxide dismutase in rabbit's blood. Adult female rabbits (n = 20) and male rabbits (n = 20) were used in experiment. Rabbits were divided into a control group (C) and experimental groups (E1 – E3). Experimental groups received quercetin (Sigma Aldrich, Saint Louis, USA) in injectable form (intramuscularly) at 10 μ g.kg⁻¹ in E1, 100 μ g.kg⁻¹ in E2 and 1000 μ g.kg⁻¹ mg.kg⁻¹ in E3 group for 90 days. Control group received injection water (Imuna Pharm a.s. Šarišské Michal'any, Slovak Republic).

Statistical analyses showed significant differences in SOD activity between E1 group in both genders where the lowest dose of quercetin was applied. We found significant lower value in female E1 group in comparison with the E1 male group. The application of quercetin resulted in decrease of SOD activity in all experimental female groups in the comparison with the control group, however differences were not significant. The lowest value in E1 after quercetin treatment was observed.

On the other hand increase of the antioxidant enzyme SOD in all experimental male groups in comparison with the control group was found, but differences were not significant. Generally, quercetin treatment increased SOD activity in male more than in female groups. Our findings suggest that the natural substance quercetin may have a beneficial effect on level of superoxide dismutase in rabbits. To prove this idea, more experiments and analysis of further parameters of animal homeostasis should be performed.

Keywords: quercetin, superoxide dismutase, rabbits.

Acknowlegments: This work was financially supported by VEGA scientific grant Vega 1/0084/12 and 1/0022/13 and Kega 030 SPU-4/2012.

SELECTED BIOCHEMICAL PARAMETERS STUDIED IN WARM-BLOODED MARES

Janka Poráčová², Janka kotosová¹, Marta Mydlárová Blaščáková², Vincent Sedlák¹, Terézia Pošiváková¹

Address(es):

¹Department of Ecology, Faculty of Humanities and Natural Sciences, Presov University in Presov

²Department of Biology, Faculty of Humanities and Natural Sciences, Presov University in Presov

Corresponding author: jankakotosova@hotmail.com

ABSTRACT

The aim of this work was to study the quantitative presence of biochemical parameters in the blood serum of warm-blooded breeds of horses. From the selected biochemical parameters the presence of AST-aspartate aminotransferase, ALT-alanine aminotransferase and LDH-lactate dehydrogenase were monitored in blood serum of warm-blooded mares. The measured minimum and maximum values of AST in the blood serum of warm-blooded mares ranged from 1.95 to 3.51 µkat.l⁻¹, the average value of AST was x = 2.77 µkat.l⁻¹. The measured minimum and maximum values of ALT activity ranged from 0.27 to 0.45 µkat.l⁻¹ and the average value of ALT activity was x = 0.36 µkat.l⁻¹. The minimum and maximum values of LDH activity in blood serum of warm-blooded mares ranged from 1.36 to 6.45 µkat.l⁻¹ and the average value of LDH activity was x = 3.52 µkat.l⁻¹.

Keywords: AST-aspartate aminotransferase, ALT-alanine aminotransferase, LDH-lactate dehydrogenase, warm-blooded mares

INFLUENCE OF INADEQUATE TRAINING ON HORSETENDONAPPARATUS

Dagmar Pospíšilová, Gabriela Klemová

Address(es):

Mendel University in Brno, Agronomical Faculty

Corresponding author: dagmar-pospisilova@seznam.cz

ABSTRACT

Quality of horse tendon apparatus is an important factor in the training exercise. A damage occurs especially if inadequate load. Differences are determined by the type of sport, different disabilities occur in case of racing horses and other ones in case of horses in show jumping. These differences are given by the way of injury, where an important role plays varieties of training and racing track surfaces. For racehorses a damage of superficial digital flexor on the left thoracic limb prevails due to unbalanced repetitive strain injury and fast work on hard uneven surface. In case of show jumping horses a damage of strangulation tendon apparatus caused by foreleg improper bandaging or both digital flexor tendon and interosseous muscle predominates after work on a deep and hard surfaces. Inappropriate surface in connection with fast movement of horse increases frequency of tendon organ damage in case of racing canter horses unlike show-jumping ones who train and compete on the sandy arenas at a slow exercise.

Keywords: training exercise, damage, horse, tendon apparatus

DYNAMICS OF SOME BIOCHEMICAL PARAMETERS IN THE BLOOD SERUM OF MOUFFLONS IN THE PERIOD OF SYNCHRONIZATION TREATMENT AND ON THE DAYS OF OBSERVATION AFTER INSEMINATION

Terézia Pošiváková¹*, Peter Smitka², Janka Poráčová³, Ján Pošivák⁴, Marta Mydlárová – Blaščáková³, Vincent Sedlák¹

Address(es):

¹Department of Ecology, Faculty of Humanities and Natural Sciences, Presov University in Presov

³Department of Biology, Faculty of Humanities and Natural Sciences, Presov University in Presov

Corresponding author: tereziap@centrum.sk

ABSTRACT

The 10 moufflons of 3-7 years of age were included in the experiment. Their weight range was from 32 to 40 kg. The animals were kept in a game preserve and their food consisted of pasture and water were unlimited. Heat is conducted in the month of November. Blood was collected from the jugular vein before synchronization (day 0) and on days 5, 8, and 12 during treatment with tampon Chronogestervag. spong. a.u.v. (Cronolone 20 mg/tampon). To induce oestrus in moufflons eCG was used during oestrus and they were fertilized. Sampling continued in the days and on days 5, 12, 18 and 33 after oestrus. Sodium concentration and K were determined in serum by absorption spectrometry method and tools AtomspecFY-

RangHigler. The concentration of Ca, P, total lipids and cholesterol were determined using BIO-Lachematests (Lachema, Brno). A statistically significant decrease was observed at the peak of oestrus (p<0.01) and also at the end of the trial (p<0.001). K value decreased, were statistically significant (p<0.01) in the days and after oestrus in the days following oestrus 5, 12, 18 and 33 (p<0.001). Concentration of Ca tended fall, were statistically significant (p<0.05, p<0.01, p<0.001, p<0.001) the investigated days. Significant decrease (p<0.05), the concentration of P is from 5 day after oestrus. Mean levels of total lipids significantly diminished after oestrus (p<0.05) and were in the reference range. Starting 5 days after oestrus and at end of the experiment the concentration in the range of 1.59 \pm 0.47 to 1.81 \pm 0.49 g/l.

SEASONAL VARIATIONS IN THE ENZYMATIC ACTIVITY OF ALP AND ALT IN CONVENTIONAL BREEDING OF SLOVAK SPOTTED CATTLE

Vincent Sedlák^{1*}, Janka Poráčová², Marta Mydlárová – Blaščáková², Janka Kotosová¹, Terézia Pošiváková¹

Address(es):

¹Department of Ecology, Faculty of Humanities and Natural Sciences, Presov University in Presov ³Department of Biology, Faculty of Humanities and Natural Sciences, Presov University in Presov

Corresponding author: tereziap@centrum.sk

ABSTRACT

In this paper we focus on the breeding of Slovak spotted cattle in conventional breeding conditions at a farm in the Prešov region and on the selected biochemical parameters. In the work we monitor the enzymatic activity values of selected enzymes (ALP alkaline phosphatase and ALT alanine aminotransferase) in the blood serum of Slovak spotted cattle. The enzymatic activity of selected enzymes were measured in the blood serum of dairy cows (n=20) in the middle of lactation. Blood samples were collected twice a year (autumn and spring). For determination of enzymatic activity we used biochemical analyzer Cobas Integra 400 plus (Roche). The ALP activity of the autumn blood samples (0.57 \pm 0.19 μ kat/L) was not significantly lower in comparison with the ALP activity of the spring blood samples $(0.64 \pm 0.29 \,\mu\text{kat/L})$. The ALT activity of the autumn blood samples $(0.35 \pm 0.08 \,\mu\text{kat/L})$ was significantly lower (p<0.05) in comparison with the ALP activity of the spring blood samples $(0.55 \pm 0.09 \,\mu\text{kat/L})$.

EFFECT OF DIFFERENT CONCENTRATION OF CAFFEINE ON TURKEY SPERMATOZOA MOTILITY IN *IN VITRO* CONDITIONS AT 41°C

Tomáš Slanina¹*, Lucia Slyšková¹, Kinga Kraska², Peter Massányi¹

Address(es):

¹Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic ²Pedagogical University of Cracow, Faculty of Geography and Biology, Institute of Biology, Department of animal physiology and toxicology, Podbrzezie 3, 31-054 Kraków, Poland Corresponding author: slaninatomas@atlas.sk

ABSTRACT

The target of this study was to evaluate the turkey spermatozoa motility in in vitro conditions and to prove the effect of various caffeine concentrations (diluted in physiological solution): 5 mg.ml⁻¹ – A; 2.5 mg.ml⁻¹ – B; 1.25 mg.ml⁻¹ – C; $0.625 \text{ mg.ml}^{-1} - D$ at various time periods at 41°C. Individual motility parameters were recorded at four time periods: 0, 60, 120 and 180 minutes. Semen samples of adult male turkeys of the line Big 6 were diluted a ratio of 1 part of semen and 200 parts of physiological solution (control sample) and with different concentrations of caffeine (experimental groups). Measurements were evaluated by the CASA system (Computer Assisted Semen Analyser). At the beginning of cultivation significantly lower values of spermatozoa motility were detected only in the sample D (p<0.05). Significantly higher values (p<0.001) of this parameter were observed after 60 minutes of cultivation in the samples A, B and the sample D comparing with control. The A sample showed significantly higher values (p<0.05) also at time 120. Spermatozoa progressive motility followed the tendency of spermatozoa motility. Significantly higher data were observed in samples A (p<0.01), B (p<0.05) and the sample D (p<0.01) compared the control after 60 minutes of culture. Non-significant differences were found in all experimental samples in other time of in vitro cultivation. Results of our study detected positive effect of caffeine on the turkey spermatozoa motility cultured at 41 °C..

Keywords: caffeine, turkey, spermatozoa, motility, CASA

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GENDER INFLUENCE ON SNIFFING BEHAVIOR IN DOGS

Michaela Šedivá, Petr Řezáč, Eva Jeřábková

Address(es):

Department of Animal Morphology, Physiology and Genetics, Mendel University in Brno, Zemědělská 1, 61300 Brno, Czech Republic

Corresponding author: sediva-michaela@seznam.cz

ABSTRACT

The objective of the study was to examine sniffing behavior in male and female dogs in open places. It was observed 468 dogs. Female dogs more often sniffed the head of another dog than male dogs. Male dogs more frequently sniffed the backside of another dog than female dogs. Female dogs more often sniffed the belly of another dog than male dogs. Further research is needed to understand the dog's sniffing behavior on walks.

Keywords: Dog, Sniffing, Behaviour

ULTRASTRUCTURAL AND ANNEXIN V-DETECTED DAMAGES IN RAM FROZEN-THAWED SEMEN

Eliška Špaleková^{1,2}, Alexander Makarevich¹, Elena Kubovičová¹, Juraj Pivko¹

Address(es):

¹Institute of Animal Genetics and Reproduction, Animal Production Research Centre Nitra, Slovak Republic ²Department of Animal Physiology, Faculty of Biotechnology

and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic

Corresponding author: spalekova.eliska@gmail.com

ABSTRACT

Semen cryopreservation and thawing processes may cause damage to sperm membrane structures, which can leads to lower viability and motility of post-thaw sperm. The aim of this study was to examine ultastructural alterations and membrane destabilization in frozen-thawed ram semen. Ultrastructural alterations were detected using transmission electron microscopy (TEM). Analyzed sperm heads were classified into 4 categories: I - sperm with intact plasma membrane and intact acrosome; II sperm with waved or cracked plasma membrane; III - sperm with swollen or damaged acrosome; IV - sperm with pseudoacrosomal reaction and loss of acrosomal content. Sperm phosphatidylserine translocation (PS, membrane destabilization) was detected by fluorescently labelled Annexin-V-Fluos kit (Roche), and evaluated under a Leica fluorescent microscope. An annexin V-positivity in spermatozoa was localized on acrosomal part of sperm head, mitochondrial segment (MIs), postacrosomal segment (PAs) and an equatorial segment (EQs). Control groups (CG) were represented by fresh sperm samples. In a fresh semen, about 77 % of the sperm cells were distributed into categories I (19 %) and II (58 %), and 23 % of the spermatozoa were classified into categories III (17 %) and IV (6 %). Oppositely, in frozen-thawed semen more noticeable membrane changes were observed. About 32 % of sperm cells were belonging to the category III and 10 % to the category IV, whilst only 11 % were classified to the category I (intact membranes) and 47 % to the category II (waved membranes). The most of the annexin-V positive sperm in CG were labelled in the acrosomal part of the head (26 %) and in MIs (31 %) or in both areas (28 %). None of sperm in CG were marked in EQs. In frozen-thawed sperm annexin-V was localized mainly in the acrosomal (31 %) and in PAs (23 %) and only 10 % of sperm were marked in MIs, whilst 3 % of sperm were labelled in the EQs. These results indicate negative effect of frozen and thawing process on sperm membrane ultrastructure and stability mainly in acrosomal and post-acrosomal part of the sperm head.

Keywords: acrosome, phosphatidylserine translocation, ram sperm

Acknowlegments: Grant support: APVV-0514-07, APVV-0137-10.

PULMONARY ARTERIAL HYPERTENSION IN ISOLATED PORTAL VEIN DISEASE MAY DIFFER FROM OTHER FORMS OF PORTOPULMONARY HYPERTENSION

Marcela Tavačová, Milan Luknár, Iveta Šimková, Peter Lesný, Eva Goncalvesová

Address(es):

National Cardiovascular Institute, Bratislava, Slovakia

ABSTRACT

Portopulmonary hypertension (POPH) is associated with a poor prognosis in comparison to some other types of pulmonary arterial hypertension (PAH). Due to various causes of portal hypertension, the subgroup of POPH can be heterogeneous and PAH is considered to be related more closely to portal hypertension than to an intrinsic hepatic disorder. We report a retrospective series of 4 consecutive patients with severe POPH due to pre-hepatic portal hypertension and no signs of liver function impairment. All of them were non-responders to acute vasoreactivity testing and were treated with specific treatment. Patient 1 (male) was diagnosed with PAH at the age of 4. At the age of 31, his status deteriorated, dysphonia and abdominal dyscomfort appeared and his PAH was linked to newly diagnosed portal vein atresia. Sildenafil treatment was initiated. After 6 years of treatment, the patient is asymptomatic. Patient 2 (female) was diagnosed with PAH associated with portal vein agenesis at the age of 27. At the age of 30, symptoms worsened and peripheral edema developed. Ambrisentan treatment was initiated. After 4 years of specific treatment, the patient is asymptomatic. Patient 3 (male) was diagnosed with PAH associated with post-traumatic portal vein thrombosis at the age of 49 and bosentan was introduced. After 6 yrs of treatment, the patient's status remains stable, he is mildly symptomatic. Patient 4 (female) was diagnosed with PAH associated with spontaneous portal vein thrombosis due to hypercoagulable state at the age of 48. Sildenafil treatment was started and her status improved. 2 yrs after treatment initiation, her condition deteriorated (WHO FC III, 6-min walking distance 200 m), and ambrisentan was added. After another 2 vrs of combination treatment, her status remains improved in WHO class II. In all cases, no major adverse effects of specific treatment have been reported. Main patient characteristics are summarized in Table 1. Specific treatment in this small case series of pts with POPH due isolated portal vein disease uniformly resulted in an improvement of clinical status, markers of prognosis, and long term survival. This supports the opinion that POPH is a heterogeneous clinical condition with POPH due to pre-hepatic portal hypertension possibly being a distinct entity. Specific treatment including ERA antagonist seems to be warranted and these patients should not be excluded from randomized clinical trials.

	before treatment						
Pt No	WHO FC	mPAP	PAW	CI	PVR	6MWD	NT-proBNP
		(mmHg)	(mmHg)	(l/min/m2)	(W.U.)	(m)	(ng/L)
1	3	85	6	2.1	12.6	520	na
2	2	65	2	4,3	7.2	570	103
3	3	70	10	3.3	10.3	390	na
4	3	50	9	2.8	8.2	300	280
	after treatment						
Pt No	WHO FC	mPAP	PAW	CI	PVR	6MWD	NT-proBNP
		(mmHg)	(mmHg)	(l/min/m2)	(W.U.)	(m)	(ng/L)
1	1	na	na	na	na	690	18
2	1	na	na	na	na	645	52
3	2	53	15	3.4	6.0	450	161
4	2	46	13	3.6	4.8	330	55

Table 1 Main characteristics of patients

Keywords: Pulmonary arterial hypertension, portal vein disease, portopulmonary hypertension

ABSTRACT

PREOVULATORY FOLLICLE DEVELOPMENT IN HIGH YIELDING COWS

Radovan Tomášek, Miroslava Škarková, Petr Řezáč

Address(es):

Department of Animal Morphology, Physiology and Genetics, Mendel University, Zemědělská 1, 61300 Brno, Czech Republic

Corresponding author: <u>skarkova.m@seznam.cz</u>

ABSTRACT

The aim of the study was to examine the development of preovulatory follicles in pregnant and non-pregnant high yielding cows. The treatment by supergestran and oestrophan was used to synchronize the estrous cycle. Ovaries were monitored by transrectal ultrasonography. The linear increase of preovulatory follicles was observed in pregnant (P < 0,001) and non-pregnant (P < 0,001) cows during 8 days before ovulation. In conclusion, preovulatory follicles in pregnant and non-pregnant high yielding cows developed similarly.

Keywords: Cow, ovary, follicle, ultrasonography.

EFFECT OF DIETARY MANGANESE ON LIPID OXIDATION OF EGG YOLK AND ANTIOXIDATIVE STATUS OF LAYING HENS

Katarína Venglovská¹, Ľubomíra Grešáková², Klaudia Čobanová²*

Address(es):

¹Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafarik University, Moyzesova 11, 040 01, Košice, Slovak Republic

²Institute of Animal Physiology, Slovak Academy of Sciences, Šoltesovej 4-6, 040 01, Košice, Slovak Republic

Corresponding author: boldik@saske.sk

The purpose of this experiment was to examine the effect of feed supplementation with various manganese sources on the oxidative stability of egg yolk during storage and some parameters of antioxidative protection in laying hens. Obviously, the performance parameters of laying hens were evaluated too. Twenty-weeks-old laying hens of Lohmann Brown breed were randomly allocated to 4 treatments with 6 replicates of 4 hens in each. For the subsequent 8 weeks the birds were fed the identical basal diet supplemented with equivalent dose of Mn (30 mg/kg) from various sources. Group 1 (control) was given the unsupplemented basal diet (BD) with only background Mn level of 51.7 mg/kg dry matter. Diets for groups 2, 3 and 4 consisted of same BD supplemented with Mn in the form of Mn-sulphate, Mn-proteinate (Mn-Prot) and Mn-glycine chelate (Mn-Gly), respectively. During the entire experiment, the daily feed intake of birds was not affected by dietary treatments. Egg production was significantly increased in group supplemented with Mn-Gly. The feed supplementation with Mn-sulphate resulted in significantly lower egg weight than in birds fed diet with equivalent Mn dose from Mn-Gly and BD only. The feed to egg mass ratio was significantly elevated in group supplemented with Mn-sulphate compared to all experimental groups. Lipid oxidation of egg yolks during cold storage was affected by dietary manganese. The malondialdehyde (MDA) values of egg yolk were started to increase in different storage time (in Group 1, 3 and 4 at 20th, 20th and 30th storage days respectively) during 40 days of cold storage. Lipid oxidation in egg yolks of hens fed diet enriched with Mn-sulphate was stable during entire storage period. Although there were no significant differences in MDA values among the treatments until 30 days of storage, Mnsulphate group showed significantly lower MDA concentration compared to Mn-Gly group after 40 days of storage. Intake of BD enriched with Mn-sulphate resulted in significatly higher activity of glutathione peroxidase in blood and total antioxidant status in plasma of laying hens. The dietary Mn intake did not influence the activity of superoxide dismutase and MDA concentration in liver. The presented results showed that diet supplementation with inorganic form of manganese may improve the oxidative stability of egg yolk. The adequate supplementation of diet with Mn has positive effect on the antioxidative status of laying hens.

Keywords: manganese; laying hens; egg; lipid peroxidation; antioxidant enzymes

Acknowlegments: This study was supported by the Grant Agency for Science, VEGA of Slovak Republic, Grant No. 2/0045/12.

DIFFERENCES IN HAEMOGLOBIN CONCENTRATION AND HAEMATOCRIT LEVEL IN RANA RIDIBUNDA PALL. UNDER A DEHYDRATION STRESS

Bartłomiej Zyśk¹, Łukasz J. Binkowski¹, Marek Guzik¹, Lucjan Schimscheiner¹, Robert Stawarz¹, Tomasz Łaciak¹, Włodzimierz Wojtaś¹, Waldemar Szaroma², Renata Muchacka²

Address(es):

¹Department of Vertebrate Zoology and Human Biology, Institute of Biology, Pedagogical University of Cracow, Podbrzezie 3, 31-054 Cracow, Poland

²Department of Animal Physiology and Toxicology, Institute of Biology, Pedagogical University of Cracow, Podbrzezie 3, 31-054 Cracow, Poland

Corresponding author: <u>bzysk@up.krakow.pl</u>

ABSTRACT

The aim of this study was to evaluate the influence of particular water loss on changes of haemoglobin content and haematocrit level in marsh frog. During these research we used only mature females, taken directly from the natural environment, in twoterms of the annual cycle, i.e. during hibernation (3rd decade of February) and during activity (3rd decade of May). The amphibians in each term were divided into three groups. The control group had unlimited access to water. The experimental groups underwent a controlled dehydration until the loss of respectively 17% and 20% of the body mass. After the planned body mass loss frogs were sectioned and during the process blood samples, directly from the heart ventricle, were taken for the analysis. The haemoglobin concentration was measured with the colorimetric method, using spectrophotometer Spekol 11 (Carl Zeiss Jena) and the haematocrit level was measured with the microhaematocritic method (modified Hedin method). The result of analysis was that the dehydration stress has definite influence on elevation of haemathological indexes in frogs. In comparison to other species it is only a slight elevation. However the grater elevation of haemoglobin and haematocrit values can be observed during the active life period than during the hibernation. It is definitely due to differences of metabolism in hibernated (ponderous) and mating (very active) specimens.

Keywords: haemoglobin, haematocrit, dehydration, marsh frog

ROLE OF INDIAN TRADITIONAL HERBS IN MODULATING REPRODUCTIVE PHYSIOLOGY

Shubhadeep Roychoudhury

Address(es):

Department of Life Science and Bioinformatics, Assam University, Silchar-788011, India

Corresponding author: shubhadeep1@gmail.com

ABSTRACT

Since time immemorial plants are being used as a rich source of effective and safe medicines in India. India has been identified as a part of the top twelve mega bio-diversity hotspots of the world and North- Eastern region of the country accounts for the largest repository of medicinal plants and has a diverse group of ethnic people. In India, plant-derived extracts have been immensely used as crude drugs in traditional medication and this has resulted in an inherited knowledge about the curing prospective of plant species. Fertility regulation with plants or plant preparations has been reported in Avurveda - the ancient Indian literature of indigenous system of medicine. It is is often translated as the "science of life". It tells us (vedayati) which substances, qualities, and actions are life-enhancing (ayusya) and which are not. A large number of plant species with anti-fertility and reproductive health effects have been screened in India beginning about 50 years ago.

Plant products have been long regarded as mainstay of drug discovery programmes. Recent failures in the discovery process of new chemical entities have made natural product researchers to look into history of herbal drug use for search of leads. Reverse pharmacology approach forms the major weapon of search of anti-fertility leads. Long documented/undocumented use of different unexplored medicinal plants by the ethnic groups forms the first step in the search of anti-diabetic lead. Expectation of low toxicity for humans from plants used traditionally can be easily accepted as these plants have been thoroughly tested owing to their use since ages. With the increasing hectic lifestyle which has made the pathogenesis of reproductive health and physiology complex. Modern medicine attempts to use a single compound to hit single target of a particular pathway for combating the related disease which may not prove to be a long-lasting management strategy. On the other hand, traditional medicines exert synergistic effects due to its galaxy of phyto-constituents hitting multiple targets and thus acting through multi-mechanistic pathway. Henceforth, drug discovery spectrum from traditional medicine has now two basic objectives a) Discovery of new chemical entities from plants mentioned in literature of traditional systems of medicine and b) Development of standardized poly-herbal formulations based on traditional medicines formulae. However, to gain global acceptance and better therapeutic management for such metabolic disorders it is essential that such drug discovery approaches be integrated with modern biology to evolve a holistic system biology approach for the management of antifertility as well as reproductive health problems. Through this integration, drug discovery and modulation of reproductive physiology from Ayurveda can make a strong impact on the western world.

Key Words: Drug discovery, Reproductive physiology, antifertility, reverse pharmacology, Indian Traditional Medicine, herbal products

THE EFFECT OF DIFFERENT CONCENTRATION OF FALLOPIAN TUBES (OVIDUCTS) SECRETION EXTRACT ON TURKEY SPERMATOZOA MOTILITY *IN VITRO*

Michal Miškeje^{1*}, Tomáš Slanina², Ida Petrovičová¹, Peter Massányi²

Address(es):

¹Constantine the Philosopher University in Nitra, Faculty of Natural Sciences, Department of Zoology and Anthropology Nábrežie mládeže 91, 949 74 Nitra, Slovak Republic ²Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic

Corresponding autor: michal.miskeje@gmail.com

ABSTRACT

Viability of turkey semen is very important in the management of turkey reproduction. It can be influenced by many factors and additives. Very important from these factors is survival of spermatozoa in female oviduct. Target of our study was to analyze the effect of different concentrations of extracts from fallopian tubes (oviducts) on the motility parameters of turkey spermatozoa in vitro at different time intervals (0, 240 and 1440 minutes) at 5°C. Oviducts were collected from 9 adult female turkeys immediately after defeat. Samples were diluted and divided into four groups A (100% oviducts extract), B (50%), C (25%) and K (diluted only with saline solution) as a control. The measurements were carried using CASA computer-assisted semen analysis. Similar values of progressive motility were found at time 0 minutes (values between 30.72% -K and 35.81% – B). After 240 minutes of culture considerable differences between the "oviducts" groups and control group were observed. The values in the all experimental groups generally increased compared to the control group. Similar results were detected after 24 hours of cultivation, but all values were decreased against 240 minutes of cultivation. Our work indicates that oviducts secret has positive effect on spermatozoa motility and viability. In all concentrations and time periods in experimental groups higher values of progressive motility compared to the control are reported.

Keywords: turkey, spermatozoa motility, oviduct secretion, CASA