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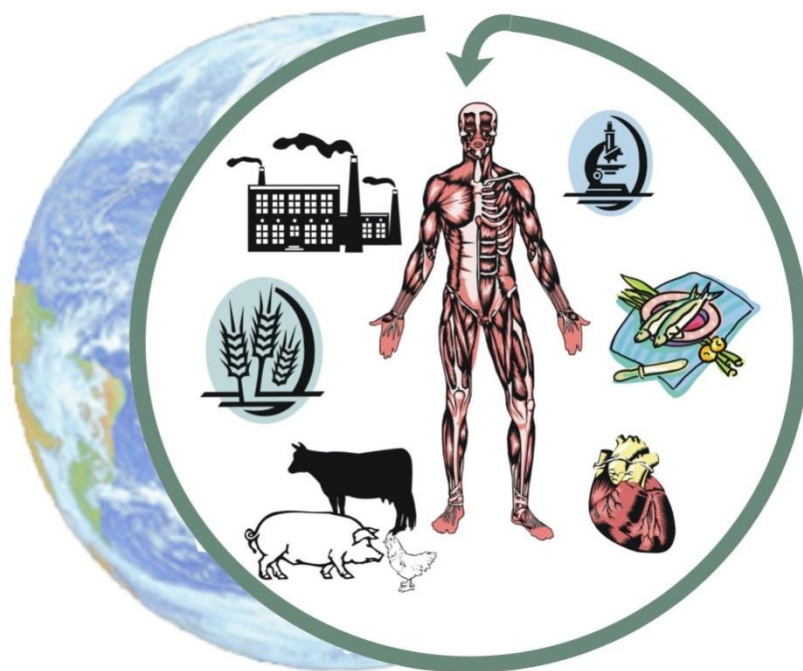
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# **XVI Risk Factors of Food Chain 2015**

Book of Abstracts

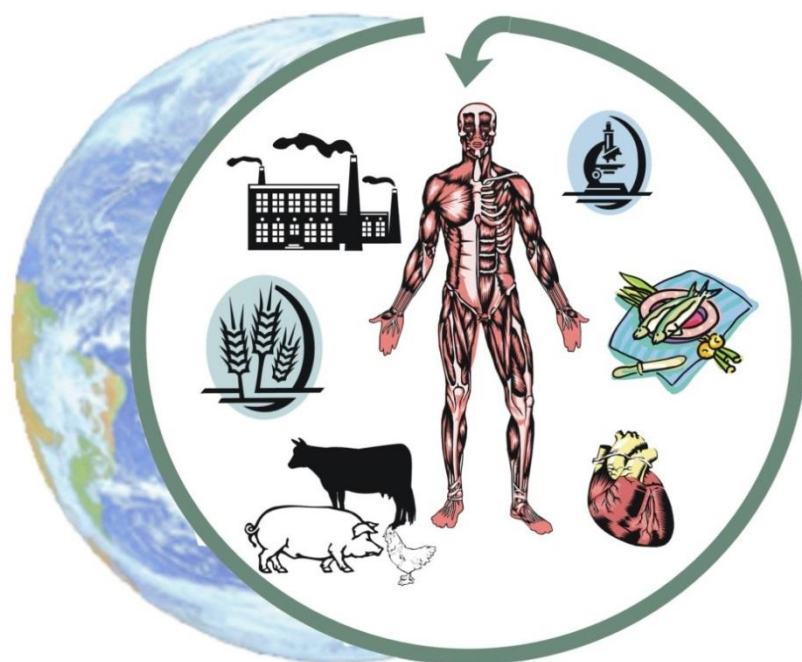


**October 19–21, 2015**

**Dudince, Slovak Republic**

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Slovak University of Agriculture in Nitra, Slovak Republic**

**Institute of Biology  
Faculty of Geography and Biology,  
Pedagogical University in Krakow, Poland**

**Department of Animal Physiology and Health,  
Faculty of Agricultural and Environmental Sciences  
Szent István University Gödöllő, Hungary**

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## **XVI Risk Factors of Food Chain 2015**

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## EFFECTS OF DIAZINON ON ALKALINE PHOSPHATASE ACTIVITY IN OSTEOBLASTS

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The effect of the organophosphate insecticide diazinon on alkaline phosphatase (ALP) activity during *in vitro* osteogenic differentiation was evaluated. Primary human osteoblasts (PromoCell, Heidelberg, Germany) were cultured for 72 hours in complete medium (control) and in complete medium with different doses of diazinon (1, 10, 50  $\mu$ M). The BCIP/NBT substrate (Sigma Aldrich) was used for colorimetric detection of ALP activity. The relative ALP activity was calculated from the absorbance values measured at 440 nm. Diazinon exposure induced a dose-dependent reduction in ALP compared to controls. The enzymatic activity decreased by 7, 24, and 31 % at a dose of 1  $\mu$ M, 10  $\mu$ M, 50  $\mu$ M ( $P < 0.01$ ), respectively. Reduced ALP activity demonstrated the inhibition of bone formation. The present study demonstrates that exposure to diazinon may impair bone health through decreased osteoblast differentiation and function.

**Keywords:** diazinon, osteoblasts, alkaline phosphatase activity

**Acknowledgments:** This study was supported by the grant KEGA 035 UKF-4/2013.



## THE EFFECT OF QUERCETIN ON FEMORAL BONE STRUCTURE IN MALE RABBITS

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Quercetin is an important phytoestrogen present in a variety of fruits and vegetables. It has beneficial effects on human health, including cardiovascular protection and anticancer activity. However, little information is available on its role of on femoral bone structure. Therefore, the aim of our study was to investigate the effect of a high dose of quercetin on bone microstructure in 5-month-old male rabbits. Five rabbits of Californian broiler line were randomly divided into two experimental groups. In the first group (E group; n = 3), animals were intramuscularly injected with quercetin at dose 1000 µg.kg<sup>-1</sup> for 90 days, 3 times per week. Two rabbits without quercetin administration served as a control group (C group). At the end of the experiment, cortical bone thickness and histological structure of femoral bones were evaluated in each group. Our results showed no adverse impact of quercetin on cortical bone thickness in investigated animals. Microscopic structure of the femurs from rabbits of the E group has to be almost identical than in rabbits of the control group. Changes were present only in the middle part of the compacta, where a reduced number of secondary osteons was observed. From histomorphometrical point of view, a significant decreased sizes of primary osteons' vascular canals (P < 0.01) and secondary osteons (P < 0.01) were found in rabbits exposed to quercetin. Our findings allow for the conclusion that intramuscular administration of quercetin has considerable impact on both qualitative and quantitative histological characteristics of femoral bones in adult male rabbits.

**Keywords:** quercetin, femoral bone, rabbit, histomorphometry

**Acknowledgments:** This study was supported by the grant KEGA 035 UKF-4/2013.



## EXAMINATION OF COLOUR, ODOR AND TASTE CHARACTERISTICS OF FRUIT JUICE PRESERVED BY HEAT TREATMENT AND COOLING

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It is often discussed what effects preservation methods take on food characteristics. Colour, odour and taste determine the character of the given product, at the same time these are subjective parameters due to different tastes and sensing capabilities. Perhaps this is why an ever increasing demand occurs for analytical or instrumental determination of these three characteristics. In our paper we compared the sensory parameters of fresh apple juice samples and those preserved by cooling and heat treatment at the Faculty of Mechanical Engineering in the Szent István University by involving agricultural and food engineering students. The parameters determining the product were verified by instrumental and analytical tests as well. The fresh and pasteurised apple juice was provided directly by RAUCH Hungaria Ltd. A portion of the fresh apple juice was cooled down and stored in the lab of the Szent István University's Faculty of Mechanical Engineering under controlled and checked circumstances at +4 °C for 7 days. Colour, odour and taste characteristics were assessed by also sensory and analytical tests as well. People's opinion is decisive in case of every food product. Sensory tests declared by standards are intended to determine the quality of a given food product. During the consumer sensory tests we may choose from a wide variety of methods. The most frequently applied procedures are the A – Not A one sample test, paired preference, triangle test, classification and free describing test. We applied the triangle test for deciding the samples different or not. During the triangle test the amateur examiners (students) received three encrypted samples. There are two identical and one different in the sample group. Task of the tester was to find and mark the different sample. The students did not participate in either the preparation works or evaluation processes. The CIE Lab coordinates of colour, the odour unit of the sample (odour concentration), the physical and chemical properties as density, pH-value, conductivity, redox-potential, dry matter content, acid content were determined by standards with analytical methods. The target of the examination was to establish whether a difference can be detected between the fresh and preserved samples by the colour, odour, and taste. Based on our results which were performed by means of sensory test we established that the odour characteristics were the most significant deviations between the samples. However this could not be justified by standardized analytical examinations. Preservation operations (cooling and heat treatment) had the least impact for the colour parameters of apple juice. Neither analytical nor instrumental tests were capable of detecting significant difference between the characteristics of the three samples. Our tests may be interpreted in preservation by cooling or heat treatment could not cause detectable deviations in the products.

**Keywords:** sensory test, colour, odour, taste, apple juice

**Acknowledgments:** Our research was supported by „Basic and industrial research in association with production of food of plant and animal origin meeting the food safety requirements” KTIA\_AIK\_12-1-2012-0012 project.





## TAURINE AND ETHANOL INFLUENCE ON ALKALINE PHOSPHATASE IN MICE SERUM

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Alkaline phosphatase (ALP) is a membrane bound enzyme which acts as a marker for the endoplasmatic reticulum and plasma membrane. The activity of ALP may be affected by cell membranes damage, therefore the measurement of its activity can be useful indicator of e.g. liver function. It is well-known that sustained heavy alcohol drinking promotes the development and progression of liver disease. Fortunately, there are substances which prevents toxin-mediated liver injuries. One of them is taurine – sulphur containing  $\beta$ -amino acid which protects from organs injuries due to several actions: modulation of calcium mitochondrial level, enhancement of mitochondrial functioning and oxidative stress reduction. The aim of present study was to determine if it is possible to reverse the effects of alcohol consumption on liver condition by taurine supplementation. In each series of the experiment (24 hours, 14 days and 56 days) 20 adult Swiss Albino males mice were used and divided into 4 groups: control (tap water), ethanol (treated with 15% ethanol added to tap water), taurine (injected intraperitoneally with 20 mg/kg of taurine) and ethanol-taurine (15 % ethanol + 20 mg.kg taurine). Serum ALP activity was determined by a kit from BioSystems, SA. Spain. We detected that taurine decline ALP activity in mice treated with ethanol in each series of our experiment, suggesting it exerts protective effect on the alcoholic liver.

**Keywords:** alkaline phosphatase, ethanol, hepatic injury, taurine



## FISH GILLS ULTRASTRUCTURAL CHANGES AS A BIOMARKER OF HERBICIDE CONTAMINATION OF SURFACE WATERS

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Using herbicides to control unwanted plant species has become a common practice worldwide. Herbicides are often used in modern agriculture and landscape management. They can enter aquatic habitats as a result of agriculture runoff, spray drift or erosion. The aim of the study was to investigate the effects of herbicide pendimethalin on the ultrastructure of common carp (*Cyprinus carpio*) gills. During the experiment fish were kept in aquaria in controlled environmental conditions. In the first period of the experiment (14 days) animals were exposed to low concentration of pendimethalin ( $2.5 \mu\text{g}\cdot\text{L}^{-1}$ ). After 14 days fish were transferred to clean water for the next 30 days (recovery period). The study showed that low pendimethalin concentration can cause some ultrastructural changes in gills. Decrease in the number of microfolds in respiratory epithelial cells of secondary lamellae in pendimethalin-exposed fish was observed. Pathological changes: hyperplasia and hypertrophy of secondary lamellae were also observed. The same changes were observed in the osmoregulatory epithelium. The tested herbicide caused increase of mucosecretory activity in gills of pendimethalin-exposed fish. Between adjacent secondary lamellae cellular infiltrations were present. It resulted in frequent merging of adjacent secondary lamellae. The recovery time of 30 days was too short for complete regeneration of epithelial cells.

**Keywords:** common carp, *Cyprinus carpio*, biomarkers, surface waters contamination, herbicides

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## CITRININ – CONTAMINANT OF FOOD AND FEED

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Mycotoxins are the secondary products of fungal metabolism and they are small and quite stable molecules which are extremely difficult to remove and enter the food and feed chain while keeping their toxic properties. Is it a global problem because more than 25 % of world grain production is contaminated by mycotoxins. Mould production of mycotoxins in food is influenced by a number of factors such as temperature, water activity, substrate composition, mould physiology, or interactions with other microbes. Citrinin is a toxic metabolite produced by several filamentous fungi of the genera *Penicillium*, *Aspergillus* and *Monascus*, which has been encountered as a natural contaminant in grains, foods, feedstuffs, as well as biological fluids. Citrinin is generally formed after harvest and occurs mainly in stored grains, but also in other plant products such as beans, fruits, fruit and vegetable juices, herbs and spices, and also in spoiled dairy products. Citrinin has nephrotoxic, hepatotoxic, genotoxic, teratogenic and immunotoxic effects. For animals, risk characterisation was based on the estimate of the citrinin concentration in grains that would result in exceedance of the NOAEL (no-observed-adverse-effect-level) of 20 µg.kg b.w. per day for pigs, which ranged between 640 and 1 173 µg.kg. In recent years, only a few studies have been published on the toxicity of citrinin. Further research needs to be focused on the generation of data dealing with epidemiological and toxicity effects, especially in humans. There is also a need for well-designed toxicological studies in laboratory animal species to further explore the toxicological potential of citrinin and to characterize the dose-response relationships. Strategies for reducing the exposure to mycotoxins is to decrease their bioavailability and degradation of mycotoxins into non-toxic metabolites by using biotransforming agents such as bacteria/fungi or enzymes.

**Keywords:** mycotoxins, citrinin, food, feed

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



## CHANGES IN HAEMATOLOGICAL PARAMETERS AFTER EXPOSURE BY ZEARALENONE IN VITRO

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Mycotoxins are secondary metabolites of fungi present in animal nutrition. Thus, health and performance of the animals may be compromised as well as the quality of animal derived food. Zearalenone (ZEN) is commonly found in widely-used ingredients for many human and animal foods, such as maize, barley, wheat, oats, sorghum and sesame seeds. Its major *in vivo* metabolite  $\alpha$ -zearalenol, classified as endocrine disruptor, exert estrogenic like action and has influence on the reproductive system in animals. Acute ZEN administration caused deleterious haematological effects in some animals, so also blood may be target for ZEN. The goal of this *in vitro* study was to analyse haematological parameters of porcine blood after mycotoxin ZEN exposure. Samples of porcine blood were incubated with ZEN:  $10 \mu\text{.ml}^{-1}$  in E1 group,  $100 \mu\text{g.ml}^{-1}$  in E2,  $1000 \mu\text{g.ml}^{-1}$  in E3 group for 6 hours at  $37^\circ\text{C}$ . The group without any addition served as the control. ZEN exposure caused significant decrease of white blood cells count and lymphocyte count in all experimental groups when compared to the control. The count of red blood cells (RBC) was significantly lower the in porcine blood followed by increase of haemolysis and decrease of haemoglobin and haematocrit. The count of platelets was significantly decreased after ZEN exposure in all experimental groups against the control. Results of this study provide a foundation for further analysis and researches on mycotoxins impact on living cells and the system of possible protection against its effects as well as evaluation of various dose dependencies on haematological parameters.

**Keywords:** rats, ovarian fragments, molybdenum, silver, SOD

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



## TIME-COURSE EFFECT OF 4-NONYLPHENOL ON H295R CELL LINE

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In our previous dose-response study we investigated the effect of 48h exposure of 4-nonylphenol (4-NP) in 0.2–50 µg.ml concentration range on the sexualsteroid production of H295R human adrenocortical cell line. We found a dose-dependent decrease of progesterone (P) and androstenedione (A) and increase of testosterone (T) production. The 17β-estradiol (E2) production was also increased, but its peak was at 10 µg.ml<sup>-1</sup> 4-NP concentration. However, 4-NP decreased the viability of cells at 25 µg.ml<sup>-1</sup> and above. The aim of this study was to clarify this discrepancy between the viability and hormonal results. Our hypothesis was that cytotoxicity of 4-NP was likely time-dependent and T and E2 levels might be strongly elevated before the cell death. This theory was partially supported by the time-course viability study of 4-NP presented here. In this study we measured the H295R cell viability after 4, 8, 12, 16, 24 or 48 h of 4-NP exposure. The cell viability was above 80 % at 25 µg.ml<sup>-1</sup> 4-NP even after 24h exposure and at 50 µg.ml<sup>-1</sup> 4-NP after 4 h exposure. As a next step we would like to justify our hypothesis with hormonal measurements of this time-course study.

**Keywords:** endocrine disruptors, reproductive toxicology, cytotoxicity, sexualsteroids, testosterone, androstenedione, progesterone, 17β-estradiol



## IMPACT OF LOAD ON SELECTED INDICATORS OF HAEMATOLOGY IN HORSES

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Evaluation of biochemical, hematological, and physical parameters of horses in relation to age, breed and sporting events is imperative for the assessment of health and fitness levels of horses. The goal of this study was to examine the effect of performance on the hematologic parameters after an endurance race. In the experiment was included 6 horses (3 operating horse, 3 sport horses), bred at the Experimental center of livestock on DAH. We studied the impact of load on a conveyer of 30 minutes on selected haematological parameters during the trial (C – control), the first day after load after 5 and 30 minutes (1a5, 1a30) and seventh day after exercise also at 5 and 30 minutes (7a5, 7a30). We collected blood from the jugular vein at specified intervals. In blood we determined the red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT) and the platelet count (PLT) immediately after the collection with an automated hematology analyzer Abacus Junior Vet. From the results, we calculated the basic statistics, and the differences were tested by t-test and ANOVA in SAS program. RBC Operating horses had an average level of leukocytes at rest (C)  $6.56 \times 10^{12}/l \pm 0.82$ , (1a5)  $7.10 \times 10^{12}/l \pm 0.77$ , (1a30)  $6.46 \times 10^{12}/l \pm 0.70$ , (7a5)  $6.99 \times 10^{12}/l \pm 0.67$ , (7a30)  $6.31 \times 10^{12}/l \pm 0.60$ . The sport horses (C)  $7.74 \times 10^{12}/l \pm 0.09$ , (1a5)  $7.40 \times 10^{12}/l \pm 0.82$ , (1a30)  $7.57 \times 10^{12}/l \pm 0.22$ , (7a5)  $7.30 \times 10^{12}/l \pm 0.78$ , (7a30)  $6.84 \times 10^{12}/l \pm 0.40$ . HGB For operating horses were recorded mean levels at rest (C) 134.33 g/l  $\pm 14.79$ , (1a5) 139.00 g/l  $\pm 7.00$ , (1a30) 129.67 g/l  $\pm 17.79$ , (7a5) 137.67 g/l  $\pm 7.02$ , (7a30) 129.33 g/l  $\pm 5.69$ . For sport horses are ranged at a level (C) 152.33 g/l  $\pm 12.58$ , (1a5) 146.00 g/l  $\pm 7.94$ , (1a30) 148.67 g/l  $\pm 10.07$ , (7a5) 142.33 g/l  $\pm 10.26$ , (7a30) 138.67 g/l  $\pm 10.60$ . HCT Levels ranged in operating horses (C) 24.92 %  $\pm 2.70$ , (1a5) 25.94 %  $\pm 0.99$ , (1a30) 24.68 %  $\pm 2.95$ , (7a5) 25.56 %  $\pm 1.16$ , (7a30) 24.19 %  $\pm 1.34$ . At sport horses (C) 28.14%  $\pm 1.44$ , (1a5) 26.60%  $\pm 1.46$ , (1a30) 27.65%  $\pm 1.45$ , (7a5) 25.96%  $\pm 1.85$ , (7a30) 25.76%  $\pm 2.16$ . PLT Operating horses had levels of PLT in the control (C)  $69.67 \times 10^9/l \pm 18.48$ , (1a5)  $68.00 \times 10^9/l \pm 15.59$ , (1a30)  $79.00 \times 10^9/l \pm 26.00$ , (7a5)  $82.67 \times 10^9/l \pm 5.69$ , (7a30)  $90.00 \times 10^9/l \pm 27.07$ . Sports horses (C)  $85.33 \times 10^9/l \pm 6.66$ , (1a5)  $49.00 \times 10^9/l \pm 26.85$ , (1a30)  $51.00 \times 10^9/l \pm 47.74$ , (7a5)  $71.33 \times 10^9/l \pm 18.77$ , (7a30)  $53.00 \times 10^9/l \pm 56.32$ . Neither among the groups nor between operational and sport horses have not seen significant differences. The highest levels of the monitored parameters, we found 5 minutes after exercise in RBC, HGB, HCT and 30 minutes after exercise in PLT.

**Keywords:** RBC, HGB, HCT, PLT, horses

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## STUDY OF THE TRANSFER OF HEAVY METALS FROM SOIL TO HONEY

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Cadmium presence in honey is caused mainly by soil contamination from where it is easily transferred to plants and then to bee products. Lead, which is generally not transported by plants, can contaminate air and then directly nectar and bee products. Honey bees (*Apis mellifera*) have a great potential for detecting and monitoring the environmental pollution. It is because they are directly depending on the toxicological conditions of environment. Moreover, honey bee body works as bio-filter and only small part of contaminants such as heavy metals migrate to honey. The aim of this study was to control the transfer of cadmium and lead from soil, through melliferous plants and honey bees to honey. Samples of honey bees and honey (n = 27) were taken directly from beekeepers localized in Podkarpacie region. Samples of soil and melliferous plants (n = 27) were collected from areas where nectar flow was collected. The ICP-OEC method for determination of heavy metals with prior microwave mineralization was used. The similar level of cadmium was detected in the tested soil and plant samples and ranged from 0.040 to 1.250 mg.kg<sup>-1</sup> and 0.040 to 1.740 mg.kg<sup>-1</sup>, respectively. The highest level of this metal (up to 3.190 mg.kg<sup>-1</sup>) was detected in the body of honey bees and the lowest in honey which ranged from 0 to 0.050 mg.kg<sup>-1</sup>. Among all tested samples, soil was the most contaminated by lead and reached in average 23.342 mg.kg<sup>-1</sup>, which was 6 times higher than in plants. In the case of honey bees and honey 30 % samples were completely free of lead and the maximum detected level was 0.767 and 0.395 mg.kg<sup>-1</sup>, respectively. Results indicate that honeys from Podkarpacie region contain heavy metals in concentrations well below allowable levels and are safe for human consumption. Moreover conducted test confirm bees are especially susceptible to cadmium accumulation.

**Keywords:** bees, *Apis mellifera*, bee honey, contamination, heavy metals, plat-soil relations



## THE IMPACT OF SODIUM L-GLUTAMATE AND $\alpha$ -LIPOIC ACID ON ANTIOXIDANT ENZYME ACTIVITY IN SELECTED ORGANS OF MICE

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The aim of this study was to evaluate the effect of intraperitoneal administration of sodium L-glutamate (MSG) and  $\alpha$ -lipoic acid (LA) on the activity of antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in the liver, kidney, pancreas and brain of mice. Forty adult male mice, line Swiss, were divided into two control and six experimental groups. The experimental animals of the first series received a single, intraperitoneal injection (i. p.) of MSG at the dose of 6 mg.kg<sup>-1</sup> b.w. Mice of the second group received a single, intraperitoneal injection (i. p.) of alpha-lipoic acid (ALA) at the dose of 100 mg.kg<sup>-1</sup> b.w. The experimental animals of the third group received MSG at the dose of 6 mg.kg b. w. and after one hour ALA at the dose 100 mg.kg<sup>-1</sup> b.w. Control and experimental animals of first series were decapitated 24 hours after the injection. Animals of the second series, which were divided into analogous groups to animals of the first series, were decapitated 48 hours after injection. The variance analysis test (ANOVA) showed a statistically significant decrease in the activity of examined enzymes in the liver and brain after administration of MSG, while LA treatment resulted in a significant increase in the activity of GPx and CAT relative to control values. Co-administration of both compounds caused a significant increase in these enzymes only after 24 hours of injection in the liver. Similarly, in the kidneys and pancreas administration of MSG caused a decrease in the activity of the enzymes after 24 hours while the LA injection caused an increase in the activity of GPx and CAT. The study showed that MSG is a compound that causes disturbances of antioxidant barrier while LA has shown strong antioxidant properties.

**Keywords:** sodium L-glutamate,  $\alpha$ -lipoic acid, superoxide dismutase, catalase, glutathione peroxidase





## MILK THISTLE SEEDS MODULATES THE PERIPHERAL BLOOD CELL COUNT IN A MICE INFLAMMATION MODEL

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Milk thistle (*Silybum marianum* (L.) Gaertn.) and its seeds contain a whole family of natural compounds, called flavonolignans. Silimarin is a dry mixture of these compounds, which are extracted after processing with ethanol, methanol, and acetone. Silimarin contains mainly silibin A, silibin B, taxifolin, isosilibin A, isosilibin B, silichristin A, silidianin, and other compounds in smaller concentrations. Silibinin, a major flavonolignan isolated from milk thistle seeds, is being used clinically as a hepatoprotective and antioxidant agent in Asia and Europe. The aim of this study was to investigate the effect of aqueous extract of the herb or extract of the seeds of milk thistle on leukocytes in an animal model of inflammation induced by Zymosan A. Male mice (Swiss) were used in the experiment. The animals were divided into four groups: one control and three experimental groups. The control group - healthy mice treated with saline; I experimental group - mice after intraperitoneal injection of Zymosan A, the second experimental group - mice after oral administration of the aqueous extract of *Silybum marianum* seeds, the third experimental group - mice after a single intraperitoneal injection of Zymosan A and after oral administration of aqueous extract of the *Silybum marianum* seeds. The experiment lasted four weeks, on day 29 of the experiment the animals were decapitated. The peripheral blood of all animals tested was determined the number of neutrophils, eosinophils, basophils, lymphocytes and monocytes. The study shows that in all treatment groups showed alterations in the total number of neutrophils, eosinophils, lymphocytes and monocytes compared to the control group. The results of this study further strengthened the earlier works on the medicinal benefits of *Silybum marianum* seeds and its virtue as a good pharmacological source of hematopoiesis.

**Keywords:** milk thistle, *Silybum marianum* (L.) Gaertn , Zymosan A, leukocytes



## THE IMPACT OF 4-NONYLPHENOL ON THE VIABILITY AND ANDROSTENEDIONE PRODUCTION OF MICE LEYDIG CELLS

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There is growing interest in the possible health threat posed by endocrine-disrupting chemicals (EDCs), which are substances in our environment, food, and consumer products. Endocrine-disrupting chemicals interfere with the synthesis, metabolism or action of hormones, resulting in a deviation from normal homeostatic control or reproduction. Continuous exposure of these types of compounds induces problems in human and animal health. Nonylphenol ethoxylate (NPEO) has been proven to be an endocrine disruptor. The primary degradation product of NPEO is nonylphenol (NP). It is a toxic xenobiotic compound capable of interfering with the hormonal system of numerous organisms. NP is hazardous to the health of human and animals, especially to male fertility. Many studies showed toxic effects of this compound in whole cells or in subcellular structures and organelles. The objective of this *in vitro* study was to determine the effect of 4-nonylphenol as an endocrine disruptor on the viability and androstenedione production of mice Leydig cells as a biological model. Leydig cells are located in the testicular interstitium and are the primary site of synthesis and secretion of testosterone, the major male sex hormone essential for the normal functioning of the male reproductive system. The use of primary cultures for the study of Leydig cells physiology is particularly difficult because of their low abundance in the testes. Our Leydig cells were obtained from mice testes, but the methods needed to isolate them are rather laborious. The cells were cultured for 44 h with the addition of 0.04; 0.2; 1.0; 2.5 and 5.0  $\mu\text{g}\cdot\text{mL}^{-1}$  of 4-nonylphenol and compared to the control. Concentrations of androstenedione in the media samples were determined using enzyme linked immunosorbent assay (ELISA). The 4-nonylphenol concentration increased the androstenedione release in three applied concentrations. The hormone production significantly ( $P < 0.01$ ) increased at 1.0  $\mu\text{g}\cdot\text{mL}^{-1}$  and higher concentrations (2.5 and 5.0  $\mu\text{g}\cdot\text{mL}^{-1}$ ) of 4-nonylphenol. Mitochondria are one of the targets of toxic compounds influence and 4-nonylphenol can act as very strong mitochondrial uncoupler. The viability of mice Leydig cells was detected by the MTT (metabolic activity) assay estimating the mitochondria structural integrity. We observed an increase at the doses of 1.0; 2.5 and 5.0  $\mu\text{g}\cdot\text{mL}^{-1}$  of 4-nonylphenol, but this increase was not significant. The obtained data indicate dose-dependent increases in androstenedione production of mice Leydig cells following a 44 h *in vitro* 4-nonylphenol exposure. The results from our experiments suggested that 4-nonylphenol at lower doses does not damage mitochondria and thus not decrease the mitochondrial activity of mice Leydig cells.

**Keywords:** nonylphenol, Leydig cells, androstenedione, MTT

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## MINERAL PARAMETERS OF BLOOD IN RABBITS AFTER APPLICATION OF PATULIN

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Patulin (PAT) is a mycotoxin produced by certain species of *Penicillium*, *Aspergillus*, and *Byssoschlamys*. However, there is little information available concerning its toxic behaviour *in vivo*. In the present study, we investigated PAT-induced changes in selected parameters of mineral profile (calcium, phosphorus, magnesium, potassium, sodium, chlorides) in rabbits. Adult female rabbits (n = 10) were used in experiment. Animals were divided into two groups: control group (C) and experimental group E1. Animals from experimental group received patulin through intramuscular injection 10 µg.kg<sup>-1</sup> (*Musculus biceps femoris*) twice a week. Experiment lasted 30 days. The blood serum was separated from whole blood by centrifugation at 3000 g for 30 min. The following parameters (calcium, phosphorus, sodium, potassium, magnesium chlorides) were determined using automatic analyser RX Monza (Randox, United Kingdom) and microprocessor-controlled analyser EasyLite (Medica, Bedford, USA) according to the manufacturer's instructions. Mineral parameters were not influenced (P > 0.05) after intramuscular injection of patulin. Details of the physiological mechanism involved in this should be further investigated.

Key words: rabbits, patulin, mineral parameters, blood

**Keywords:** rabbits, blood samples, mineral parameters, patulin (PAT)

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## EFFECT OF COENZYME Q10 ON THE MALONDIALDEHYDE CONCENTRATION IN MICE MODEL OF INFLAMMATION

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Coenzyme Q10 is a natural antioxidant present in animals in every cell of the body. The greatest concentration of Coenzyme Q10 are characterized by tissue with the greatest demand for energy. Primordium is involved in electron transport in the respiratory chain, accelerates metabolism, preventing the formation of free radicals, prevent initiation and propagation of peroxidation of polyunsaturated fatty acids, lipids and phospholipids in mitochondrial membranes. The concentration of Coenzyme Q10 with age, poor diet, drug abuse or alcohol is lowered. The result of the shortage of the compound is a disturbance of function of various organs and systems especially the cardiovascular and immune systems. Inflammation is the body's response to infections, caused by damaging agent: biological, physical, chemical. The aim of this study was to analyze the levels of malondialdehyde after the administration of Coenzyme Q10 as well as in the course of inflammation caused by injection of Zymosan A. The study was conducted on 48 male Swiss white mice, in two series after 3 and 6 hours of each series of animals were divided into four groups, namely one control and three experimental = 6 in each group. Animals of the control group were given *per os* olive oil at a dose of 100  $\mu$ l, the first experimental group were dosed intraperitoneally with Zymosan A at 75  $\text{mg}\cdot\text{kg}^{-1}$  of body weight, the second experimental group *per os* – Coenzyme Q10 at a dose of 30  $\text{mg}\cdot\text{kg}^{-1}$  of body weight, while mice third test group Zymosan A and Coenzyme Q10. Were taken the pancreas, liver and kidneys. The studies it can be concluded that Coenzyme Q10 significantly reduces the concentration of malondialdehyde or protective functions and indicates the antioxidant properties of this compound. In contrast, administration of Zymosan A causes inflammation which increases oxidative stress, increased lipid peroxidation by the increase in the concentration of malondialdehyde, which is one of the markers of oxidative stress.

**Keywords:** coenzyme Q10, inflammation, MDA



## REDUCTION OF DON-TOXIN CONTENT IN WHEAT

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Across the whole food chain – from the production of raw material to processing and sale of final product - the adequate technical background is important factor to fulfil the food safety criteria determined by laws and market requirements. Therefore it is essential that the suppliers can deliver machinery and equipments which must correspond to the food safety requirements and these conditions can be guaranteed for a long time. Adequate technical conditions increase food safety. It help food business operators and their suppliers to be able to ensure that all stage of production, processing and distribution of food satisfy the relevant hygiene, chemical and microbiological requirements laid down in the regulations. The objective of my research work is to investigate: Is there any, justifiable way to decrease the level of mycotoxin - within this deoxinivalenol (DON) content – of breadmaking wheat during the milling process. The source of food safety in food chain is that the primary products suit the food safety requirements. Basic requirement is that these products don't contain microbiological, chemical and other contaminations, or at least not more than the maximum allowable limits. It is a very difficult or sometimes it is not possible to correct food safety risk factors – which got into the products during cultivation – in the course of processing. Such factor is fusariotoxin in fodder and bread wheat. DON-toxin is the most frequent toxin in cereals. This is a very unvarying chemical substance which stands out heat effects and doesn't decompose in the manufacturing, storage and handling procedures. It follows that, if the milling industry process such raw materials, which is infected and polluted by toxin, there is this mycotoxin in processed cereals, too. The place of investigation was at the Júlia Malom Ltd, where I studied how is possible to decrease toxin content of durum wheat, and what extent by application of Sortex Z optical sorter.

**Keywords:** DON-toxin, food safety, technical conditions, breadmaking wheat, food chain

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## DIFFERENCES IN LIPID PARAMETERS OF RABBIT'S BLOOD FOLLOWING ADMINISTRATION OF T2 TOXIN: GENDER COMPARISON

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The aim of the study was to determinate differences after application of T2 toxin on selected lipid parameters (cholesterol, TAG ) of rabbit's blood. Adult male rabbits (n = 10) and female rabbits (n = 10) were divided into two groups (n = 5 in each group), control group (C) and experimental group. The rabbits in the experimental group received T2 toxin in injectable form (intramuscularly) at 10 µg.kg<sup>-1</sup> for 90 days 3 times a week. Control group received injection water. Blood samples were analyzed by automatic clinic analyzer Microlab 300. Higher values of cholesterol and triacylglycerol in the control groups in comparison with all experimental groups were found, however without significant differences (P > 0.05). Based on these results further investigation is needed to obtain a final answer concerning the effect of T2 toxin on lipid metabolism of rabbit's blood.

**Keywords:** T2 toxin, rabbit, cholesterol, TAG, gender comparison

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## OPTIMIZING FED-BATCH CULTIVATION OF RIBOFLAVIN OVERPRODUCING YEASTS *CANDIDA FAMATA* #91 STRAIN USING DIFFERENT ANTIFOAMS

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Riboflavin, also known as vitamin B<sub>2</sub> or additive E101, is an easily absorbed micronutrient with a key role in maintaining health in humans and animals. It serves as biosynthetic precursor of flavin nucleotides FMN and FAD used as coenzymes by flavoproteins. As such, vitamin B<sub>2</sub> plays a key role in energy metabolism, for metabolism of fats, ketone bodies, carbohydrates, and proteins. Recently, applying approaches of random mutagenesis and metabolic engineering, a riboflavin overproducing strains of the flavinogenic yeast *Candida famata* were constructed. The aim of this work was optimization of cultivation conditions for maximal accumulation of riboflavin by the isolated recombinant strain *C. famata* #91. A series of experiments were carried out to study the effects of different antifoams for the riboflavin production by the *C. famata* #91 strain during fermenter cultivation. For this, foaming was suppressed by addition of four sterile antifoams: Structol 673, Silicone Antifoam 30 % (ROTH), Silicone Antifoam (SIGMA), Antifoam 204 (SIGMA); each to the final concentration of 20 mg.L<sup>-1</sup>. The experiments were conducted in the 1.3 L laboratory glass fermenters (BioFlo®/CelliGen® 115, New Brunswick Scientific Co.). The batch operation mode was adopted to obtain a high cell density and high concentration of riboflavin. The inoculum in a concentration of 20 % was added to 0.5 L of optimized medium OP 5 m. The dissolved oxygen (DO) concentration was monitored using the fermenter control unit based on polarographic DO probe. Air was used for culture aeration (1.1 vvm). The bioreactor was operated at 28 °C and 600 – 1100 rpm. The pH 5.5 of the medium was automatically adjusted (Mettler – Toledo pH electrode) by 1 N HCl or 0.1 N NaOH. Samples were withdrawn twice a day, biomass and riboflavin concentrations in the medium were determined. The following results on riboflavin accumulation were obtained after 120 h cultivation using different antifoams used: Structol 673, 8.2 g.L<sup>-1</sup>, Silicone Antifoam 30 % (ROTH), 7.18 g.L<sup>-1</sup>. Silicone Antifoam (SIGMA), 6.9 g.L<sup>-1</sup>, Antifoam 204 (SIGMA), 7.4 g.L<sup>-1</sup>. Thus the antifoam Structol 673 appeared to be the most appropriate for maximal riboflavin accumulation. During accumulation of 8.2 g of riboflavin per L with Structol 673, biomass production was achieved 60.4 mg.mL<sup>-1</sup>. Thus, optimization of cultivation conditions led to a noticeable improvement in riboflavin production by the recombinant *C. famata* strain #91 during fed-batch cultivation.

**Keywords:** riboflavin, *Candida famata* #91, antifoams



## ASSESSMENT OF *LUTEINIZING* HORMONE LEVEL IN BLOOD PLASMA OF MALE RABBITS AFTER SHORT-TERM AMYGDALIN AND APRICOT KERNELS TREATMENT

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Luteinizing hormone (LH) is a hormone produced by gonadotropic cells in the anterior pituitary gland. LH in males had also been called interstitial cell-stimulating hormone (ICSH), its role is to stimulate Leydig cell production of testosterone. It acts synergistically with follicle stimulating hormone (FSH). Cyanogenic glycoside as an amygdalin was detected in apricot kernels, bitter almonds and peach, plum, pear and apple seeds. Previous studies related to amygdalin have primarily focused on antitumor mechanism, and on its toxicity caused by the release of cyanide. Previous studies describe the effects of natural compound amygdalin on male reproductive systems focused on process of steroidogenesis, spermatozoa motility and morphological abnormalities of spermatozoa. Objective of this *in vivo* study was to evaluate the potential effect of amygdalin (as a bioactive component of apricot kernels) and apricot kernels on the plasma levels of LH using rabbits as animal model. Adult rabbit males (n = 20, age: 150 days) were divided into to 5 groups: control without amygdalin addition, 2 experimental groups received intramuscular injection of amygdalin (at dose 0.6 and 3.0 mg.kg<sup>-1</sup> b. w.) during 2 and 4 weeks and other 2 experimental groups were fed by apricot seeds (at dose 60 and 300 mg.kg<sup>-1</sup> b. w.) mixed with feed during the same period. After this period the blood was collected and plasma levels of the LH was assayed by ELISA. Our results showed no significant (P ≥ 0.05) differences in LH plasma level after 2 and 4 weeks of amygdalin and apricot kernel application in comparison to untreated control group. In conclusion, amygdalin and apricot kernels did not affect the plasma LH level in male of rabbits after short-term exposure.

**Keywords:** amygdalin, apricot kernels, LH, rabbit.

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## ASSESSMENT OF FUSARIUM TOXINS COMBINED WITH LUTEINIZING HORMONE ON PROGESTERONE SECRETION BY PORCINE OVARIAN GRANULOSA CELLS *IN VITRO*

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*Luteinizing* hormone (LH) is a hormone produced by gonadotropic cells in the anterior pituitary gland. In females, an acute rise of LH triggers ovulation and development of the *corpus luteum*. Mycotoxins are contaminants of animal feed that can impair fertility and cause reproductive alterations in farm animals. The aim of the study was to examine the LH-induced progesterone secretion by porcine ovarian granulosa cells after addition of *Fusarium* toxins deoxynivalenol (DON), zearalenon (ZEA) and T-2 toxin each at the dose of 100 µg.ml<sup>-1</sup> for 24 h. Progesterone levels in culture medium were detected by ELISA. The doses 0.1, 1 and 10 µg.ml<sup>-1</sup> of LH added to culture medium significantly ( $P \leq 0,05$ ) increased progesterone secretion by GCs. The dose 100 ng.ml<sup>-1</sup> of T-2 toxin but not DON and ZEA added to culture medium significantly ( $P \leq 0,05$ ) increased progesterone secretion by GCs. The combination of the lowest LH dose (0,1 µg.ml<sup>-1</sup>) and 100 ng.ml<sup>-1</sup> of ZEA (but not DON a T-2 toxin) significantly ( $P \leq 0,05$ ) increased progesterone release by GCs. Progesterone secretion was significantly ( $P \leq 0,05$ ) stimulated by 1 µg.ml<sup>-1</sup> of LH addition in combination with DON, ZEA and T-2 toxin at the dose 100 ng.ml<sup>-1</sup>. DON and ZEA (but not T-2 toxin) each at the dose 100 ng.ml<sup>-1</sup> in combination with 10 µg.ml<sup>-1</sup> of LH addition significantly ( $P \leq 0,05$ ) increased progesterone secretion by GCs. Our findings suggest that *Fusarium* toxins can affect the process of steroidogenesis in porcine ovarian cells.

**Keywords:** DON, ZEA, T-2 toxin, LH, progesterone, granulosa cells, pigs

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## EFFECT OF THE ANTIBIOTIC DIHYDROSTREPTOMYCIN ON THE CELL METABOLISM *IN VITRO*

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Dihydrostreptomycin is an aminoglycoside antibiotic (derivative of streptomycin), used against many types of bacterial diseases and Gram-negative organisms. Their antimicrobial effects stimulated their large usage in human and also in veterinary medicine. In our study the effect of various concentrations of Dihydrostreptomycin (0, 7500, 15000, 20 000  $\mu\text{g}\cdot\text{ml}^{-1}$ ) were tested on the Vero cells (The 'Vero' lineage was isolated from kidney epithelial cells extracted from an African green *Chlorocebus aethiops*) *in vitro*. The concentrations of tested antibiotic were derived from the LD50, dose that kills 50 % of treated animals. The viability of treated cell cultures was assessed microscopically after 24 hour incubation. The culture medium removed from wells was used for biochemical assays for monitoring of the influence of chemicals on cell metabolism (levels of total proteins and cholesterol). For the comparison of analyzed data in each group ANOVA Scheffe's test was used ( $P < 0.05$ , 0.01 and 0.001). The observed parameters were affected by antibiotic only at higher concentrations. Decreasing content of total proteins showed effects of dihydrostreptomycin on the Vero cells, however without significant differences ( $P > 0.05$ ). Significant decrease ( $P < 0.01$ ) of cholesterol level was observed only in the highest concentration of antibiotic. Acquired knowledge is possible to apply in toxicity evaluation of pharmacological effective substances *in vitro*. Eukaryotic cell *in vitro* respond to chemical substances influence sensitively, therefore these techniques could replace examination *in vivo*, which require great numbers of animal.

**Keywords:** dihydrostreptomycin, VERO cells, total proteins, cholesterol

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## OPTIMIZATION OF PHYSIOLOGICAL PROCESSES IN DAIRY COWS AS FUNDAMENTAL PRINCIPLE OF HEALTH

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The nutritional and metabolic status during onset of lactation is the main factor influencing the fertility of dairy cows which is the reason of poor reproduction and economics of dairy herds. The energy requirements of lactating cows are met through a combination of dietary intake and mobilization of body reserves. Postpartum reproductive functions depend on nutrient energy intake linking to milk production what depends on nutrient energy intake and rapid recovery of energy balance. Energy deficit (gluconeogenesis and lipolysis) at early lactation reflects in energy and nitrogen parameters of metabolic profile. The objective of this paper was to analyse the changes of selected energy and nitrogen metabolism parameters in blood and urine of dairy cows with fertility disorders. The following indicators of the metabolic profile were determined: glucose, total lipids, triacylglycerol, cholesterol, total protein and urea in blood and urine, ketone bodies in urine. Acid–base homeostasis has a close relationship to the process of absorption of substance into their metabolic transformation and excretion and gas exchange in organism. Our results confirmed tendency in higher metabolic turnover during early lactation manifested with lipolysis, ketonuria and metabolic acidosis.

**Keywords:** biochemical parameters, blood serum, milk production, Acid-base homeostasis

**Acknowledgments:** This work was financially supported by APVV- 0304-12.



## CHANGES IN FEMORAL BONE MICROSTRUCTURE OF MALE RABBITS INTRAMUSCULARLY ADMINISTERED BY AMYGDALIN

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Amygdalin is one of many nitrilosides abundant in the seeds of apricots, almond, peaches, apples and other rosaceous plants. Amygdalin itself is a non-toxic, but its component hydrocyanic acid (HCN) decomposed by some enzyme is the poisonous substance. Many studies have shown that amygdalin plays a supporting role in the treatment of cancer, diabetes, asthma, bronchitis and other diseases. However, its effect on bone microstructure has not been investigated to date. In our study, nine four-month-old male rabbits were randomly divided into three groups of three animals each. Adult rabbits from the experimental groups (E groups) were intramuscularly administered with amygdalin at doses  $0.6 \text{ mg.kg}^{-1}$  body weight (b. w.) (group E1) and  $3 \text{ mg.kg}^{-1}$  b. w. (group E2) one time per day for one month. Three rabbits without amygdalin intoxication served as a control group (group C). At the end of the experiment, cortical bone thickness and histological structure of femoral bones of rabbits from all groups were determined. We found no significant differences in cortical bone thickness between all analysed groups. However, intramuscular application of amygdalin induced an absence of the primary vascular longitudinal bone tissue in some areas of endosteal border. This part of the bone was formed by irregular and/or dense Haversian bone tissues. Also, the middle part of *substantia compacta* was composed of primary vascular longitudinal bone tissue, extending from *periosteum* in these rabbits. The measured values for area, perimeter and minimum diameter of the primary osteons' vascular canals were significantly decreased ( $P < 0.05$ ) in rabbits from E1 and E2 groups. In addition, all values (area, perimeter, maximum and minimum diameter) of the secondary osteons were significantly decreased ( $P < 0.05$ ) in the E1 group. Our results indicate that intramuscular administration of amygdalin demonstrably influences only histological structure of femoral bones in adult male rabbits.

**Keywords:** amygdalin, femoral bone, rabbit, histomorphometry

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## TEACHING STYLE OF UNIVERSITY TEACHER

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Nowadays constantly emphasizes autoevaluation of teacher and the need to change the relationship teacher - student, so we have chosen this topic using a questionnaire interaction styles teacher. In the work we described the questionnaire and to submit the results of its own investigation, where we used a questionnaire interaction styles teacher. The questionnaire we have entered the second year student of bachelor study Slovak University of Agriculture. The questionnaire was completed a total of 50 students, including 22 boys and 28 girls. Students evaluated a total of five randomly selected teachers Slovak Agricultural University. Most teachers that the questionnaire appeared, has over ten years' experience, some even more than twenty years' experience. Each student could choose one of the five teachers who want to evaluate. The condition was that this teacher he taught at the university. Apart from the results we have described the progress of work with a questionnaire atmosphere, questions and subsequent discussion in the classroom. Questionnaires were evaluated and the results were compared. We compared different teachers here, the differences between teachers in terms of students. The results obtained suggest that the evaluation of university teachers in the eyes of their students were considered as a good organizer who helps his students to be successful, understand their problems and try to keep them accountable. At the same time, these teachers do not act on the students very strictly. In my view they are both teachers exercising their profession responsibly acting in their students positively and shifted their knowledge and development forward. Comparison of ratings of boys and girls, we found that there was no significant gender difference in the perception of teachers.

**Keywords:** teacher's personality, teaching style, interaction styles

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## DETERMINATION OF MERCURY AND CADMIUM IN BLOOD OF WILDLIFE ANIMALS

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Monitoring of contaminants in wildlife animals has great importance for the animals and also people. Wildlife animals occurring in the natural environment is the best bioindicators. Heavy metals are among the most important pollutants in the environment. Toxic actions may occur with low concentrations. A wide range of adverse effects that can cause in the body, including such major changes as carcinogenic activity, effects on reproduction and violation of hormonal balance. Most harmful to living organisms are considered mercury, cadmium, chromium and lead. The organism can get by inhalation, transdermal or oral. The aim of our study was to determine mercury and cadmium in the blood of wildlife animals. For the experiment were used 11 sexually mature animals (wild boar, *Sus scrofa* L.) of which 5 males and 6 females. The animals were blasted in the Nitra region, area Topolčianky. After shooting an animal's blood was collected and in cold boxes transported to the laboratory (Department of Chemistry, FBP SPU). For the evaluation of mercury in the blood plasma was used AMA (Advanced Mercury Analyzer) method using the AMA 254. Cadmium levels were measured by FAAS (Flame Atomic Absorption Spectrometry) method using AAS 3. The concentration of mercury in the blood ranged between 0.00073–0.000571 mg.kg<sup>-1</sup>. The concentration of cadmium in the blood accounted range 0.00139 to 0.00723 mg.kg<sup>-1</sup>. The obtained results were compared with the maximum permitted levels (for mercury 0.005 mg.kg<sup>-1</sup>, for cadmium 0.008 mg.kg<sup>-1</sup>) in the blood of the animals. We found that the animals have not been exceeded recommended values, with the exception of one female to 0.00869 mg.kg<sup>-1</sup> which was measured the increased content of Cd. Comparison of mean content of mercury in the blood of male and females, we found that in females the content of mercury was higher (0.00239 mg.kg<sup>-1</sup>) than in males (0.00232 mg.kg<sup>-1</sup>). Similarly, the content of cadmium in females was higher (0.00520 mg.kg<sup>-1</sup>) than in males (0.00398 mg.kg<sup>-1</sup>).

**Keywords:** wild boar, *Sus scrofa* L., mercury, cadmium, wildlife animals, blood, AMA method, FAAS method

**Acknowledgments:** This work was financially supported by KEGA grant no. 006/SPU-4/2015. This work was co-funded by European Community under project no 26220220180: Building Research Centre "AgroBioTech".





## ANTIOXIDANT ENZYMES ACTIVITY AND GLUTATHIONE LEVEL IN EGGS FROM HERITAGE BREED AND COMMERCIAL CROSSES HENS

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The experiment was carried out with 180 hens of three heritage breeds (Yellowleg Partridge, Sussex, Leghorn) and 180 commercial crosses of laying hens (Hy-Line, ISA Brown, Lohmann). Layers were reared in the litter system at a stocking density of 9 birds per m<sup>2</sup> and had no outdoor access. Birds were assigned to groups I to VI (Yellowleg Partridge, Sussex, Leghorn, and commercial layers Hy-Line, ISA Brown and Lohmann, respectively). Each group was subdivided into 3 subgroups, each having 20 birds. Birds were fed *ad libitum* standard diets based on concentrates for laying hens. Birds had free access to water throughout the experiment. All the groups were managed under uniform environmental (air humidity and temperature, lighting programme) and feeding conditions. At 48 weeks of age (peak of lay), 6 eggs from each group were collected. In the samples of egg yolks and albumens the concentration of reduced glutathione, and activity of superoxide dismutase, glutathione peroxidase and catalase were estimated. The results were statistically analysed by one-way analysis of variance and significant differences were estimated with Duncan's multiple range test. The egg yolks and egg albumens showed a statistically significant differences in the activity of SOD and GSH levels. The highest activity of SOD and the lowest level of GSH were observed in eggs from Sussex hens (of heritage breeds) and in eggs from Hy-Line (of commercial crosses hens). It is concluded from the present results that the origin of laying hens has an impact on the activity of antioxidant enzymes and glutathione levels in eggs, which may affect their quality.

**Keywords:** GSH, antioxidant enzymes, yolk, albumen, eggs, laying hens



## EFFECT OF METHOD OF THE BREWING ON CAFFEINE CONTENT IN THE INFUSIONS OF COFFEE

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Coffee is the most popular, but also controversial beverage in the world. Coffee was consumed for centuries in order to improve mental and physical efficiency. The coffee is rich in charitable but also harmful compounds. Caffeine in a coffee has a positive effect on the human body – provided that it is in the right amounts. Antioxidants protect the body against effects of free radicals. Coffee also contains adverse compounds, as oxalic acid, chlorogenic acid and irritants. The power of the brew is not constant: the first caffeine passes to the brew and subsequently tanning agents, but if the brewing temperature is higher, the more tannins is in brew. There are many species of coffee prepared in various ways. The most common way coffee brewing is flooded with water at a temperature of 95–98 °C portion of coffee, but in the last years significantly has increased the demand for more expensive coffee prepared in coffee makers. The aim of this study was to determine the caffeine content in coffee infusions prepared by various methods making. Caffeine was determined in infusions of granular and minced coffees (Arabica and Robusta species) using the HPLC method. Used were as follows methods of brewing: flooding of boiling water of ground coffee portion, brewing in the jug coffee machine Moka, brewing in the overflow espresso machine, brewing in the pressure espresso machine. The caffeine content depends on the type of coffee. In the Robusta coffees caffeine level was higher than in Arabica coffees. Brewing method influences the level of caffeine in coffee, these differences are statistically significant. The highest content of caffeine was found in coffees brewed in the jug coffee machine Moka, while the lowest in coffees flooded with boiling water.

**Keywords:** coffee, caffeine content, brewing methods





## TOMATOES STORAGE CONTAINER FOR EXTENSION OF FITNESS FOR CONSUMPTION

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The work includes the test of the comparative analysis of selected varieties of tomatoes, stored and treated with Earth's magnetic field. Deprivation fruit external influence factor of the geomagnetic field will be carried out by placing the selected fruits in the prototype pipe windings Helmholtzta. The mechanism of action is based here on offsetting resultant vector Earth's magnetic field inside the coil through the induced vector directed opposite. This type of approach to the process of storing and replacing traditional methods of extending the shelf life of fruit intake (e. g. fruit storage of gases in the atmosphere) is a new, innovative and forward-looking approach to the process extending the life of the plant material. The prerequisites for the possible success of the proposed method are extremely interesting observations Balyavskaya (2004). The alleged impact of reduced natural, Earth's magnetic field, with which we are dealing min. in an open space, as well as spacecraft, which act as a kind of shell screens for magnetic fields. This solution makes it possible to store fruit for a longer time without influence of an external magnetic field, which makes the fruit respiration is reduced and thus there is no possibility of evaporation of water and other valuable nutrients to the tissues of stored fruits and vegetables.

**Keywords:** magnetic compensation, tomatoes, storage container



## CONCENTRATIONS OF CALCIUM AND MAGNESIUM IN MOUSE BLOOD SERUM AFTER ACRYLAMIDE APPLICATION

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Major role in prevention against acrylamide (AA) toxicity may be played by controlling of production of free radicals. This role may be played by diet supplements. The production of free radicals may be attenuated by magnesium supplementation. It also seems that some of the potentially toxic AA effect on blood may be reversed by Mg application. Magnesium is a macroelement necessary for the normal development and functioning of the body. It is naturally present in many foods, added to other food products, available as a dietary supplement, and present in some medicines. Thus the aim of our study was to indicate the effects of AA combined with Mg ions on magnesium and calcium content. Our analysis indicated increase of magnesium concentration in AA20 and AA40 group compared to control group of mice. The highest magnesium concentration was observed in mice supplemented with Mg. Mg supplementation after exposure to acrylamide indicated decrease in the concentration of Mg ions. We have also observed high Ca concentration after exposure to AA. Increased Ca content occurred in the AA20 and AA40 groups of mice compare to control group. We did not observed clear changes in calcium content after application of acrylamide and magnesium.

**Keywords:** mouse, blood serum, calcium, magnesium, acrylamide application



## THE ACTIVITY OF LIVER ENZYMES FOLLOWING APPLICATION OF PATULIN IN THE RABBITS BLOOD

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In today, patulin belongs to a short list of mycotoxins (aflatoxins, ochratoxin A, zearalenone, fumonisins and trichothecenes) whose level in food is regulated in many countries around the world, with European countries being among the first to propose limits in the levels. The aim of the present study was to determine the effect of intramuscular application of patulin on the activity of liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), *alkaline phosphatase* (ALP), *gamma-glutamyl transferase* (GGT) in rabbit's blood. Adult female rabbits ( $n = 10$ ) were divided into two groups ( $n = 5$  in each group), control group (C) and experimental group (E1). Experimental group received patulin through intramuscular injection  $10 \mu\text{g}\cdot\text{kg}^{-1}$  (*Musculus biceps femoris*) twice a week. Blood was collected on 30<sup>th</sup> day of the experiment. Automatic analyzer RX Monza (Randox, United Kingdom) was used to measure the samples according to manufacturer conditions. No significant higher values ( $P > 0.05$ ) of AST were observed in experimental group E1 ( $0.55 \pm 0.14 \mu\text{kat}\cdot\text{l}^{-1}$ ) in comparison with the control group ( $0.46 \pm 0.09 \mu\text{kat}\cdot\text{l}^{-1}$ ). The activity of ALP were no significantly higher in group E1 vs. control group. Other parameters were unaffected. Patulin application had no significant effect on the activity of liver enzymes.

**Keywords:** rabbits, patulin, AST, ALT, ALP, GGT

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



## CHANGES CADMIUM AND COPPER LEVELS AFTER ADMINISTRATION OF SODIUM HUMATE IN THE TISSUES OF BROILER

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A study was conducted to determine the efficacy of natrium humate in counteracting of the selected trace elements in tissues and organs broiler chicks. An experiment with broiler chicks was used to evaluate the effects of natrium humate (HuNa) on the changes of levels of copper (Cu) and cadmium (Cd) in liver and muscle. Broiler chicks were fed on diet containing HuNa and divided into 3 experimental groups: G1) control group; G2) 3.0 mg.kg<sup>-1</sup> b.w. CdCl<sub>2</sub>; G3) 1 % HuNa feed mixture and 3.0 mg.kg<sup>-1</sup> b.w. CdCl<sub>2</sub>. The samples of liver and muscle of poultry were analysed for the presence of Cd and Cu on the AAS (Unicam Solar 939). Higher Cd contents (muscle 0.47 and liver 1.93 mg.kg<sup>-1</sup>) were recorded in group (G2) after Cd dietary application. After addition of HuNa positive effect on significantly decrease of Cd levels in liver (from 1.93 to 1.62 mg.kg<sup>-1</sup>) and in muscle (from 0.47 to 0.36 mg.kg<sup>-1</sup>) were noticed. Concentrations of Cu were significantly decreased in liver and muscle (1.68 and 0.25 mg.kg<sup>-1</sup>) in group (G2), after Cd application in comparison to control group (G1). Similarly, lower of Cu levels have been detected in group (G3) in liver and muscle (1.92 and 0.196 mg.kg<sup>-1</sup>), respectively. However, decreases in Cu concentration in muscle (G3) due to the complexation of Cd by HuNa was not efficient. On the other hand, Cu concentration in liver (G3) was enhanced. Humate showed a protective effect against the toxic effects of heavy metals decreased levels of copper and cadmium in the liver and muscle.

**Keywords:** cadmium, copper, liver, muscle, natrium humate, broiler, AAS



## EFFECT OF ADDING HERBAL MIXTURES TO WATER ON DURATION OF TONIC IMMOBILITY AND HETEROPHIL TO LYMPHOCYTE RATIO IN BROILER CHICKENS

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One of the criteria for determining stress and welfare levels in birds is to measure the duration of tonic immobility and the blood heterophil to lymphocyte ratio. The aim of the study was to determine the effect of adding drinking water to mixed herb extracts with antistress effects on the duration of tonic immobility and the blood heterophil to lymphocyte ratio in broiler chickens. The experiment was conducted at a poultry farm in Aleksandrowice with 360 Ross 308 broiler chickens. In group 1 (control), broiler chickens received drinking water without herb extracts throughout rearing. In groups 2 and 3 water drinkers were supplemented from 21 to 35 days of rearing, for 5 h per day (from 800 to 1300 h), with alcoholic extracts from 3 herb mixtures: mixture 1 (20 % of chamomile (*Matricaria chamomilla* L.), 20 % of oregano (*Origanum vulgare* L.), 20 % of peppermint (*Mentha piperita* L.), 20 % of yarrow (*Achillea millefolium* L.), 20 % of knotgrass (*Poligonum aviculare* L.)); mixture 2 (50 % of chamomile (*Matricaria chamomilla* L.), 25 % of valerian (*Valeriana officinalis*), 25 % of large-leaved lime inflorescence (*Tilia platyphyllos* L.)). For 42 days, birds were kept on litter at a stocking density of less than 33 kg per m<sup>2</sup>. At 21, 28, 35 and 42 days of growth, tonic immobility (TI) was measured according to Akşit et al. (2006) in 10 birds from each group, blood was collected and blood smears were prepared, and stained leukocytes were counted under a Nikon YS 100 microscope. Thereafter, the heterophil to lymphocyte ratio (H:L) was calculated. The results were statistically analysed using analysis of variance by estimating significant differences with Duncan's multiple range test. No statistically significant differences were observed between the groups in the duration of TI. On day 21 of the experiment, a highly significant difference in H:L was noted between groups II and III. On day 35, the highest H:L percentage was found in the blood of broilers from group II compared to groups I ( $P \leq 0.01$ ) and III ( $P \leq 0.05$ ). In summary, in terms of the studied parameters the herbal extract supplemented to the drinking water had no effect on improving broiler welfare.

**Keywords:** herbal mixtures, duration of tonic immobility, heterophil to lymphocyte ratio, broiler chickens



## EFFECT OF VARIOUS TAURINE CONCENTRATIONS ON TURKEY SPERMATOOZOA MOTILITY: IN VITRO CULTURE AT 5 °C

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The target of this study was to investigate the effect of different concentration of taurine on the turkey spermatozoa motility and progressive motility during the *in vitro* incubation at 5 °C. Semen samples of adult male turkeys of the line Big 6 were diluted a ratio of 1 part of semen and 100 parts of physiological solution (control sample – C) and with four different concentrations of taurine: 0.078125 mg.ml<sup>-1</sup> – sample T1; 0.15625 mg.ml<sup>-1</sup> – sample T2; 0.3125 mg.ml<sup>-1</sup> – sample T3; and 0.625 mg.ml<sup>-1</sup> – sample T4 (diluted in physiological solution). Selected motility parameters were monitored at six time periods: 0, 1, 2, 3, 4 and 5 hours. Measurements were evaluated by the CASA system (Computer Assisted Semen Analyser) with the program Sperm Vision®. Spermatozoa motility at the beginning of incubation showed equal values in all experimental samples. The same tendencies were measured in the all tested time of *in vitro* incubation. However at the beginning of incubation significant decrease of progressive motility was detected in samples T2 (26.79 %), T3 (26.32 %) and T4 (27.30 %) compared to control (32.20 %). After 1 hour of incubation all tested samples showed balanced values of spermatozoa progressive motility. Non-significant differences were found in all experimental samples in other time periods of *in vitro* incubation. It can be concluded from this study that addition of taurine (from 0.078125 to 0.625 mg.ml<sup>-1</sup>) to turkey spermatozoa have no positive effect on monitored spermatozoa motility parameters at 5 °C.

**Keywords:** turkeys, taurine, spermatozoa, motility, CASA

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## STRESS INDICATORS OF CHICKENS FROM THREE COMMERCIAL LINES HOUSED IN MODIFIED CAGES

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The aim of the study was to analyse corticosterone and adrenaline levels, the blood heterophil to lymphocyte ratio, and the duration of tonic immobility in chickens from three commercial lines housed in modified cages. The experiment used 18-week-old commercial Hy-Line (group I), ISA Brown (group II) and Lohmann Brown pullets (group III), which were raised in modified cages (750 cm<sup>2</sup> per bird) equipped with a nest, perch and claw-shortening device. Birds were fed standard layer diets and had free access to feed and water drinkers throughout the study. During the experiment, the laying hens had uniform microclimate conditions. At 32 and 56 weeks of growth, tonic immobility (TI) was measured according to Akşit et al. (2006) in 10 birds from each group, blood was collected and the level of corticosterone and adrenaline was determined by enzyme immunoassay and radioimmunoassay, respectively. Blood smears were prepared and the heterophil to lymphocyte (H:L) ratio was determined. The results were statistically analysed by one-way analysis of variance and significant differences were estimated with Duncan's multiple range test. The longest tonic immobility, at both 32 and 56 weeks of the experiment was observed for Lohmann Brown hens compared to hens from the other two commercial lines, but the difference was not significant. There were no statistically significant differences in blood corticosterone levels between birds. At 32 weeks of growth, a significant difference was observed in blood adrenaline concentration between groups I and III. During the same growth period, Lohmann Brown layers exhibited significantly the highest heterophil to lymphocyte ratio compared to Hy-Line and ISA Brown layers. At 56 weeks of the experiment, a significant difference was found in the H:L ratio between groups II and III. It is concluded from the present results that the housing system used in modified cages was most stressful to Lohmann Brown layers compared to commercial Hy-Line and ISA Brown hens.

**Keywords:** laying hens, corticosterone, adrenaline, heterophil to lymphocyte ratio, duration of tonic immobility





## EXPOSURE TO 2.45 GHz MICROWAVE RADIATION INTERRUPTS TESTICULAR STRUCTURE OF PUBERTAL TESTIS IN RATS

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In the experiment, 21 days old Wistar rats (at the onset of puberty), were irradiated by whole body pulse electromagnetic radiation at frequency 2.45 GHz and mean power density 2.8 mW per cm<sup>2</sup> 3 hours a day for 3 weeks. Following the last exposure the tissue samples were histologically processed. The light microscopy showed diffuse degenerative changes in the testes of examined animals. The testes comprised of irregular seminiferous tubules and enlarged interstitium. The seminiferous epithelium contained abnormal features such the degenerating sex cells, sloughing of immature sex cells, accumulation of sloughed cells within the lumen of tubule and also occasional necrotizing sex cells. A special feature was the presence of giant multinucleated cells in the adluminal compartment of tubule. This investigation proved harmful effect of EMR on juvenescent testicular tissue and spermatogenesis as well as confirmed the fact, that the testes are highly sensitive to non-ionizing radiation.

**Keywords:** electromagnetic radiation, giant cells, testes, rats





## HEALTH SAFETY OF EDIBLE MUSHROOMS COLLECTED FROM THE INDUSTRIAL AREA

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Mushrooms as food accompanied mankind from immemorial time. However, despite the presence of many nutrients in mushrooms, scientists are divided about their positive impact on the human body. Therefore it was found that different species of mushrooms may accumulate toxic elements, consumption of large amounts of fungi can be associated with the risk of high heavy metals intake. Due to this ability fungi can be used as bioindicators of environmental contamination with toxic metals. The aim of the study was to assess the health risk associated with consumption of edible mushrooms derived from area surrounding the metallurgical plants. Three species (*Xerocomus badius* n = 6, *Suillus leteus* n = 5 and *Leccinum scabrum* n = 4) of mushrooms have been studied. Samples were collected at different distances from the border of an industrial area in order 200 m, 1 km, 5 km, 10 km, 15 km, and 30 km (as control sample). After collecting, samples of mushrooms were dried and then transported to laboratory. To determine the content of 13 elements ICP-OES method with prior microwave mineralization was used. Among studied metals only Al concentration in mushrooms was distance-dependent. The content of Al in tested samples ranged from 2.8 – 39.6 mg.kg<sup>-1</sup> d. m. and significantly (P < 0.05) decreased with increasing distance from the industrial plant. High levels of Fe, Ni and Cu were observed in the sample near to metallurgical plant (up to 5 km). For other studied elements (K, Mg, Zn, Mn and Se) no effect of pollutants emitted by foundry for mushrooms was observed. Despite the presence of high aluminum concentration in mushrooms collected near metallurgical plants, average consumption does not pose a health risk for human.

**Keywords:** edible mushrooms, quality, health safety, industrial area



## SELECTIVITY OF THE MAGNETIC FIELD STIMULATION APPLIED TO GROWING APPLE FRUITS

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Stimulation of plant growth by means of magnetic fields is an important factor of modern agriculture. In the paper is presented the system for alternating magnetic field generation to stimulate biological objects. The system is controlled by computer and allows for an independent control of up to 10 induction coils inducing magnetic fields with amplitudes from 0.1 to 150  $\mu\text{T}$  and frequencies from 0.1 to 100 Hz. The proposed set-up was used during three growing seasons for stimulation of the strawberry plants. An increase in the total solids content was observed in the case of the alternating magnetic field stimulation with the parameters: frequency – from 50 to 100 Hz and amplitude – from 50 to 100  $\mu\text{T}$ . This selectivity of the magnetic field influence (especially in terms of the frequencies) suggests the resonant mechanism of this impact. Magnetic fields with certain sublimated frequencies (50–100Hz) and of 100  $\mu\text{T}$  may cause increased enzymatic activity resulting in enhanced transformation of polysaccharides to monosaccharides, and as a consequence in increased total extract in fruits.

**Keywords:** magnetic fields, apples, monosaccharides, firmness



## EVALUATION OF SOME ANTIOXIDANT PARAMETERS AFTER TWO WEEKS EXPOSURE OF PATULIN IN RABBITS

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Mycotoxin patulin (PAT) is a metabolite produced by a large number of fungi within several genera such as *Byssochlamys*, *Eupenicillium*, *Penicillium*, *Aspergillus* and *Peacylomyces* in a variety of food products, e.g. apricots, grapes, grape fruit, peaches, pears, apples, olives and cereals. Patulin has been reported to be a genotoxic, reprotoxic, embryotoxic, and immunosuppressive compound. In this study antioxidant parameters (superoxide dismutase – SOD, catalase – CAT, glutathione peroxidase - GPx, bilirubin and albumins) in blood of rabbits after patulin administration during two weeks were analysed. Animals were divided into two groups: control group (C) and experimental group (E). Experimental group received patulin in injectable form at  $10 \mu\text{g}\cdot\text{kg}^{-1}$  for 14 days two times a week. Activity of SOD was measured in lysate from erythrocytes and activity of GPx was analysed in whole blood using commercial assay kits (Randox, Bratislava). Activity of CAT was performed according to photometric method (Tvrda et al., 2013). Content of bilirubin and albumins in blood serum were measured using commercial assay kits DiaSys (Diagnostic Systems GmbH, Germany). The all parameters were measured by spectrophotometer Genesys 10 (Thermo Fisher Scientific Inc., USA). Insignificant ( $P > 0.05$ ) lower activity of SOD, GPx and CAT in E group in comparison with the control group was observed. In the case of bilirubin, higher content in the experimental group against the control was observed, however without significant differences ( $P > 0.05$ ). Content of albumins was not affected by patulin. Patulins affinity for SH-groups could explains its inhibition of many enzymes. In recent years, only a few studies have been published on the *in vivo* toxicity of patulin. Research in this field is a necessity as there is every possibility that the toxins will enter the human food chain. Further research needs to be focused on the generation of data dealing with epidemiological and toxicity effects, especially in humans.

**Keywords:** patulin, antioxidant parameters, rabbits, blood

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