Risk Factors of Food Chain

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Content

Plenary lecture

Risk factors in low-processed food chain

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The 'From Farm to Fork' strategy developed by EU pursues the issue of food sustainability. Specific target areas include e.g. increasing the availability of health food options, developing knowledge of the health rating of products and sustainable packaging as well as reducing the use of chemical pesticides.

It is well known that consumption of high-processed food is positively correlated with increased evidence of some diseases such as cancers, diabetes 2-type, inflammation, obesity, and cardiovascular diseases. Fortunately, a significant increase in the consumption of low-processed food, e.g. sprouts, cut fruit and vegetables, mixed salads, has been observed in recent years. Such "ready-to-eat" products are characterized by high nutritional value and desirable content of pro-health components, e.g. vitamins, phenolics, and dietary fibre. In contrast to high-processed food, they do not contain artificial additives, e.g. colorants or preservatives. Health benefits observed after consumption of this food are also well-documented.

The consumer quality of low- and unprocessed food should be controlled in each step of production and distribution. Some risks are associated with the applied farming systems. These aspects may have an impact on the accumulation of some contaminations such heavy metals, fertilizers, pesticides, or mycotoxins; thus, their content should be monitored. On the other hand, the conditions of cultivation (e.g. biofortification) may tailor the phytochemical profile of raw products, which is usually reflected in enhanced pro-health properties. In organic farming, some desirable effects are also reached by application of biopesticides or biostimulants able to use the neutral mechanism of plant resistance. During minimal processing (washing, cutting etc.), some risks may be limited by post-harvest treatments. Unfortunately, due to the inapplicability of advanced techniques for preservation of low-processed food, products of this type are characterized by a short shelf-life and usually need refrigeration storage. This is mainly related to the presence of endogenous microorganisms and the elevated activity of endogenous enzyme systems. At this step, threats resulting from these aspects may be limited by treatments that reduce microbiota growth and/or limit the degree of enzymatic browning. At all these points, particular attention should be also paid to the effect of modifications on the nutritional value and safety of these new "designed" food products.

Although increased consumption of low-processed food brings many benefits to developed societies, one should keep in mind a number of risks associated with these products. Some problems related to low-processed food have already been solved; however, there are still many new huge challenges for modern agriculture, food technology, and dietetics.

Keywords: low-processed food, shelf-life, microbiological contaminations, post-harvest treatments, sprouts, ready-to-eat products

Plenary lecture

Animal testing - necessary evil?

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The discussion on the relevance of using animals in scientific research and/or for educational purposes has been held for many years and evokes extreme emotions in the society. A fundamental question to be addressed is whether it is ethically and morally acceptable to use animals to develop science, achieve progress and expand knowledge? We can clearly see the conflict of interests. On the one hand, animals' life and welfare is at stake, but on the other hand one must face people's expectations regarding novel medical technologies, progress in pharmacology or food and cosmetic industries. The opponents of animal testing provide multiple reasons explaining their stance. Besides ethical and moral dilemmas that arise from the conviction that animals are badly treated during testing and applied procedures are painful as well as cause suffering, there are other arguments, like species differences, what represents a serious challenge for the relevance of extrapolating research results obtained in the course of animal testing on people.

The supporters of using animals in scientific research and educational programs highlight that animal testing directly contributed to the development of human and veterinary medicine, including devising vaccinations, antibiotics and innovative medical procedures that save and improve the quality of life of both people and animals. Almost every significant medical advancement in the 20th century depended largely on experiments involving animals. Moreover, the development of preventive healthcare and treatments for Alzheimer's disease, AIDS and cancer also covers animal testing.

It must be highlighted that due to inspiring ethical and moral considerations, animal testing is governed by a series of strict legal regulations aimed at limiting the use of animals in research to experiments that are conducted for clearly defined purposes that cannot be achieved in other way. These regulations are included in documents such as Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and are intended at strengthening protection of animals subjected to research procedures and promoting alternative methods.

Keywords: animal testing, legal regulations

Metal Risk Assessment in Iranian Canned Tuna Fish

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This study aimed to estimate the concentrations of cadmium (Cd), mercury (Hg), nickel (Ni), and lead (Pb) in canned tuna fish produced and consumed in Iran and calculate risk assessment parameters eating these fish. In many countries, fish is a high proportion of food, are an important source of proteins, and provide a good many nutrients that are beneficial for our health. Due to significant deposits of xenobiotics in the water environments, however, fish may also be a source of non-essential metals, causing a variety of disorders. Iran is the largest fishery producer in the region reaching nearly 1 million tons. The main challenges to Iranian fisheries are environmental pollution and quality control. To determine the risk assessment parameters, the metal concentrations in canned fish of four popular brands, 20 cans each, were estimated using ICP MS. Was found that the lowest concentration was observed for Cd (18 µg/kg) and the highest for Ni (132 µg/kg). Among the brands studied, Pb concentrations differed most (42.0 to 113.3 µg/kg) and Hg levels were more consistent (24.0 and 39.4 µg/kg). The concentrations of Cd, Hg, and Pb in all the brands tested were below EU permissible thresholds. The intake estimation risk assessment parameters (EDI, contribution to PTWI, and CR) and non-cancer risk assessment parameters based on reference doses (THQ and HI) demonstrated the safety of tested products in respect to all metal concentrations studied, while the parameters regarding the toxic effects (MoE, and ILCR) showed that the consumption might cause health risks in terms of Cd (ILCR), Ni (ILCR), and Pb (MoE). The consumption of the canned fish studied should therefore be maintained at a reasonable level (2–5 meals containing fish weekly), so that it may provide necessary nutrients, while avoiding the health risk due to metal content.

Keywords: food safety, THQ, MoE, cancer risk, non-cancer risk

The effect of pomegranate peel extract on growth factors and their receptors in human ovarian cells *in vitro*

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Plant extracts present a rich source of bioactive substances, such as polyphenols, which possess antioxidant, anti-inflammatory and anti-proliferative activities. It has been reported that pomegranate peel could be a valuable source for nutraceutical applications with protective effects against degenerative diseases in cancer prevention or treatment. Using *in vitro* approaches, we investigated the effects of a pomegranate peel extract on growth factors and their receptors in lysates of human ovarian carcinoma cells OVCAR-3 at the concentration range 12.5 - 200 μg/mL for 24 hours. The release of epidermal growth factor (EGF) and transforming growth factor β2 (TGF-β2) and presence of their receptors (EGFR, TGFβR2) was assayed by Enzyme-linked Immunosorbent assay (ELISA). Our results showed a significant decrease in EGF release (200 μg/mL; P≤0.05), as well as in TGR-β2 release (200 μg/mL; P≤0.001). Moreover, the level of EGF receptor at the concentration 50 (P≤0.05), 100 (P≤0.01), and 200 (P≤0.01) μg/mL of pomegranate peel extract was significantly decreased in comparison to control. In addition, a significant decrease of the TGR-β2 receptor at the concentration of 200 μg/mL (P≤0.01) was observed. Although further studies will be determinant to assess the efficacy of pomegranate peel extract, our results suggest the effect on the secretory activity of ovarian cancer cells and new promising opportunities to employ natural extracts for the development of potential chemopreventive agents in ovarian cancer.

Keywords: pomegranate, ovarian cells, growth factors, cancer

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Color analysing of IPA brewing phases, including daily measuring of fermentation process

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Over the last decade, the brewing revolution has shown a whole new face to brewing. Beside the large-scale production, small-scale, artisanal-hand crafted and home breweries have also become important and relevant. This change has forced large-scale companies to change their usual methods and brew other types of recipes as well.
One of the most popular ALE-type beer is the Indian Pale Ale (IPA), which, due to its hop content, gives the beer a completely fruity-citrus flavor. It is especially popular with novice brewers as it has no enormous machine requirements compared to LAGER type beers.
In the study, we made our already known and popular recipe, a New England IPA, in a 30-liter brewing pot. This type requires only one type of malt, however, we added barley flakes, oat flakes, and wheat flakes to enhance the flavor. Hops are very dominant in the taste of this type of beer, five of which were used during brewing and later at "dry hopping" during fermentation. ALE beer types are also characterized by a simple thermal step; simultaneous activation of alpha and beta amylases followed by simultaneous cessation of activity.
Sampling was performed every minute during mashing, brewing, hopping, cooling, and yeasting with a calibrated NIX-type measuring device, with repetition to avoid measurement errors. During the fermentation stage, the color change was observed by daily sampling. The aim of the study is to be able to seperate the stages of brewing by color and to identify the stages of the already known biological and chemical activities by color. Learning about colors can even help large-scale companies to identify chemical and biological parameters based on colors, so they can get an accurate and immediate data of the condition of their product with chemical-free sampling.
This study is the first part of a longer series of experiments where we will perform similar experiments on several types of beer (LAGER, STOUT, APA, WHEAT, RED, etc.). We would also like to know about the possible effect of carbonization on color, the possible effect of storage in a bottle on color, and possible deviations from a similar decoction.

Keywords: color measuring, brewing, fermentation

Hg concentrations in "non-suspected" herbivorous mute swans

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Birds can serve as good bioindicators of environmental mercury (Hg) exposure. Various materials and tissues collected from birds have already been used in Hg biomonitoring, among which avian blood seems to be convenient in interpretation. Most current Hg studies focused mainly on piscivorous, predatory, or insectivorous waterbird species, such as herons, loons, or gulls. Herbivorous avian species are significantly less studied, which represents a significant knowledge gap.

Thus, we aimed to measure concentrations of Hg in the blood of mute swan (one of the most common herbivore waterfowl species in Europe). Specimens were caught separately on rural breeding sites and urban wintering areas in southern Poland, Europe. They were classified into three age groups: cygnets, juveniles, and adults. The potential impact of Hg on birds was evaluated based on haematocrit (Ht), reduced glutathione (GSH) levels, and morphometric measurements.

All the birds sampled, according to the results of morphometric measurements, were in good condition. Hg concentrations were low and differed between birds from rural breeding and industrialized wintering areas (means 2 ng/mL and 7 ng/mL, respectively). Sex factor did not influence Hg concentrations, contrary to age group factor, which occurred to be significant (concentrations in cygnets 2 ng/mL, juveniles 7 ng/mL, and adults 6 ng/mL). No significant relationship between Ht levels, GSH, and Hg concentrations was found.

We found that the Hg concentrations in blood may be influenced by a combination of industrialization, season and age. Still, generally, low concentrations such as those found by us should be considered safe for those birds. Additional variation in Hg concentrations may be caused by the amount of feed ingested, the rapid growth of cygnets, depuration of Hg into developing feathers, and lifetime accumulation.

Keywords: waterbirds, waterfowl, wetlands, mercury

Acknowledgments: Birds were captured for ringing purposes under the ringing permit 1/2017. Blood sampling was done with the license from the 1st Local Ethics Committee for animal experiments in Krakow, permit No 15/2015 from 21.01.2015.

Ginkgo affects the release of ovarian hormones and response to leptin, ghrelin, and obestatin

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The study aimed to investigate the role and interrelationship of medicinal plant ginkgo (*Ginkgo biloba* L.) and metabolic hormones (leptin, ghrelin, and obestatin) in the control of ovarian hormones release. In the first series of experiments, human ovarian granulosa cells were cultured in the presence of ginkgo extract (0, 1, 10, 100 μg/ml). In the second series of experiments, cells were cultured with leptin (0, 1, 10, 100 ng/ml), ghrelin (0, 1, 10, 100 ng/ml) and obestatin (0, 1, 10, 100 ng/ml) alone or in combination with ginkgo extract (10 μg/ml). The release of ovarian hormones (progesterone, IGF-I, oxytocin, and prostaglandin F) was evaluated by EIA/ELISA. Ginkgo increased progesterone (at a dose 1 μg/ml) and prostaglandin F (at doses 1 and 10 μg/ml), and decreased progesterone and IGF-I (at doses 10 and 100 μg/ml) and oxytocin (at doses 1, 10 and 100 μg/ml) output. Leptin stimulated progesterone, oxytocin, and prostaglandin F (at doses 10 and 100 ng/ml), and IGF-I release (at doses 1, 10 and 100 ng/ml). Ghrelin promoted oxytocin and prostaglandin F (at a dose 1 ng/ml) and reduced progesterone (at doses 1, 10 and 100 ng/ml), IGF-I (at a dose 100 ng/ml) and prostaglandin F (at doses 10 and 100 ng/ml) output. Obestatin increased progesterone (at a dose 100 ng/ml), IGF-I and oxytocin (at doses 1, 10 and 100 ng/ml), and decreased prostaglandin F (at doses 10 and 100 ng/ml) release. The presence of ginkgo suppressed the stimulatory action of leptin on progesterone and IGF-I (at doses 1, 10 and 100 ng/ml), oxytocin and prostaglandin F (at doses 10 and 100 ng/ml), and supported its stimulatory effect on prostaglandin F (at a dose 1 ng/ml) release. Furthermore, ginkgo mimicked and supported the inhibitory action of ghrelin on progesterone and IGF-I (at doses 1, 10 and 100 ng/ml), perverted and even inverted its stimulatory effect on oxytocin and prostaglandin F (at a dose 1 ng/ml), and its inhibitory action on prostaglandin F (at 10 and 100 ng/ml) release. The presence of ginkgo mimicked and supported the stimulatory effect of obestatin on progesterone (at doses 10 and 100 ng/ml), suppressed its stimulatory action on IGF-I and oxytocin (at doses 1, 10 and 100 ng/ml), supported its inhibitory effect on prostaglandin F (at doses 1 and 10 ng/ml). These observations (1) provide the first demonstration of a direct, mostly inhibitory effect of ginkgo on progesterone, IGF-I, and oxytocin, and its stimulatory action on prostaglandin F release by human granulosa cells, (2) confirm the involvement of leptin, ghrelin, and obestatin in the control of these ovarian hormones and (3) demonstrate the ability of ginkgo to affect not only ovarian hormone release but also to suppress and support the responses of ovarian cells to the upstream hormonal regulators (leptin, ghrelin and obestatin). These observations suggested the inhibitory action of ginkgo on human ovarian granulosa cells functions were mediated through various regulatory mechanisms: alteration of steroid hormones, IGF-I, oxytocin, and prostaglandin F release and prevention of the action of the hormonal stimulators leptin, ghrelin, and obestatin on ovarian cells.

Keywords: ginkgo, leptin, ghrelin, obestatin, ovarian hormones

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Effect of in-series and in-parallel flow heater configuration of solar heat system for industrial processes

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The boiler is an enclosed vessel that transfer the energy from the fuel combustion or electricity into hot water or steam. Then, this hot water or pressurized steam is used for transferring the heat to a certain heat process. Usually, the required hot water or steam keep on varying throughout the day which also may imply on the heat load on daily or monthly basis. Therefore, several configurations of connecting the boiler into the solar heating system to ensure the temperature of the final output. Boiler can be connected in-series or in-parallel to improve the efficiency of the overall process as well as to reduce the running costs. This paper presents simulation study of a solar heating system for industrial heating processes. Two flow-heater system configurations are designed for covering the heat demand of a pasteurising factory exist in Budapest, Hungary. The configuration "A" consists of a solar heating system for hot water preparation using in-series flow heater to ensure the temperature of the final output. While configuration "B" consists of the same solar system but in-parallel flow heater. These system configurations are modelled using T*sol software for evaluating the system performance under the Hungarian climate from five different aspects: required collector area, glycol ratio, volume flowrate, relative tank capacity, and tank height to diameter ratio. According to the optimum design parameters, in-series configuration is better that in-parallel one by 3.14% at 45 m^2 collector area, 0.45% at 25% glycol ratio, 0.42% at 50 $l/h.m^2$ volume flowrate, 2.05% at 50 l/m^2 relative tank capacity, and 0.42% at 1.8 tank height / diameter ratio respectively. The results show that in-series configurations are better in terms of solar fractions than in-parallel boiler configuration from all five aspects.

Keywords: solar thermal, industrial processes, in-series, in-parallel, process heat

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The possibility of use of honey against COVID-19 infection

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Coronavirus disease 2019 (COVID-19) is a new infectious respiratory disease caused by the SARS-CoV-2 virus, a pathogen belonging to the Coronaviridae family, which is a causative agent of worldwide pandemic. Currently available treatment protocols have not been effective in managing COVID-19 and the only way to prevent pandemic is mass vaccination. At the same time the search for a cure for COVID-19 is ongoing all over the world; there are mainly tested the known therapeutic substances, including natural products. In this context, honey – a natural product, known for its antibacterial, antiviral and immunomodulatory properties is beeing investigated as an option for patients with COVID-19. Honey inhibits the adhesion and penetration of the virus into the host cell and virus replication and modulates the inflammatory cascade. Naturally occuring components in honey, mainly flavonoids, phenolic acids and hydrogen peroxide have shown potential antiviral effects. The variety-dependent antioxidant activity of honeys is correlated with the total content of polyphenols. Dark monofloral honeys (buckwheat, heather, phacelia) show stronger antioxidant properties compared to multifloral honeys and light-coloured monofloral honeys (goldenrod, dandelion, rape, acacia). In *in vitro* and *in silico* studies honey and its bioactive ingredients have been shown to be effective in killing SARS-CoV-2, but there is still no evidence that honey is effective in fighting COVID-19. Potential mechanisms of honey action in COVID-19 therapy include: reducing oxidative stress, strengthening the immune response, preventing secondary infections and alleviating the symptoms of comorbidities. Moreover, clinical trials of honey on COVID-19 patients are currently undergoing.

Keywords: honey, COVID-19, SARS-CoV-2, antiviral potential

Comparison of the effects of bisphenol A and its analogs on plasma membrane integrity of TM3 Leydig cells

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One of the most studied endocrine-disrupting chemicals are bisphenols. Bisphenols have been associated with many human diseases including reproductive disorders in both sexes. Testicular Leydig cells are the predominant source of the male sex steroid hormones that provide reproductive function in males. The objective of the present *in vitro* study was to evaluate and compare toxic effect of bisphenol A, B, S and F at concentrations 0.04-50 $\mu g \cdot ml^{-1}$ on the plasma membrane integrity in TM3 Leydig cell line after 24 h of exposure. The integrity of the cytoplasmic membrane of exposed Leydig cells was monitored in a comprehensive three-parameter testing of cytotoxicity using fluorescent 5-CFDA, AM (5-Carboxyfluorescein Diacetate, Acetoxymethyl Ester), a cell-permeant esterase substrate that can serve as a viability probe that measures both enzymatic activity, which is required to activate its fluorescence, and cell-membrane integrity, which is required for intracellular retention of their fluorescent product. Obtained results showed the same tendency of evaluated parameter for all tested substances after 24 h cultivation. In the case of bisphenol A was detected cytotoxicity with significantly decreased values in experimental groups with TM3 Leydig cells cultivated in the presence of 10 $mg \cdot ml^{-1}$ (*P<0.05*), 25 $mg \cdot ml^{-1}$ (*P<0.01*), and 50 $\mu g \cdot ml^{-1}$ (*P<0.001*). Exposure to bisphenol B led to significantly lower plasma membrane integrity values at the same concentrations as for bisphenol A (*P<0.05* for 10 $\mu g \cdot ml^{-1}$, *P<0.001* with respect to 25 and 50 $\mu g \cdot ml^{-1}$). The highest experimental doses of bisphenol F (25 and 50 $\mu g \cdot ml^{-1}$) also showed cytotoxicity with significantly decreased (*P<0.001*) values of monitored parameter. Bisphenol S significantly reduced (*P<0.001*) plasma membrane integrity of Leydig cells after 24 h of cultivation with 10, 25 and 50 $\mu g \cdot ml^{-1}$. Taken together, these partial results of our study reported, that bisphenol analogs increasingly used today as alternatives to bisphenol A may have similar or higher toxicological effects on male reproduction. Therefore, it is important to examine the toxicological profile of these compounds and focus on risk assessment of bisphenol A analogs to estimate the relationship between exposure and toxic outcomes.

Keywords: bisphenols, male reproduction, Leydig cells, plasma membrane integrity

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Histological structure of mammary gland of sheep from heavy metal contaminated area

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In this work influence of feed from industrial emissions contaminated area on the histological structure of mammary glands of sheep during lactation period was investigated. The study was conducted on six Merino's sheep. Animals were from Agriculture Farm Oslany (industrial polluted area). Animals were fed with grass, hay, straw, corn and grass silage, barley and pelletized meal. Histological and toxicological methods were used for evaluating of glandular tissue and feed samples. The relative volume of glandular parenchyma was 69.56 ± 4.86 %. The relative volume of epithelium accounts for 29.03 ± 1.34 % and alveolar lumen 40.53 ± 4.75 %. Total connective tissue forms 30.24 ± 6.95 %, but out of it the collagenous tissue represents 4.23 ± 3.07 % and loose tissue forms 26.01 ± 2.18 %. The relative volume of adipose tissue was decreased up to 0.20 ± 0.19 %. Alveoli are ovoid in shape. The average size of alveoli is 70.70 ± 5.06 µm, but size fluctuated from 30 µm to 110 µm and some large alveoli were 150 µm in diameter. Alveoli of one lobulus have the same size approximately, but the alveoli of various lobules can be different or are different in size, respectively, what means that alveoli presents different secretory cycles of milk production. The average number of alveoli is 1 295 724.3 ± 306 734.3 per 1 cm^3 of the mammary gland tissue. There is very strong negative correlationship (- 0.96642) between the size and the number of alveoli. The average dimension of intralobular ducts was 95.0 ± 37.42 µm, interlobular ducts had 207.5 ± 27.8 µm in diameter and lobular ducts were 356.42 ± 48.1 µm in diameter. The average dimensions of glandular and teat sinuses were investigated as well. Lobules of glandular parenchyma with alveoli at the teat connective tissue were found. These lobules surround and enter directly into teat sinus. The toxicological analyses of feed of sheep show higher levels of both cadmium (Cd) and mercury (Hg) than normal. This fact can be a reason of histological morphometric changes of mammary glands from contaminated area.

Keywords: sheep, mammary glands, histological structure, heavy metals

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Effect of environmental conditions on milk production of dairy cows

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The aim of this study was to evaluate the influence of environmental housing conditions on milk yield of dairy cows. Measurements were taken in the summer from June to September and in the winter from December to February on a large-capacity farm of Holstein Friesian cattle. Dairy cows were selected during second or third lactation, in the summer period from the 51st day to the 135th day and in the winter period from the 64th day to the 120th day of lactation. The temperatures in the housing ranged from 15.35°C to 27.52°C in summer, and from -1.78°C to 21.34°C in winter. The highest temperature in the stall during summer was 33.50°C. The average THI value in summer was 70.43, but during the day the THI values sometimes reached 75.86. The dairy cows were therefore exposed to heat stress during summer. Increasing THI and temperature values negatively affected milk yield, as there was a negative correlation between both THI and milk yield ($r = -0.641$; $p \leq 0.01$) and temperature and milk yield ($r = -0.637$; $p \leq 0.01$). Milk production decreased over the course of our summer measurements, from 53.83 $l.cow^{-1}.day^{-1}$ on the first day to 42.94 $l.cow^{-1}.day^{-1}$ on the last day. Milk production in winter was at 56.78 l.cow-1.day-1. Differences in milk yield between summer and winter were statistically significant ($p \leq 0.001$). In the summer, the milk had a significantly lower content of fat ($p \leq 0.05$), proteins ($p \leq 0.001$), lactose ($p \leq 0.001$), minerals ($p \leq 0.001$) and, conversely, a higher number of somatic cells ($p \leq 0.01$). These results show that worse environmental conditions during the summer negatively affected the level of milk yield and the composition of the cows' milk. Analyses of cow performance data in relation to environmental conditions are an important tool to enable farmers to take corrective measures.

Keywords: dairy cows, environmental conditions, milk yield

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Nutritional and sensory properties of chocolate pralines enriched with essential oils

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Pralines made from dark chocolate with higher proportions of cocoa (at least 50 percent of the dry substance of cocoa components), are attracting considerable scientific and public interest, compared with milk chocolates, because dark chocolates contain increased amounts of bioactive compounds. The aim of this study was to evaluate nutritional (total dry matter, ash and fat content), sensory (9 point hedonic scale) and antioxidant profile (antioxidant activity, total polyphenols and flavonoids) of chocolate pralines with filling produced directly from cocoa beans variety *Criollo* (Peru). Pralines (filling) were enriched with the addition of essential oils - mint, lime, orange and lavender. Pralines without addition of essential oils to filling was used as a control sample. The dry matter content in evaluated pralines ranged from 88.16 % (sample with lime) to 94.5 % (sample with mint). Ash content was in line ~ 2 % in all tested samples. Total fat content ranged from 39.11 % (sample with orange) to 47.17 % (sample with mint). Antioxidant activity tested by DPPH method ranged from 1.09 mg TEAC.g^{-1} (control sample; TEAC - Trolox equivalent antioxidant capacity) to 1.97 mg TEAC.g^{-1} (sample with lavender). The total polyphenols were the highest in samples enriched with lavender (2.64 mg GAE.g^{-1}; GAE - gallic acid equivalent) and the highest amount of total flavonoids were determined in chocolate pralines with lime - 0.27 mg QE. g^{-1} (QE - quercetin equivalent). Sensory properties of prepared chocolates were evaluated overall as good with the best score in a taste and overall acceptability in chocolate pralines with lavender and orange. The consumption of enriched chocolate pralines as a part of healthy diet of consumers due to health benefits is recommended. These products can also increase assortments of products in confectionery industry.

Key words: coca beans, addition, ash, fat, polyphenols

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Effect of post-harvest storage on apple's weight

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"Golden Delicious" is a historical and popular apple cultivar that is planted across the world due to its outstanding eating qualities, such as flavor, juiciness, environmental tolerance, and high yield. Its fruit peel, on the other hand, tends to become russet in high-humidity areas. Apples, like other fruits, are exposed to stress throughout their growth and development in the field, as well as during harvest and the post-harvest environment (processing, storage and transport). The refrigeration system allows for the bulk handling of foodstuffs from the time they are harvested to the time they are offered to the marketplace, thus ensuring that the foodstuffs are maintained in their fresh and undamaged condition for an extended time period by carefully managing storage temperature and humidity. It is critical to have the temperature sufficiently kept low otherwise there is a risk of cold injury to the food. Likewise, the relative humidity inside the warehouse must be regulated between 80-90% for the vast majority of products that are subject to perishability, unless the quality of the product's preservation is to be adversely impacted. As standards for the quality of life continue to grow, so does the desire for a natural, healthy and nutritious fresh fruit and vegetable supply. On the other hand, the highly sensitive and highly perishable properties of fruits and vegetables once they are collected and harvested render their conservation a challenge that must be faced at all times. In recent years considerable improvements have been made in the use of cold storage for foodstuffs, which has helped to better maintain the sensory properties, to reduce spoilage risks and to lengthen conservation periods.

Keywords: Golden Delicious Apples, storage, weight , cold storage

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Utilization of degenerate primers in legume allergens screening

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Different types of allergies became a part of life of many people around the world. The research activities connecting to allergens are actually not oriented only for protein and immunological interactions, but to the genomic and transcriptomic background of them, too. Analysis and description of genomic variability of allergens in plant food resources will help to manage the allergen-based strategies in the future. Here, the bioinformatic approach was used to develop and validate the degenerate primers for genomic screening of polymorphism of profilins (Profilin Based Amplicon Polymorphism; PBAB) and vicilins (Vicilin Based Amplicon Polymorphism; VBAB) among the legumes. The alignment of existing public databases data for these allergens in the group of legumes was performed. Subsequently, degenerate primers were designed and their ability to generate polymorphic amplicons were tested for three legumes - bean, lentil and chickpeas. In all cases, amplicons were generated and a polymorphism was detected in all the three species for profilin as well as for vicilin.

Keywords: legumes, profilins, vicilins, degenerate primers, polymorphism

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Analysis of the microbiological quality of pork meat with the use of molecular methods

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Pork is one of the most valued types of meat among the four main types of meat consumed in the world. The essential oils (EOs), formed in the secondary metabolism of aromatic plants, consist of volatile substances and generally they have a low molecular weight. However, some natural factors such as physiological variations, environmental conditions, geographic variations, genetic factors and also plant evolution can alter the chemical composition of these oils, as well as their yield. The extraction of EOs usually occurs with the use of conventional techniques such as hydrodistillation using a Clevenger type extractor, which is the most widespread technique for the isolation of volatile EOs. In the industry, these oils are widely studied, mainly for their potential applications as agents promoting biological activities. The volatile compounds have presented over the years several pharmacological applications, such as antioxidant, antitumor, antiprotozoal, antimicrobial and antiinflammatory activities. The aim of this work was to analyze the microbiological quality of pork meat treated with essential oils (basil and rosemary) in order to extend using by classical and molecular analytical methods. The examined material was pork (pork neck, pork loin, shoulder). The following methods were used in the research: determination of the total number of microorganisms, identification of pathogenic microflora with the use of selective media (VRBGA, SS, ENDO Agar, PCA, *Pseudomonas* Agar) and identification of microorganisms using the PCR method.
On the basis of the conducted PCR tests, the following *E. coli* bacteria were identified in: pork loin, neck and shoulder from the 3rd day of storage, *P. areuginosa* in: pork shoulder. However, the presence of *Salmonella* bacteria was not confirmed, despite the fact that its growth was observed when grown on selective media.
The PCR method is a fast and reliable method of identifying microorganisms at the molecular level. Identification by means of the PCR method showed the presence of bacteria: *E.coli* and *P. areuginosa*. Rosemary oil has better antimicrobial properties than basil oil.

Keywords: analysis, essential oils, identification of microorganisms, molecular metods, PCR, pork meat

Evaluation of the concentration-dependent *in vitro* effect of bisphenol S on viability and steroid hormone production in H295R cells

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Many epidemiological studies have focused on the potentially harmful consequences of a wide range of substances found in the environment. Many compounds with endocrine-disrupting effects are now recognized, and bisphenol A is one of the most studied. In recent years, due to the continuing usage of BPA, its analogues, such as bisphenols S (BPS), have been developed as alternative substances to replace its use. Since the use of plastics in day-to-day life is increasing, exposure to bisphenols will also increase. Therefore, this study was undertaken to determine the effect of bisphenol S on human adrenocortical carcinoma cell line H295R, which is widely used as a model for *in vitro* assessment of endocrine disruption. H295R cells were cultured with experimental doses of BPS (0.1 – 100 μM) for 24 h and compared to the non-treated cells. Viability was determined using mitochondrial activity (MTT) assay and the level of testosterone and progesterone in cell culture media were determined by enzyme-linked immunosorbent (ELISA) assay. The results of our study confirmed that 24 h treatments of BPS caused a significant ($P<0.05$) decrease in mitochondrial activity at 1, 50, and 100 μM. The results showed that testosterone production was significantly reduced at 50, 75, and 100 μM of BPS, and significantly increased at 0.1 and 0.5 μM, while a significant reduction of progesterone production was recorded at 25, 50, 75, and 100 μM of BPS, and significant increase of progesterone production was recorded at 0.1 μM. These results suggest that BPS has the potential to interfere with cellular steroidogenesis in humans, however, more detailed and systematic research is needed to have a better understanding of the risks associated with BPS effects on steroidogenesis.

Keywords: bisphenol S, cytotoxicity, H295R cells, progesterone, testosterone, viability

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Effect of forage sources as a potential risk factor on winter honey bee colony losses

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The western honey bees (*Apis mellifera*) play an important role in the production of honey and bee products such as pollen, wax, royal jelly, propolis, and others that are widely used in the food, agricultural and pharmacological industries, as well as in plant pollination including various crops. In this study, the effect of forage crops (oilseed rape and sunflower) as potential risk factors for honey bees was evaluated. The data of winter honey bee colony losses in Slovakia in the seasons 2017/2018, 2018/2019, and 2019/2020 2017/2018 and 2019/2020. In conclusion, crops may present potential risks for honey bee colonies were obtained from the COLOSS survey. The results showed the effect of foraging on oilseed rape in the season 2018/2019 and 2019/2020 leading to significantly ($p<0.05$) higher winter honey bee losses. In addition, colonies reported to be foraging on sunflower showed significantly ($p<0.05$) higher winter loss rate in season 2018/2019 compared to colonies that did not. On the other hand, foraging on sunflower did not have a significant ($p>0.05$) impact on winter honey bee losses in season and foraging on crops (oilseed rape and sunflower) may have a negative impact on the vitality of honey bee colonies due to contact with pesticides. However, further studies are needed.

Keywords: winter colony losses, risk factors, forage crops, bee vitality

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Levels of selected biochemical parameters in semen of *Iguana iguana* received by electroejaculator

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An increasing number of endangered animals, especially reptiles, underlines the need to develop appropriate strategies for their assisted reproduction in order to preserve and protect the genetic heritage. In general, it is known that for the long-term storage of germ cells, it is important to use a suitable medium, which we obtain only if we find out the exact specifications and composition of the ejaculate of the animal. In the presented study we focused on the acquisition of the male reproductive fluid from a reptile (*Iguana iguana*) using an electroejaculation method and after a successful semen collection, to investigate the levels of the selected biochemical parameters from the obtained samples. The two animals (green iguanas) were housed at the Vivarium of the University of Agriculture in Nitra. The first was 10 years old and weighed 2.012 kg and the second was 6 years old weighed 1.715 kg. The concentrations of total protein (TP), cholesterol (Chol) and glucose (Glu) in the samples were analyzed using specific Randox reagents (Total protein, Cholesterol, Glucose) (Randox Laboratories Ltd., UK) and the RxMonza automatic photometer (Randox Laboratories Ltd., UK). Our results showed that electrostimulation can be performed to obtain semen samples from green iguanas. Our results showed that the main concentrations of the selected biochemical parameters were: TP – 24.08±7.14 (g/L), Chol – 1.55±0.49 (mM/L) and Glu – 0.00±0.00 (mM/L). The electrostimulation was found as an effective method for semen collection in reptiles. Finally we can state, that the levels of these biochemical parameters can play a big role in ideal preservation media to be created which will help us to produce the right long-term conditions for sperm storage.

Keywords *Iguana iguana*, reptile, semen, biochemical profile

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In vitro effect of PCB118 and PCB153 and their hydroxylated metabolites on T_4 and T_3 synthesis and metabolism in chicken thyroid gland

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With industrialization, the production of chemicals and their introduction into the environment has increased massively. Some of these chemicals act as endocrine disruptors (EDs) that disturb endogenous hormone signalling pathways and alter the normal function of the endocrine system of both humans and wildlife. The group of EDs includes polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCBs). To assess the effect of PCBs and their OH-PCBs on thyroid hormone [TH: thyroxine (T_4) and triiodothyronine (T_3)] secretion, the concentrations of iodothyronine deiodinases (DIO1, DIO2, DIO3) and mRNA expression of genes involved in TH synthesis (*NIS, TPO, TG*) and metabolism (*DIO1, DIO2, DIO3*), chicken thyroid explants were incubated for 6 h at 39ºC in Eagle's medium without (control groups) and sumplemented with TSH (250 mU/ml), PCB118, 4-OH-PCB107, PCB153, 3-OH-PCB153 (at doses of 0.5 x 10^{-8} M) and TSH together with each PCB and OH-PCB. T_4 and T_3 secretion, concentrations of DIOs and mRNA expression were determined by RIA, ELISA and qPCR, respectively. The results obtained were statistically evaluated by means of one-way analysis of variance (ANOVA); differences between means were analysed by post-hoc Tukey's test at $p<0.05$. The results of the *in vitro* experiment revealed that, except for 4-OH-PCB107, all applied PCBs and OH-PCBs inhibited basal and TSH-stimulated T_4 secretion. Moreover, they increased basal and reduced TSH-stimulated T_3 secretion. Following PCB and OH-PCB exposure, significant changes in mRNA expression of *NIS, TPO*, and *TG* were observed. PCBs and OH-PCBs affected *DIO1* and *DIO3* transcript levels and protein abundances of each DIO. In conclusion, both PCB118 and PCB153 and their OH-PCBs affect TH synthesis and deiodination processes in the chicken thyroid gland. In addition, the effects of PCBs and OH-PCBs depended mainly on the type of PCB congener and the exposure time. These results indicate that not only parental PCBs but also OH-PCBs are hazardous for the thyroid gland and may disrupt its endocrine function. Further studies are necessary to explain a mechanism of PCB and OH-PCB action in the avian thyroid gland.

Keywords: PCBs, OH-PCBs, thyroid hormones, deiodinase, chicken

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The effect of eggs disinfection method on the hatching process of domestic duck (*Anas platyrhynchos f. domestica*)

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The standard hatching technology of waterfowls recommends washing duck eggs. However, this procedure removes a mucin layer of eggshell that reduces the gas exchange and the risk of microbiological contamination during egg storage and incubation. Therefore, there is a need to test alternative "dry" disinfection methods and/or water-soluble formulations capable of restoring the protective layer of the eggshell. Such solutions must not, however, be embryotoxic and also disturb the hatching process.
The hatching Pekin duck eggs (line Cherry Valley, 640 eggs per group) were washed in 1% water solution of silicone (Dergall®) or polysaccharide formulation (Chitosan 650, ICB Pharma) or ozonized (concentration 0.17 mg/m3 10 minutes). Next eggs were incubated in standard condition in laboratory incubators (Iglotech).During the last 4 days of incubation the hatching process was monitored every 6 hours. The eggs rejected during candling and unhatched were embriopatological analized.
The hatch analysis showed that hatchability was 68.7 and 67.5% in ozonized and Chitosan group but 64.6% in Dergall group ($P<0.05$). Ducklings in eggs treated by Chitosan or Dergall solutions accelerated hatching by c.a. 5 hours ($P<0.05$) compared to ozonized group ($a=625.2$ h). However the hatching synchronisation was highest in ozonized groups ($b=98.9$ compared to 116.5 h, $P<0.05$) Conclude, the high hatchability results and the correct course of hatching occurred in ozonized groups allow to recommend this method for practical use in duck eggs incubation.

Keywords: domestic duck, hatchability, hatch synchronization; disinfection,

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Changes of duck eggshell temperature in a single-stage incubator

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Bird embryos at the end of incubation intensively produce energy. It is necessary to cool the eggs daily during artificial incubation. In traditional duck breeding technology, the eggs are taken out of the incubators for several minutes and sprinkled with water. The procedure is time consuming and imperfect. A new type of incubator was tested in the project. The cooling process by sprinkling with water and airing is performed automatically inside the apparatus (a single-stage incubator). Pekin duck eggs (Cherry Valley, Heavy line) in the period from 10 to 23 days of incubation (DI) were cooled inside the hatching apparatuses by winding and spraying with water (T=16°C).
The research was carried out in nesting devices (SOMMEN) with a capacity of 28224 eggs using the FLIR E50 thermal imaging camera. Temperature analysis of duck eggs' shells at various stages of embryo development was carried out using the thermal imaging technique and further analysis of the obtained thermal images. In the 2nd DI, the egg surface temperature (EST) was found to reach the temperature of the air in the incubator (about 37.8°C) and remained at this level up to the 6th DI. In the following days of incubation, the EST is higher than the temperature in the incubator. An increase in EST over 38°C was observed locally (max 38.2-38.9°C in places where the radiation reflected from many adjacent eggs accumulates. EST in 13 DI reaches an average value of 38.2-38.3°C), and in 23 DI it reaches 39.2°C . After cooling the eggs, the EST dropped by 2-3°C. At the same time, it was found uneven cooling of the eggs depending on the position of the egg on the tray. In the upper trays, the more chilled area (EST average 34.4°C) was closer to the edge of the trays, while in the lower trays the more chilled area was in the centre of the trays. The EST difference between these areas was about 1.5°C. Conclusion: despite the satisfactory results of the brood, it is advisable to improve the sprinkling and egg cooling system.

Keywords: duck, incubation, thermovision

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Effect of xylene, *Tribulus terrestris* and their combination on bovine ovarian cell functions

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The aim of our *in vitro* study was to examine the effect of xylene and the potential influence of the medicinal plant *Tribulus terrestris* L. (puncturevine) on basic bovine ovarian cell functions (proliferation, apoptosis and hormone release) their interrelationships, and mechanisms of action. For this purpose, we analyzed the effect of puncturevine extract (0, 10, and 100 μg/ml) alone or in combination with xylene (0, 10, and 100 μg/ml) on cultured bovine ovarian cells. Cell proliferation (PCNA accumulation), apoptosis (accumulation of bax) were analyzed by quantitative immunocytochemistry and the release of progesterone (P4), testosterone (T) and IGF-1 were analyzed by ELISA. The addition of xylene alone increased the accumulation of proliferation marker but did not affect the marker of apoptosis, promoted the release of IGF-1 and inhibited P4 and T. The addition of puncturevine alone increased cell proliferation, decreased apoptosis and inhibited P4 release and promoted T and IGF-1 release. Moreover, puncturevine inverted the stimulatory effect of xylene on granulosa cell proliferation and change it to an inhibitory action, induced the stimulatory action of xylene on apoptosis and promoted the effect of xylene on the release of P4 but not T and IGF-1. These observations show that xylene can directly regulate (mostly inhibit) basal bovine ovarian cell functions. The mostly stimulatory action of tribulus on these functions suggests, that tribulus can be a promoter of ovarian cell proliferation and steroidogenesis. Furthermore, our results show, that tribulus may promote or prevent xylene effects on the ovarian cells. These observations indicate the usefulness of tribulus against the potential negative effects of xylene on female reproduction.

Keywords: xylene, tribulus, proliferation, apoptosis, steroid hormones, IGF-1

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Effect of endocrine disruptors on male reproductive cells

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Endocrine disruptors are substances that may affect the endocrine system and produce adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife. Endocrine disruptors get into environment mainly through anthropogenic activities and include a very heterogeneous group of chemicals, which is not yet complete). Common environmental contaminants classified as endocrine disruptors are alkylphenols. They consisting of 80% nonylphenol ethoxylate and 20% octylphenol ethoxylate. The primary degradation products are nonylphenol and octylphenol. Nonylphenol has been found in polyvinyl chloride, which is used in the food processing and packing industries and may contaminate water flowing through polyvinyl chloride pipes. Octylphenol is frequently used in detergents, paints, pesticides, and other industrial products. Especially vulnerable to damage by endocrine disruptors is reproductive system. Sensitive compartment of the testes like spermatozoa or Leydig cells may be affected by nonylphenol and octylphenol. All stages of spermatogenesis and steroidogenesis are potential targets of disruption. Endocrine disruptors are able to bind to the nuclear receptors (estrogen, androgen) and interfere with the synthesis, metabolism or action of reproductive hormones. The first aim of our *in vitro* study was to detect progesterone receptor in spermatozoa and evaluate the ability of nonylphenol bind to progesterone receptors. Bovine spermatozoa was incubated with 10 μg/mL of progesterone and 10 or 200 μg/mL of nonylphenol during 30 min. The detection of the presence and the localization of the progesterone receptor was performed using the primary rabbit antibody and the secondary antibody conjugated to FITC. Analysis was performed with fluorescent microscope LEICA IM 500.

The second part of *in vitro* study was focused on specific mechanism that could be involved in nonylphenol and octylphenol toxicity, namely on *GJIC* through connexin channels using Lucifer yellow microinjection. It has been suggested that Cx43 *gap junctions* in Leydig cells may coordinate the androgenic secretory activities of these cells. TM3 Leydig cell line was cultured with addition of 2.5; 5.0; 10.0 and 25.0 μg/mL of nonylphenol and octylphenol during 30 min. Scrape-load/dye transfer method is direct and rapid technique with which to study this cellular phenomenon. It employs scrape-loading to introduce a low molecular weight fluorescent dye, Lucifer yellow into cells in culture and allows the monitoring of its transfer into contiguous cells. The results of spermatozoa experiments suggest that progesterone receptor was detected in the equatorial segment and post-acrosomal region of bovine spermatozoa. Progesterone (10 μg/mL) exhibited a higher binding affinity (31.11%) for the progesterone receptor compared to concertation of nonylphenol (10 and 200 μg/mL). The results suggest that 17.87% of spermatozoa receptors were occupied by nonylphenol and in the highest doses (200 μg/mL) was observed 21.95% of occupied receptors. In case of TM3 cell line was observed significant ($P<0.05$) decrease in *GJIC* after 5.0 μg/mL of nonylphenol treatment. The higher concentrations of nonylphenol (10.0 and 25.0 μg/mL) significantly ($P<0.001$) inhibit *GJIC* after 30 min cultivation. Similar tendency was observed in octylphenol addition. All experimental doses of octylphenol (2.5; 5.0; 10.0 and 25.0 μg/mL) inhibited *GJIC* but not significantly. In the highest concentration (25.0 μg/mL) was observed significant ($P<0.001$) decrease (V). Both of tested compounds caused GJIC inhibition in the TM3

cells after 30 min treatment. Based on our results we can confirm that the spermatozoa and Leydig cells are sensitive to the influence of endocrine disruptors associated with loss of natural functions due to the decrease of their activity.

Keywords: octylphenol, steroids, spermatozoa, Leydig cell,

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The somatic cell count effect on milk yield and composition of Jersey cows

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The aim of the study was to analyse the somatic cell count (SCC) of the individual cows milk samples (N = 681) under practical condition, and its relationship with milk yield and milk composition. The study was carried out on farm with 42 Jersey cows from December 2017 to October 2020. The measurements of milk yield and milk sampling were carried out at monthly intervals. The milk composition was analyzed for percentage of fat, protein, lactose, and total solids with MilkoScan FT120. Somatic cells count (SCC) was analyzed with Somacount 150 analyzer. The animals were divided into five groups according to SCC (cells.mL^{-1}) per milking: first group (FG) - animals with SCC ≤ 2 x 10^5, second group (SG) - animals with SCC from 2 x 10^5 to 4 x 10^5, third group (TG) - animals with SCC from 4 x 10^5 to 6 x 10^5, fourth group (FUG) animals with SCC from 6 x 10^5 to 1 x 10^6, and fifth group (FIG) ≥ 1 x 10^6. Three stages were stated according to the days in milk: Stage 1 (0-100 d); Stage 2 (100 - 200 d); Stage 3 (≥ 121-200 d). As the SCC increased the lower milk yield was observed except in SG. The lowest milk yield (11.82 ± 0.49 kg) was found in SG and the highest (13.19 ± 4.32 kg) in FG. There were no significant differences among the SCC groups in milk composition (the percentage of fat and protein) except lactose. The percentage of lactose was highest in the FG (4.83 ± 0.03 kg) and the lowest in FIG (4.58 ± 0.04 kg). Milk yield decreased with increased stage of lactation (13.31 ± 0.36 kg, 12.77 ± 0.45 kg, 11.50 ± 0.57 kg). The percentage of fat and protein were the highest in Stage 3 (5.94 ± 0.28 and 4.45 ± 0.07%) and the lowest in Stage 1 (5.47 ± 0.17 and 3.76 ± 0.05%). The percentage of the lactose had opposite tendency (4.77 ± 0.04 and 4.63 ± 0.04%; Stage 1 and Stage 3, resp.). Stage of lactation had not effect on the SCC. In conclusion, milk yield and percentage of lactose differed in according to SCC.

Keywords: cow, somatic cell count, milk composition

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Biogenic and risk element in selected organs and tissues of fallow-deer

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Monitoring the concentration of biogenic and hazardous elements in the organs and tissues used for consumption is crucial for possible contamination of the food chain. This study is focused on the analysis of selected biogenic and risk elements in the organs and tissues of fallow-deer caught in a defined area. The risk elements based on an international program in relation to public health were identified. The concentration of cadmium was highest in the kidneys, the second highest content was in the liver. The highest average nickel content was in the diaphragm and similar values were in muscle. There were differences in lead concentrations between all samples. The highest lead content was in the heart with an average of 0.8202 mg/kg. The lowest average lead content was in the liver and diaphragm. The highest arsenic content was measured in the diaphragm, then in the kidney and liver. The lowest average concentration of arsenic in the heart, lungs and spleen was found. The lungs have the highest iron content, similar values are in spleen. Kidneys, heart, diaphragm, and muscle have the lowest iron content. The average sodium content in the organs and tissues of fallow-deer is highest in the lungs, followed by the kidney, liver, spleen, heart muscle, skeletal muscle and diaphragm. The calcium concentration was highest in the lungs and the diaphragm had the lowest calcium concentration. The highest zinc content was measured in the diaphragm and liver. The lowest mean concentration was in the lungs. Large differences in copper concentrations were found in the liver and kidneys compared to other samples.

Keywords: biogenic elements, xenobitics, organs, fallow deer

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Green tea extract effects on human ovarian granulosa cells *in vitro*

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Epigallocatechin-3-gallate (EGCG) is the major catechin in green tea with numerous health benefits. The cancer-preventive effects of EGCG are widely supported by results from epidemiological, cell culture, animal and clinical studies. EGCG from green tea is a known antioxidant compound and suppresses the inflammatory processes that lead to transformation, hyperproliferation, and initiation of carcinogenesis. The aim of this *in vitro* study was to determine the effect of green tea extract at concentrations 5; 10; 20; 50; 100 µmol/mL (24 h) on the viability of human ovarian granulosa cell line (HGL5), as well as secretion of steroid hormones (17ß-estradiol and progesterone). Cell viability was evaluated by *AlamarBlue™* cell viability assay, and the release of steroid hormones was assayed by ELISA methods. In our research, we observed significant changes in the cell viability of granulosa cells. The number of viable HGL5 cells was significantly ($P \leq 0.001$) decreased at 50 and 100 µmol/mL green tea extract concentration. Progesterone and 17ß-estradiol secretion were not affected by green tea extract. According to our research, despite the many protective effects of green tea, it can be potential risk in higher concentrations for healthy ovarian cells *in vitro*. Further study is needed to safely determine the bioactivity of green tea and its compounds.

Keywords: green tea, EGCG, ovarian cells, steroid hormones

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Iodine concentration in pasteurized cow's milk in Slovakia

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Milk and dairy products are important sources of iodine in many countries. Therefore, the aim of this study was to assess concentration of iodine in pasteurized milk available in Slovak food stores from chosen commercial brand. The samples (n=40) of organic (whole, semi-skimmed milk) and conventional milk (whole, semi-skimmed milk) were collected monthly from June 2020 to March 2021. Iodine concentration of acquired samples was determined by spectrophotometric method of Sandell-Kolthoff. The average iodine concentration of examined conventional whole milk (n=10) was 282.9±21.5 µg/l. Median of conventional whole milk was 299.2 µg iodine/l. The average iodine concentration and median of conventional semi-skimmed milk (n=10) were 329.3±12.3 µg/l and 353.9 µg/l, resp. The average iodine concentrations of organic whole and semi-skimmed milk were 252.9±36 µg/l and 298.3±14.7 µg/l, resp. Medians of organic whole and semi-skimmed milk were 284.4 µg iodine/l and 299.2 µg iodine/l, resp. The differences between conventional, organic milk and whole, semi-skimmed milk were not significant. To conclude exammined pasteurized cow's milk with average milk iodine concetration 290.9±11.9 µg/l might be considered sufficient to cover substantial portion of recommended human iodine intake in Slovakia. Morover, observed iodine concentrations in milk suggest sufficient intake of iodine in Slovak dairy cows.

Keywords: iodine concentration, cow's milk, pasteurized milk

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The content of bioelements and toxic metals in green fruits and leaves of walnut (*Juglans regia*)

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The aim of the research was to determine the mineral composition of leaf samples and unripe walnut fruit. Five samples of leaves and green fruits from wild walnut clones growing in different locations of the Podkarpackie Province were examined. The content of 17 elements was analyzed, including toxic metals: cadmium and lead, using the ICP-OES method and expressed on the dry mass (DW) of tested material.

The macronutrients: potassium, calcium, phosphorus, magnesium and sulphur dominated in the elemental profiles for both tested raw materials. These elements accounted for over 99% of the total minerals. A higher potassium content (on average) was noted in the case of leaves than fruits (61.67% vs. 40.77%), whereas the opposite relationship was observed in the case of calcium (16.04% vs. 36.79%) and magnesium (6.18% vs. 11.89%). The specific accumulation of aluminum in leaves was observed (on average 50.46 mg/kg DW; 0.12% of total elements). Among micronutrients, interesting differences were observed for manganese and strontium (5 times more in leaves). The low content of selenium, an element with antioxidant properties, was found mainly in green nuts (0.40 mg/kg DW). Importantly, toxic cadmium and lead were present only in trace amounts in each of the samples tested.

The presented results prove that both the leaves and unripe walnut fruits are an important source of macro- and microelements, they also are not contaminated with harmful heavy metals. These observations made for the first time, are very promising in terms of using walnuts leaves and unripe fruits as pharmacognostic raw materials, which are famous of their beneficial antioxidant and antimicrobial properties..

Keywords: walnut, leaf, unripe fruit, mineral composition, lead, cadmium

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Effect of subclinical mastitis on milk composition in organic and conventional dairy herds

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It is well established that subclinical mastitis (SCM), characterized by somatic cell count (SCC) >400 thousands of cells per mL of milk, has a negative effect on the productivity and quality of milk. In organic herds, where the use of antimicrobial drugs is restricted for the treatment and control of intramammary infections (IMI) in dairy cows, little is known about the effect of SCM on the milk composition and the share of valuable fatty acids in milk. The aim of this study was to evaluate the effect of subclinical mastitis (SCM) on milk composition in organic and conventional dairy herds (ODHs and CDHs, respectively). A total of 466 milk samples were collected in the summer and winter seasons from cows kept in 3 ODHs and 2 CDHs. Samples of fresh milk were analyzed to determine their proximate chemical composition (protein, fat, lactose, and dry matter content) by the infrared spectrophotometric method with the MilkoScan FT 120 (FossElectric), and the somatic cell count (SCC) by flow cytometry with the BactoCount IBC (Bentley). Milk fat was extracted and the concentrations of 43 fatty acids were determined by gas chromatography, in the Varian CP 3800 chromatograph. Udder health was evaluated based on SCC, in thousands of cells per mL of milk: ≤200 - healthy udder, 201–400 - risk of mastitis; 401 – 1000 - subclinical mastitis, >1000 - clinical mastitis. In ODHs, milk contained around 700 000 somatic cells per mL on average, i.e. twice as many ($p<0.01$) as milk in CDHs. Cows from CDHs produced more milk ($p<0.01$) with higher fat content ($p<0.05$) and higher casein, lactose and urea content ($p<0.01$). The higher level of urea in milk and the better condition of cows in CDHs testify to better nutrient supply. The higher SCC was associated with lower milk yield and lower lactose content. Cows from the SCM group produced similar quantities of milk to cows from the >1000 group (clinical mastitis). Minor differences in the fatty acid profile of milk were found between ODHs and CDHs. They resulted from the fact that fat was assessed only in milk samples collected in winter, when cows were fed only preserved fodder (silage, hay). In ODHs, milk fat contained higher ($p<0.01$) concentrations of polyunsaturated fatty acids (PUFAs), including n-6 PUFAs (linoleic acid and arachidonic acid), conjugated linoleic acid (CLA) and decosapentaenoic acid (DPA).

Keywords: organic milk, somatic cells, fatty acids, CLA

Effect of potato varieties and the culinary treatment on the content of metals in tubers

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The aim of the study was to evaluate the effect of potato variety and culinary processing on the level of selected metals in tubers. The potatoes for testing came from local crops from the Podkarpacie region. Potato tubers were cooked using the traditional method, in a pressure cooker, in microwave oven and baked in the oven. Processed samples were wet mineralized in closed system using the Berghof Speedwave mineralizer and their Fe, Mn, Cu, Zn, Pb, Cd content was determined by atomic absorption spectrometry using an atomic absorption spectrophotometer (Hitachi Z-2000).

The content of metals in potato tubers depended on their variety. The highest amounts of metals were determined in variety Lord, and the lowest - in variety Bellarosa. The exception was cadmium, where the highest level for the traditionally cooked potatoes of the Tajfun variety (0.031 mg · kg^{-1}), and the lowest in the Lord (0.010 mg · kg^{-1}) were found.

The method of culinary processing significantly influenced the level of metals in potatoes. For all potato varieties, the lowest amounts of metals were found in products cooked using the traditional method, slightly higher in those cooked in a pressure cooker, higher in those prepared in a microwave oven, and the highest in baked ones. In potato variety Lord, the lead content, depending on the preparation method, was ammounted: 0.046, 0,049, 0,050 and 0,053 mg · kg^{-1}, respectively

The permissible levels of cadmium and lead were not exceeded in all tested potato varieties, regardless of the cooking method.

Keywords: potatoes, variety, culinary processing, metals

Research of the internal environment of the military camp buildings

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One of the key tasks of our research work is to assess the carbon dioxide (CO_2) pollution in the resting areas of military camps under the current deployment conditions and to assess its impact on soldiers. In the process, we became aware of the environmental impacts that affect the CO_2 concentration in different rooms in different ways.
Fresh air contains about 450 ppm CO_2. Inside building, Max von Pettenkofer defined the criterion of good air as 1000 ppm. Hungarian health standard allows a maximum 5000 ppm concentration of CO_2. In our measurements in the temporary camp containers, we measured 2500 ppm during the sleep of 2 people and 3700 ppm of CO_2 during the 'sitting-work' of 2 people. The camp shelters are furnished for 4 people, so the fresh air supply system is just as important as the heating or air conditioning equipment in the tight container shelters in the camps.
In addition to the rest areas of a military camp, enumerating the major camp facilities that affect the "welfare" feeling of soldiers on foreign service, we arrived at the kitchen complex and the work environment of the kitchen staff. One of the key parts of the camp kitchen complex is the food storage, where the raw materials needed for the supply are stored. Storage is very important in a crisis situation, when you have to be prepared to stay away from the homeland, to prevent any supply or procurement problems. A particularly important task in providing food raw materials is the so-called "Fresh" storage.
Our aim is to examine the changes in the CO_2 concentration of the camp storage room, which is of key importance in food supply, in the vegetable (fruit) storage places. By modeling at a measurement site set up in the laboratory of the host institution, we measure the CO_2 composition of the indoor air in the warehouse by placing various vegetables and fruits.
We assess the typical vegetable and fruit varieties to be stocked according to the camp supply standard and we examine the change in CO_2 concentration for typical vegetable and fruit varieties stored in closed storage rooms, taking into account the degree of effective storage capacity. The change of CO_2 concentration is examined separately for certain types of vegetables and fruits stored in closed storage rooms, taking into account the degree of effective storage capacity and determine the required fresh air value to ensure proper storage conditions. Finally we'll create a mathematical model to simulate changes in storage conditions, what offer helping to plan of military camp.

Keywords: Military camp, inside air quality (IAQ), temporary facilities

Effect of addition of freeze-dried fruits and vegetables on the quality of kefirs

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Dietary fiber supports healthy body weight, and when consumed in adequate amounts, it reduces the risk of cardiovascular disease, cancer, and diabetes. The fiber content in many popular food products is insufficiently high, therefore, a more varied diet is recommended.
The aim of this study was to evaluate the possibility of using raspberry, plum and beet dietary fibers in the concentration of 1.5 % in kefir manufacture. The scope of the research included the production of kefirs with the addition of dietary fiber and the evaluation of the physicochemical and organoleptic properties of kefirs.
The evaluation of kefir quality was performed after seven days of refrigerated storage at 5°C. In kefir, the texture, pH, total acidity (°SH), lactic acid content (g of L^{-1} lactic acid), syneresis (%) and organoleptic evaluation were carried out. The obtained results were presented as the mean and standard deviation, and the simple correlation coefficient (r) was estimated statistically using the Statistica v. 13.1 software (StatSoft, USA). The significance of differences between the averages was evaluated with Tukey's test ($P \leq 0.05$).
Kefirs with fibers are novel functional products that may interest consumers by affecting their health and reducing the risk of disease. The addition of fibers to the processing milk decreased the pH value, which may result in reduced thermal stability of milk proteins during pasteurization. Beet, raspberry and plum fibers did not affect the fermentation process of kefirs. In all groups of kefirs, after seven days of storage, pH was determined in the range of 4.62-4.65. The addition of beet fiber reduced syneresis by 4% compared to the syneresis occurring in the control kefir. The addition of dietary fiber to milk did not significantly affect the texture parameters of kefirs: hardness, cohesiveness, springiness, gumminess and chewiness. The results of the organoleptic evaluation showed that consumers preferred the most natural kefirs and with the addition of beet fiber.

Keywords: kefir, fermented milk, dietary fiber, texture, syneresis, organoleptic evaluation

Changes in the concentrations of selected toxic and essential elements due to the lactation stages in an area with a potentially undisturbed environment of Slovakia

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The aim of this study was to evaluate the effect of lactation on the concentration of selected essential and toxic elements in sheep milk feom area of Slovakia with potentially undisturbed environment and to find the actual contamination of selected area, in view of its environmental character, and to refer to the suitability of the use of milk from this area to other food processing. The research was conducted with 400 sheep (Tsigai breed), average of 7 years, where the milk samples were taken during the lactation periods (early lactation stage, middle lactation stage, late lactation stage). Sheep were reared on the extensive pastures, reared indoors afterwards, fed with pasture *ad libidum*. Milk samples were collected after morning and afternoon milking. Despite the fact that there was large number of animals on the farms, average milk samples were obtained from milk tanks at the end of milking. The samples of milk were analyzed by method of AAS. Significant increase ($P<0.05$) of Ca, Mg, Se and Fe concentrations were found in the milk during the lactation. In case of Mg, Se and Fe increase of concentration was found only in the last stage of lactation. Statistically significant difference ($P<0.05$) in concentration of Zn were found during the lactation stages, but the concentration of Zn decrease between the spring and summer lactation and increased between the summer and autumn lactation. Concentration of essential element (Cu) and toxic elements (As, Cd, Hg, Ni, Pb) in milk was low, below the LOQ. It can be concluded, that the use of milk of sheep from this area for direct use or for dairy products processing is appropriate, safe and poses no health risk for the consumers.

Keywords: essential elements, toxic elements, sheep, milk, environmental burden

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The concentration level of PM10 in southern Poland (Katowice, Kraków, and Rzeszów) during the year 2018

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Human health and food quality are greatly affected by the state of the ambient air. In the European Union, Poland is considered as a country that has the most polluted air. The level of PM10 concentration exceeds the EU limit value in almost all the areas of Poland, but higher concentrations are registered in the southern regions, which are considered as the coal heartlands. Katowice, Kraków, and Rzeszów are three big cities in the southern part of Poland and are surrounded by Coal mining industries. High PM10 concentrations are usually registered in these three cities, especially in the winter period. In 2018, the peak PM10 daily concentration occurred in the three cities at the same period (04/03/2018 in Rzeszów, 05/03/2018 in Krakow, and 05/03/2018 in Katowice). The aim was to identify the effect of each of the 8 coal mines that exist in Poland on the level PM10 concentration for the first week and March where the highest daily PM10 concentration for the year 2018 was registered. Using HYSPLIT Frequency analysis, the results showed that 100% of the particles coming from Bełchatów, Bolesław Śmiały, Halemba, Jas-Mos and Marcel Coal Mines hit Katowice region, and 10% from Bogdanka and Pniówek Coal Mines. While for Kraków, it was affected by 100% of the particles that are originated from Bolesław Śmiały, Pniówek, Halemba and Jas-Mos Coal Mines and 10% Bogdanka, Bełchatów, Jas-Mos and Marcel Coal Mines. Moreover, Rzeszów was the least affected city by the coal mines, 10% of the particles coming from Bogdanka, Bełchatów, Jas-Mos and Marcel, Halemba, and Pniówek Coal Mines attributed to high PM10 concentration during the first week of March 2018. Katowice and Krakow are more affected by the Coal mines industry, Particulate Matter particles originated from the coal mines sites contribute to the high level of PM10 concentration.

Keywords: PM10, Coal Mines, Frequency analysis, Katowice, Krakow, and Rzeszow

Relationship between gestational diabetes and plasma trace element levels in pregnant women from Eastern Iran: A multivariate approach

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The prevalence and frequency of gestational diabetes mellitus (GDM) have increased rapidly in populations that are becoming more westernized. Exposure to environmental pollutants may be a risk factor for the development of GDM, but this is heavily dependent on particular circumstances. Studies linking various factors are therefore needed. In our study, we examined the associations between plasma metal levels (linked to particulate matter air pollution) such, as As, Cd, Cu, Hg, Mn, Ni, V, and Zn and incidents of GDM among 102 pregnant women (diabetic n=60 and healthy n=42) living in Birjand (Iran). The concentrations of As (8.58 vs. 3.14 µg/L), Cd (6.74 vs. 0.52 µg/L), and Hg (2.60 vs. 0.90 µg/L) were significantly higher in women with GDM. Risk difference (RD) estimation revealed that As, Cd, and Hg increase the possibility of the incidence of GDM, while V lower that risk. We were also able to separate subjects according to their GDM status based on element levels with the principal component analysis (PCA). Such classification revealed very high efficiency with a true positive rate of 93%, according to linear discriminant analysis (LDA). Higher levels of As, Cd, Hg in subjects with GDM, indicating that these elements may disturb insulin metabolism and promote GDM development. Therefore, the systematic monitoring of trace elements followed by multivariate modeling in women planning pregnancy should be carried out to prevent the development of GDM.

Keywords: Cd, GDM, Hg, PCA, pregnancy.

Drone brood as a dietary supplement - benefits and limitations

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Drone brood homogenate (DB) is a little - known bee product obtained by the collection of drone larvae from drone cells of honeycomb. It is often called the male equivalent of royal jelly, the most appreciated bee product, because of its similar chemical composition. In many countries (Japan, China, Russia, Romania, Ukraine) drone brood is applied for preparing snacks in the fried or baked form. The aim of the study was to determine the benefits and risks of introducing drone brood into the diet on the basis of the analysis of selected parameters of its chemical composition.
Drone brood was obtained at various stages of development (7-, 11-, 14th day of development). It was analyzed in terms of polyphenolic profiles using HPTLC method. The content of selected minerals and the toxic metals were determined by (ICP-OES) and the activity of hormone (testosterone, estradiol) in tested drone brood samples was demonstrated with the use of immunoenzymatic ELISA test kits.
Based on the analysis of the color intensity and the number of bands on HPTLC chromatograms, it should be concluded that the richest source of phenolic compounds is the 7-day-old drone brood, and the weakest - the 14-day-old one. Ferulic and ellagic acids were found as specific polyphenols in drone brood. Among the identified macroelements, the highest content was found for phosphorus (302.17 mg/100 g) and potassium (288.75 mg/100 g) whereas the lowest value for sodium (8.04 mg/100 g) was detected. The significant quantities of zinc, manganese and iron were found. In addition, a low amounts of contaminants such as lead (0.0033 mg/100 g) and cadmium (0.0021 mg/100 g) were identified and a higher content in the case of DB aluminium (0.62 mg/100 g was found. Drone brood is a bee product containing on average 4.2 nmol/100 g of testosterone, whose level increases significantly with the development phase of the drone brood ($p<0.05$). A comparative analysis of drone brood estradiol showed a higher level of estradiol at the earlier stages of DB development.
The rich chemical composition of the drone brood contributes to the high degree of biological activity and leads to a beneficial effect on the human body. Due to high mineral composition and hormone levels, drone brood effectively prevents the processes of cellular aging and many diseases like treating androgen deficiency in women and for preventive action against the osteoporosis and in men infertility. However it should be serve in limited dose to avoid hormone overdosing. Moreover, due to reported contamination with toxic metals, the drone brood should be carefully examined before being introduced as a dietary supplement.

Keywords: drone brood, dietary supplement, polyphenols, minerals, hormonal activity, toxic metal contamination

Reproductive toxicity of metal nanoparticles can be prevented by their chemical modification and functional food plants and plant isoflavones

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The application of metal nanoparticles in modern society is growing, but their application is hampered by their general and reproductive toxicity. The possible approaches to reduce this toxicity could be the chemical modification of the used nanoparticles, as well as the application of some medicinal and functional food plants or their constituents. The aim of the present studies was to examine the direct influence of silver and titanium dioxide nanoparticles (AgNPs and TiO_2NPs), and unsupported and supported copper nanoparticles (CuNPs) of different morphologies, on ovarian granulosa cell functions, to understand the character and mechanisms of their action, as well as to determine whether their toxic effects could be mitigated by the chemical modification of the nanoparticles and some medicinal and functional food plants or plant isoflavones. It has been shown that the addition of these nanoparticles to cultured porcine ovarian granulosa cells is able to reduce the expression of markers of their proliferation, apoptosis, viability and hormones release. The addition of plant extracts (buckwheat *Fagopyrum Esculentum*, and vitex *Vitex Agnus-Castus*) and plant phytochemicals (resveratrol, diosgenin, rutin and apigenin) is able to mitigate and to prevent the action of AgNPs, TiO_2NPs and CuNPs on ovarian cell functions. The comparison of the activity of unsupported spherical, triangular and hexagonal CuNPs, as well as of spherical CuNPs supported on TiO_2, zeolite Y and activated charcoal, demonstrated that the morphology and support of CuNPs can affect, mitigate and even invert the toxic action of CuNPs on ovarian cells.
These observations suggest some reproductive toxicity of metal nanoparticles, which should be taken into account for their application. Nevertheless, the adverse effects of these nanoparticles can be mitigated and prevented by modification of their morphology, support on inorganic substances and action of some medicinal and functional food plants or their derived isoflavones.

Keywords: nanoparticles, ovary, toxicity, proliferation, apoptosis, hormone

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Content of micro- and macroelement in drones of Rožňava region

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The external environment directly affecting the health of the whole bee community is nowadays strongly negatively affected by anthropogenic activities. The fact that pollen and nectar from entomophilous plants present the main part of bee's food allows perceiving bees to be local natural bioindicators. And whereas drones depend on food that is produced directly in the hive, can be considered as an internal environment indicator of the hive. The aim of the present study was to determine the content of selected chemical elements in drones of the honey bee from Rožňava region of south-eastern Slovakia. Biological samples (the drones) were collected at the beginning of the bee production season in the months of May and June. Based on the required minimum weight of the methodical analysis, the drones from the hive were divided into twelve separate samples containing 2-3 individuals. The samples were mineralized in the high-performance microwave digestion system Ethos One in a solution compound from HNO_3 and H_2O_2. Levels of selected elements in samples were determined using the inductively coupled plasma optical emission spectrometry (ICP-OES) method. The concentrations of tested elements in drones body were: Al 7.44 ± 1.79 mg kg^{-1}, Ba 0.21 ± 0.07 mg kg^{-1}, Ca 265.30 ± 24.49 mg kg^{-1}, Cu 10.04 ± 1.21 mg kg^{-1}, Fe 34.25 ± 3.87 mg kg^{-1}, K 5217.00 ± 280.30 mg kg^{-1}, Mg 520.20 ± 29.90 mg kg^{-1}, Mn 4.12 ± 1.44 mg kg^{-1}, Na 357.90 ± 28.05 mg kg^{-1}, Sr 0.14 ± 0.03 mg kg^{-1}, Zn 40.35 ± 5.96 mg kg^{-1}. Based on acquired knowledge and the obtained results we can recommend using drones as an appropriate testing material of environmental bioaccumulation to determine the environmental quality and vitality of the hive as well as the potential quality of chemical composition of honey without negative impact to the hive in the production season.

Keywords: bee, drones, elements, ICP-OES

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The risk assessment of coumarin overdosage as the result of consumption honey enriched with *Melilotus plant*

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Honeys enriched with herbs are a new product appearing more and more often on the Polish market. The addition of herbs to honey allows to enrich it with bioactive compounds derived from the plant material. The sweet clover (*Melilotus*) is a plant with high biological potential, due to the occurrence of specific secondary metabolite – coumarin. This chemical exhibit anticoagulant properties, however its overdosing can act hepatotoxic.
The aim of this study was to assess the possibility of exceeding the daily limit for coumarin (0.1 mg/kg) as the result of consumption of enriched honey. For comparison, natural white sweet clover (*Melilotus albus*) honey and 4 enriched honeys were, including rape honey added with dried flowers (HF) and leaves (HL) of *Melilotus albus* plant as well as honey enriched with dry extract of flowers (HeF) and leaves (HeL) of the same plant. The coumarin content was determined by HPLC-PAD.
All honey enriched with the addition of sweet clover (*Melilotus albus*) shows significantly higher coumarin content (from 0.64 to 7.31 mg/100g) compared to natural sweet clover honey (0.04 mg/100g). Among enriched honeys, the highest coumarin level was found for honey with 1% additive of dried flowers (7.31 mg/100g), the lowest for additive of dry exract and flowers (1.52 and 0.64 mg/100g, respectively). Taking into account acceptable daily intake for adults (7 mg per day) it was found that in most cases the overdosing of coumarin resulting from honey consumption is impossible. However, the most active HF honey should be supplemented with caution and appropriate information on limiting consumption should be placed on the product label.

Keywords: honey, *Melilotus albus*, enrichment, coumarin, health risk

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BBAP amplification profiles of apple varieties

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Several types of allergies are currently known and are characterized by an exaggerated response of the immune system to substances from various sources called allergens. One of them is food allergy, which is becoming more common in the population. For this reason, it is necessary to describe the issue from several aspects including genomic variability of plant allergens. The objective of this study was to analyse intraspecific variability of 10 different varieties of apple species (*Malus domestica* Borkh.). BBAP technique for genomic determination of the presence of Bet v 1 homologs at the DNA level was performed. Degenerate primers that anneal a variable and conserved part of PR-10 protein homologues genes were used in the analyse. Amplicons were generated and formed relatively monomorphic profiles, indicating the stability of the given isoforms within the apple varieties.

Keywords: Bet v 1, allergens, degenerate primers, apple varieties

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The influence of cocoa and coconut fiber on the quality of kefirs

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Adequate consumption of dietary fiber, depending on age, sex, and physiological state, guarantees confirmation of its pro-health properties. Dietary fiber is mainly used to prevent diseases of the gastrointestinal tract and metabolic disorders.
This study aimed to develop a recipe and the production of kefir with fibers (cocoa, coconut in the amount of 2 %) with the evaluation of its quality.
The quality assessment was carried out after seven days of storage (5°C). In the fermented milk pH, total acidity (°SH) and lactic acid content [g of L^{-1} lactic acid), syneresis (%), color L * a * b *, texture were determined. Furthermore, the organoleptic evaluation was performed.
The obtained results were given as the mean and standard deviation, and the simple correlation coefficient (r) was calculated statistically using the Statistica v. 13.1 software (StatSoft, USA). The significance of differences between the averages was estimated with Tukey's test ($P \leq 0:05$).
Coconut and cocoa fibers belong to the group of natural plant additives. Introducing them to the processing milk in the amount of 2% resulted in a decrease in the pH value of the milk. Therefore, increasing the amount of added fiber before pasteurization may lower the thermal stability of the milk. The total acidity in kefirs with fibers was higher by about 3-7° SH and the lactic acid content by 0.06-0.12 g L^{-1}. Kefirs with fibers were characterized by a higher level of syneresis and a darker color than natural kefirs. The addition of fiber shaped the texturometric profile: it decreased hardness, adhesiveness, and increased resilience and stinginess length. In the opinion of the evaluators, kefirs with the addition of fiber were more desirable than natural kefirs. Cocoa and coconut fiber are dietary fibers of plant origin, and their appropriate consumption, i.e. at the level of 35-40 g/day/adult, guarantees a therapeutic effect.

Keywords: milk, kefir, fiber, cocoa, coconut

Milk yield and its composition: possible effects of parity

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It is well known that parity influences the milk yield and its composition but at present the most of the cows are culled very soon indicating the average culling rate at 1.8-2.2 lactation. The evaluation of cows on higher parities are missing. The aim of the study was to evaluate the effect of dams' parity on milk yield and on chosen milk components (fat, protein and lactose). The data, 92 records of dairy cows, were collected in years 2014-2017 from the farm located in Lower Váh region (western Slovakia). The studied herd consisted of Slovak spotted breed. The mean 305-d milk yield of the exammined herd was 8133±1380 kg. The effect of dairy cows' parity was divided into three groups (1st parity cows, 2nd-3rd parity cows, 4th and higher parity cows). The dams' parity in our study significantly influenced lactose content in milk ($P < 0.01$). Dairy cows in the 4th and higher parity showed lower lactose content (4.75±0.03%) compared to 1st (4.86±0.03%) and 2nd-3rd parity cows (4.86±0.02%). Nevertheless, other milk components (fat, protein content) as well as milk yield did not reach significance ($P > 0.22$; $P > 0.27$; $P > 0.50$; resp.). However, we observed that fat and protein content were, similarly to lactose, numerically lower in 4th and higher parity cows compared to 1st, 2nd and 3rd parity cows. In this respect 1st and 2nd-3rd parity dams produced numerically less milk compared to 4th and higher parity dams (8122.95±264.15 kg; 7884.46±222.66 kg; 8480.71±259.39 kg; resp.). This might be related to the already mentiond lower concentration of milk components (lactose, fat and proteins) in 4th and higher parity dairy cows. Therefore, our results might point out important soure of information for farmers to manage cows in proper ways to increase the average age of cows at culling.

Keywords: parity, milk yield, composition

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The use of chemometric analysis to verification of botanical origin of Polish honey

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The objective of this study was a comparative analysis of four honey types from the area located in south-east Poland, based on the content of the main phenolic acids and proline, the mineral composition, and selected physicochemical properties. The study material comprised 48 honey samples from apiaries located in mountainous areas within the Carpathian Foothills region. The honey classification based on the investigated parameters was conducted with the use of multivariate statistical analysis. Most analyses were performed using chromatographic methods. A detailed analysis of the results of comprehensive physicochemical research on the selected types of Polish honey showed that multifloral honeys exhibit almost a two-times-higher content of phenolic acids compared to linden honey, but half the amount compared to honeydew honey; furthermore, caffeic acid was not identified in any honeydew honey. Honeydew honeys were the richest in phenolic acids and minerals, as well as oligosaccharides, of all the studied honey types. Dark-colored honeys were characterized by the highest proline content. The dominant elements in all types of honey were potassium and calcium. The analyzed honeys also contained a significant amount of magnesium. Additionally, honeydew honeys contained the highest amount of toxic metals. The results of the present study showed that the specific phenolic acids, minerals, proline, and sugar content, in combination with chemometrics analysis, may successfully differentiate between the biological origins of honey samples and allow the preliminary verification of samples before performing time-consuming pollen analysis.

Keywords: chemometrics, honey, bioactive compounds, proline, minerals

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Toxicity of bisphenols altering lysosomal functions in TM3 Leydig cells

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Bisphenol A (BPA), an important additive for polycarbonate plastics and epoxy resins, is widely used in producing baby bottles, food coatings and other products, with an estimated production above 4.5 million tons per year. Its wide application exposes nearly all people to BPA and several studies have focused on its health implications and toxic effects. In consideration of BPA toxicity and limits on its use, efforts to find appropriate and safer substitutes are essential. Bisphenol B (BPB) is increasingly replacing BPA in daily supplies, increasing the exposure levels in various environments. Lysosome is regarded as an organelle with distinct sorting, secretory and proteolytic function; however, newer data suggest that the lysosome is a metabolically active organelle dictating cellular homeostasis. In the present study, comparative effects of 24h and 48h exposure to BPA and its analog BPB in concentrations 0.04 - 50 $\mu g.ml^{-1}$ on the lysosomal function of TM3 Leydig cells were determined. To achieve the goal of our experiment, neutral red cell cytotoxicity assay was used. This is one of the common methods used to detect cell viability or drug cytotoxicity. Neutral red is a eurhodin dye that stains lysosomes in viable cells. Results in our study showed that BPA significantly ($P<0.05$) stimulated lysosomal function after 24h of its exposure in concentration 2.5 $\mu g.ml^{-1}$ in comparison with the control group without addition of BPs. After 48h of BPA exposure, we determined significant ($P<0.01$) stimulation of lysosomal function in the concentration of BPA 1.0 $\mu g.ml^{-1}$ in comparison with the control group. The highest used concentrations in the experiment caused a significant decrease of lysosomal function in comparison with the control group - in concentration 10 $\mu g.ml^{-1}$ ($P<0.01$) and in concentrations 25 and 50 $\mu g.ml^{-1}$ ($P<0.001$) as well. Significantly higher lysosomal function was observed after BPB addition in concentrations 0.2 and 2.5 $\mu g.ml^{-1}$ ($P<0.01$); 1.0 $\mu g.ml^{-1}$ ($P<0.05$) after 24 h of its exposure in comparison with the control group. Two-days exposure of BPB caused significant decrease of lysosomal function in concentrations 5.0 and 10 $\mu g.ml^{-1}$ ($P<0.01$); 25 and 50 $\mu g.ml^{-1}$ ($P<0.001$); in comparison with the control group. Based on our results we could conclude that analog BPB increasingly used today as an alternative to BPA may have similar or higher toxicological effects on male reproduction and further research on cells of male reproductive system is needed.

Keywords: bisphenols, Leydig cells, male reproduction, lysosomal function

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Essential and non-mutagenic elements in raw ewe milk

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The aim of this work was to determine the concentrations of selected essential (Ca, K, Mg, Mo, Na, Zn) and non-mutagenic elements (Ag, Al, Ba, Li, Sb, Sr) in ewe milk from Orava region in northern Slovakia. Twenty milk samples were analysed in June and August using an inductively-coupled plasma optical emission spectrometry. The differences in elements concentrations between the seasonal periods were not significant except of lithium ($p<0.05$). The concentrations of essential elements were within the recommended levels. The non-mutagenic and potentially toxic metals concentrations were under the permissible limits. The elements occurred in the following order: Ca > K > Na > Mg > Zn > Sr > Ba > Al > Li > Mo > Ag > Sb in June, and Ca > Na > K > Mg > Zn > Sr > Ba > Li > Al > Mo > Ag > Sb in August. The significant correlation relationships were found between the elements. Very strong positive correlation between Al:Zn ($p<0.05$), Ba:Sr ($p<0.001$), Ca:Mg ($p<0.001$), Ca:Na ($p<0.05$), K:Mg ($p<0.05$), Li:K ($p<0.05$) were found in June. More relationships were found in August. The very strong positive correlations were found between Ag:Mo ($p<0.001$), Ba:Ca ($p<0.05$), Ba:K ($p<0.05$), Ba:Mg ($p<0.05$), Ba:Sr ($p<0.01$), Ca:K ($p<0.001$), Ca:Mg ($p<0.01$), Ca:Sr ($p<0.001$), Ca:Zn ($p<0.001$), K:Mg ($p<0.001$), K:Sr ($p<0.001$), K:Zn ($p<0.05$), Li:Na ($p<0.05$), Mg:Sr ($p<0.01$), and very strong negative correlations were found between Na:Ca ($p<0.05$), Na:K ($p<0.05$), Na:Zn ($p<0.05$) in August. This study reveals that the ewe milk from the northern part of Slovakia in Orava region contains essential elements in normal range. The risk, but non-mutagenic elements were also in low concentrations which do not pose risk for the consumer. There were very strong and significant relationships between the elements which may suggest the synergistic/additive or antagonistic effects of some elements. The monitoring of metals and other chemical elements in the basic sources of diet, mainly for children, is very important for preventing the health issues.

Keywords: essential elements, risk elements, non-mutagenic elements, ewe milk

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Assessment of mercury contamination in honeys traded in Poland using thermal decomposition amalgamation atomic absorption spectrometry (TDA AAS)

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The aim of the study was to quantify mercury contamination of honeys traded in Poland using the Direct Mercury Analyser DMA-80. The analysis were done in accredited laboratory according to procedure PB / HŻ / R-28 in measuring range from 0.001 to 1.0 mg / kg. The level of mercury was correlated with the content of 5-hydroxymethylfurfural (HMF) determined by the Winkler method and for selected honeys with the diastase activity assayed by Phadebas test. 45 honey samples differed in geographical (20 domestic and 25 imported) and botanical (22 multifloral, 6 lime, 4 buckwheat, 3 acacia, 3 honeydew, 2 rape and 5 others) origins were tested.
All the analyzed honeys contained mercury in an amount not exceeding the applicable standard levels (0.01 mg/kg of honey). The highest value for honeydew honey was observed (0.73 µg/kg) and the lowest for lime honey (0.08 µg/kg), however the observed varietal differences were not significant. The mercury level in honey was not depended on the country of origin ($p>0.05$). In all honeys, the HMF content was within the acceptable standard levels (40 mg/kg) and the highest content in buckwheat and acacia honeys were found. Diastase number activity varied significantly between honey varieties, the lowest in acacia and the highest in buckwheat honeys was determined. Statistically significant relationship between the Hg and the HMF content ($r=-0.80$, $p = 0.000 <0.05$) was observed whereas between the Hg content and the diastase number weak possitive correlation was calculated ($r=0.43$). It was found, that honeys commercially available in Poland are of good quality in terms of mercury contamination and other tested indicators of quality. In general, domestic honeys were of better quality compared to imported honeys.

Keywords: honey, mercury, variety, country of origin, DMA-80, HMF, diastase number

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Comparison of the minerals' content in the shell of chicken and duck eggs

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The avian embryo absorbs all the minerals needed for proper ossification of the skeleton mainly from the eggshell, and to a lesser extent from the egg yolk and protein. It is well known, that the egg of various poultry species differ the incubation parameters and period and also a yolk composition. For this reason, it seemed interesting to compare the mineral composition of two poultry species: chicken and duck.
Eggshell samples (10 g) were taken from the hatching eggs of parental stock of broiler chicken (Ross 308 line) and Peking ducks (Cherry Valley line). The content of elements (Ca, P, K, Mg, Na) was determined by an atomic emission spectrometer (ICP -OES Optima 7300 DV by PerkinElmer), while C, N, S with using an elemental analyzer (Vario Max Cube). Determinations in each of the analyzed samples were performed in triplicate.
It was found that the mineral composition of the chicken and duck eggshell is different. The eggshell of these poultry spiecies contained a similar value of calcium (229.9 and 213.6 g Ca/kg, respectively) and carbon (144.0 and 162.9 g C/ kg). The chicken eggshell contented: nitrogen 8.4 g N/kg); phosphorus (1.0 P g/kg), natrium 0.7 g (Na/kg) and sulfure (0.8 g S/kg), while duck eggshell was 1.9- 1.4- 1.9- 1.8- times richer in these minerals, respectively. On the other hand, the chicken eggshell contained 2.44 g Mg/kg and 0.63 g K / kg, which is 3.5-1.5 times more than that of duck.
The obtained results indicate differences in the demand of minerals of the organism of these birds in the embryonic period.

Keywords: duck eggs, chicken eggs, mineral composition, eggshell

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Influence of dairy heifer birth season on learning ability in the 12th and 19th month of their life

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The aim of this work was to evaluate the influence of the birth season and the method of rearing in the period of milk nutrition, on the ability to learn at the age of 12 and 19 months (m). Dairy female calves (51) were placed in one of three groups after birth. In the group with restricted sucking (RS), there were calves (18) born in the season S1=January-March n=9; S2=April-June n=3; S3=July-September, n=3 and S4=October-December, n=3. In the unrestricted sucking (US) group, calves (16) were born in the seasons (S1:S2:S3:S4) 5:3:3:4 and in the group of the conventional rearing (CR) calves (17) were born in the seasons 7:7:1:2. Season averages of minimum, mean and maximum temperature (in °C) on the farm were as follows: S1 (-2.3; 1.5; 5.5), S2 (8.1; 13.9; 20.0), S3 (12.6; 17.7; 24.2), S4 (1.2; 4.6; 8.4). Group RS - pen with mother to 21st d, suck 3 times daily, then group pen (6 kg milk); US - pen with foster cow, 6 kg milk daily; CR - hutch to 56th d, milk replacer (MR) 6 kg, then loose housing pen to weaning, MR 6 kg daily. After weaning (84th d), calves were kept in groups with the same ration. Learning ability was tested at the 12th and 19th month of age with a maze test. Mazes were constructed from 2 m high walls. Animals solved six tests (different arrangement of maze walls) during three consecutive days. Each test was performed twice (four runs per day) in a random order during each of these tests. The motivation to finish the problem was access to a 0.5 kg CM at the exit. In the maze, we measured the time of pass through, the time of standing and the number of vocalizations. Learning speed was assessed using the time until the animal achieved the goal. At the age of 12m, the animals of the US group needed a significantly shorter time to go through the maze than the animals of the CR group (mean ± SE; RS 1220.17±87.17 s; US 891.75±106.36 s; CR 1345.47±118.90 s; P=0.0369; US:CR*) and animals born in season S4 vocalized significantly less than animals born in S1 (S1 29.29±3.05; S2 23.57±4.16; S3 16.0±4.26; S4 11.78±2.03; P=0.0068; S1:S4 The same situation repeated itself at the time of crossing the maze at the age of 19m (RS 1198.50±94.39 s; US 909.31±102.10 s; CR 1290.13±97.60 s; P=0.0485; US:CR*). Animals born in S3 went through the maze significantly faster than those born in S1 (S1 1298.68±91.67; S2 1138.43±101.02; S3 797.0±158.67; S4 1021.88±133.01; S1:S3*). Animals from the RS group vocalized significantly more than other animals (RS 20.37±4.01; US 9.75±1.50; CR 8.38±2.63; P=0.0022; RS:US,CR*).

Keywords: dairy heifer, birth season, learning ability

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Evaluation of antioxidant activity of natural extract mixture in intensively reared growing rabbits: preliminary data

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In the last decades, the scientific community has deeply focused studies on food safety and animal welfare, searching for innovative and natural feeding strategies capable of improving animals health and production performances. The purpose of this study was to investigate the *in vitro* on human erythrocytes and *in vivo* on growing rabbits antioxidant activity of brown seaweed (*Laminaria* spp) and plant polyphenols extracts mixture. In human red blood cells we studied the antioxidant activity and the capacity to inhibit hemolysis of water extract at different concentrations of the mixture by the Cellular Antioxidant Activity (CAA-RBC) test. Further, an in vivo experiment was performed on two hundred and forty clinically healthy New Zealand White growing rabbits that were allotted into 5 dietary treatments containing 0 (Con-Con group), 0.3% (Con-Mix1 and Mix1 groups), and 0.6% (Con-Mix2 and Mix2 groups) of brown seaweed and plant polyphenols mixture supplemented to basal rabbit diet for 42 days. Rabbits from Mix1 and Mix2 groups belonged to rabbit does that were fed with diet supplemented with the same feed additive at the same doses, assuming a long-term exposition of supplementation. Whereas animals from Con-Mix1 and Con-Mix2 belonged to rabbit does that were not fed with the feed additive and received the supplement in feed from the weaning moment (35d of age) to the slaughter moment, assuming a normal term of supplementation. After slaughter, samples of liver and kidney, from rabbits of each group, were analysed for the evaluation of selected antioxidant biomarkers to monitor the animal welfare of intensively reared animals. Results on the cellular antioxidant activity and on the hemolysis inhibition of the water extract evidenced both a good antioxidant power and a marked hemolysis inhibition in a dose-dependent manner. Furthermore, the *in vivo* experiment showed an antioxidant effect of the natural extracts mixture on liver and kidney tissues, underlined by the lower value of protein carbonylation of experimental groups compared to the control group. In conclusion, the brown seaweed (*Laminaria* spp) and plant polyphenols extracts mixture could be considered a potentially useful material for both nutraceutical and animal feed industries because of its *in vitro* and *in vivo* antioxidant effects on intensively reared growing rabbits.

Keywords: feed supplement, animal welfare, rabbit, biomarker

Washing duck eggs and the risk of microbial contamination of their content

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The shell of the duck egg is covered with a thick layer of mucin. Therefore, there is recommended to remove the mucin layer by washing the eggs in order to improve gas exchange of the embryos during artificial incubation. However, this procedure may increase the risk of microbial contamination of the egg contents. With this in mind, the aim of the study was to analyze the quantitative and qualitative composition of the microflora of washed and fumigated eggs.
The Pekin duck hatching eggs (Cherry Valley line), randomly divided into two groups were subjected to the following procedures: W (n = 6330) - two-stage washing in water (T 40°C) and 1% benzalkonium chloride solution in an automatic egg washer (MST Master Matic 5000); D (n=927) - ozonizing for 30 minutes at a concentration of 0.5% O_3 (generator BT-NT18). On the day of setting, the eggs were additionally fumigated (FUMAGRI OPP) and next incubated in a single-stage incubators (type Sommen). Eggs were candled at 10. and 23. day of incubation (d.i) and eggs not developing were rejected. Microbial contamination of the eggshell surface and egg contents was analyzed for the number of mesophilic aerobic microorganisms, the presence and number of *Enterobacteriaceae*as well as coagulase-positive staphylococci.
Hatchability in groups W and D were 55.1% and 75.4% ($P<0.05$) and the percentage of organoleptically changed eggs was 11.6% and 3.1% ($P<0.05$), respectively. Analysis of the quantitative and qualitative composition of the eggshell microflora did not reveal the presence of *Enterobacteriaceae*, *Listeria monocytogenes* and *Bacillus cereus*. In the group of staphylococci identified on the surface of eggshells, coagulase-negative, lipase-negative, and lecithinase-negative strains were present before washing and disinfection. No coagulase-positive strains, including *Staphylococcus aureus*, were found. The content of organoleptically unchanged duck eggs collected at the following incubation stages were free from microbiological contamination. Organoleptically changed eggs were the site of the development of *Enterobacteriaceae* family, including *Escherichia coli*, non-fermenting *Pseudomonas*strains, including *Pseudomonas aeruginosa*, aerobic spore *B. cereus* and *B. subtilis*, coagulase-positive cocci, including *S. aureus* and micrococci.
Summarizing, the obtained results indicate that the disinfection methods used are satisfactorily effective. Bacteria multiplication mostly occurs in eggs infected vertically or horizontally on the farm. Washing the eggs also can contribute to this.

Keywords: duck eggs, incubation, microbiological contamination

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Influence of air pollution with PM_{10} and $PM_{2.5}$ particles on spatial differentiation of death rates in the Małopolska province

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Air pollution is a serious health problem to human populations. Inhaled pollutants exhibit toxic properties mainly to respiratory system, but some of them may be absorbed from lungs into circulatory system and next distributed into the whole body. Special attention is payed to particulate pollution referred to as $PM_{2.5}$ and PM_{10}. PMs include particulates of different diameter ($PM_{2.5}$≤ 2.5μm; PM_{10}≤ 10μm), and different chemical and toxicological properties: metals, organic compounds, ammonia, nitrates, carbon, bioaerosols etc. Their toxic effects may be different according to concentration in the air, particles diameter and chemical composition. Nonetheless there are recognized health problems which may be strongly related to inhaled PMs. Respiratory disorders are recognized as critical effects of PMs. Other PMs effects include cardio-vascular disorders, renal disfunction and/or neoplastic transformation of different tissues. Most of the above effect were confirmed in animal model or in vitro laboratory studies. The relation between PMs air concentration and health effects in human is still open question. This is why we decided to perform statistical analysis of the relation between PMs air concentration and mortality rate from malignant neoplasm of bronchus and lung in Małopolska citizens. For the analysis we have used a general additive model (GAM) and spatial analysis with GIS maps. The applied models enabled us to perform spatial and time related mortality with the consideration of air pollution with PMs. We have analysed data on mortality and air PMs pollution from 229 sites in Małopolska district in the years 2015-2018. The highest mean year mortality from malignant neoplasm of bronchus and lung was found in 2015 (52,34/10 thousand), with the highest mortality rate in Szczawnica town (202,29/10 thousand). The highest average year PMs concentration was found in 2018 ($PM_{2.5}$ – 26.67 μg/m^3; PM_{10} – 36.38 μg/m^3). The highest average year $PM_{2.5}$ concentration was found in Skawina-countryside (31,54 μg/m^3) and the highest average year PM_{10} concentration was found in Brzeszcze (43,72 μg/m^3). GAM analysis showed significant correlation between mortality rate from malignant neoplasm of bronchus and lung and PMs air concentration in Małopolska. The performed analysis predicts similar mortality rate in the upcoming years. We also suggest that GAM analysis applying Poisson's distribution of variables linked with logarithmic function is a good tool for statistical analysis of the relation between environmental contamination and health effects. Spatial analysis with GIS techniques showed higher concentration areas of $PM_{2.5}$ and PM_{10} in urban areas and in north-western part of the Małopolska province with particular high accumulation in Krakow. The spatial distribution of deaths caused by malignant neoplasm of bronchus and lung was similar with clusters of high mortality on the northern and north-western parts of the region.

Keywords: PM2.5, PM10, Małopolska, lung and bronchial cancer, mortality rate, GAM analysis, spatial distribution

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