

The XLVI ESNA Annual Meeting

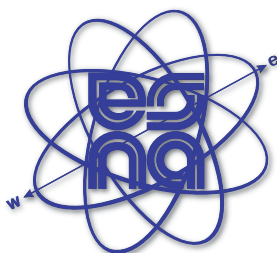
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Book of abstracts

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“Future of agriculture:
Between biotechnology and sustainable farming”

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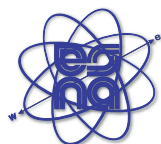
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PLENARY LECTURES



Precise editing of plant genomes

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The discovery of gene transfer between *Agrobacterium* and plants revolutionized genetic research and boost development of engineered organisms in next decades. Nowadays, a vast number of modifications contrasts with only few that were introduced into agricultural production. Among disadvantages of commonly used plant genetic transformation techniques is uncontrolled insertion of transferred sequences to the host genome. This drawback has been partially overcome by the development of novel techniques utilizing nuclease complexes targeting predefined genome sequences. Systems relying on meganucleases, zinc finger nucleases or transcription activator-like effector nucleases opened new possibilities of precise genome editing but their use is still troublesome. The breakthrough came with recent discovery of bacterial natural immune system against phages and involving clustered regularly interspaced short palindromic repeats (CRISPR) that interact with Cas9 endonuclease. The advantage of all genetic tools utilizing CRISPR-Cas9 system is their ability to target plant genome sequences recognized by the user defined short guide RNA (gRNA) thus the construction of CRISPR-Cas9 vectors is now simplified and has opened new prospects in plant genetic engineering. Moreover, a new class of Cpf1 nucleases has been recently discovered and are alternative to Cas9. Cas9/Cpf1-gRNA complex cleaves plant target DNA and then non-homologous end joining or homology directed repair mechanisms lead to nucleotide substitution, short indel or insertion of a new heterologous sequence, if it is additionally delivered to the host cell. Thus the host organism acquire a mutation at the user defined target site that may results in a new trait. Application of editing techniques using CRISPR-Cas9/Cpf1 systems implies consequences in authorization process of genetically modified organisms as in many cases there is no conclusive molecular methods distinguishing between spontaneous mutants and GM organisms.



Agricultural heritage and agroecology: a pathway to sustainable food systems

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The international efforts to implement and assess the values of the heritage of agriculture undoubtedly arise from a legitimate social and scientific demand: the need to protect all significant natural and cultural heritage properties generated by agrarian activity in the course of history (country houses, orchards, mills, terraces, crops, irrigation channels, wells, farmyards, traditional festivities, gastronomy, indigenous species, landscapes...). However, the establishment of criteria to guide the identification and formal recognition of the heritage of agriculture, in all its meanings and manifestations, is a relatively recent and pressing subject of study. In contrast to what has been achieved for other heritage categories, such as industrial or vernacular heritage, the latter being in fact frequently part of the agricultural heritage, the absence of a particular and appropriate recognition of agricultural heritage, as a multidimensional heritage which embraces different types of tangible and intangible, cultural and natural properties, has led to its underestimation and a lack of consideration.

The term agroecology refers to ecosystems. Ecosystems are characterized by aspects such as nutrient cycling, population regulation, energy flows and a dynamic equilibrium. These characteristics apply to natural ecosystems, and, in a much altered form, also to manmade or agricultural ecosystems. The magnitude of the differences between natural and agricultural ecosystems depends mainly on the human manipulations, generally characterized by the intensity of management or intervention, and the level of disturbance vis-à-vis the (natural) equilibrium. Both the intensity of management and the level of disturbance have to be considered as a result of social and economic pressures on the human actors in the agricultural ecosystem. This increasing intensity of management is clearly visible in technical innovations in agriculture, often invented under the economic pressure to become more efficient. The innovations often require a uniformity (in crops, in soil management) by which the agro-ecosystem moves further and further away from the natural system.

Agro-ecological concepts are primarily based on traditional and local knowledge, and its corresponding cultures. Agroecology combines this knowledge with the findings and

methods of modern science. The strength of agroecology lies in the combination of ecological, biological and agricultural sciences, along with medicine, nutritional and social sciences. It incorporates the knowledge of all stakeholders. Their practical contribution to solving complex problems with the help of locally available resources is crucial. Apart from water, soil and sun, other resources that are particularly important are the natural and cultivated diversity of plant species and varieties, along with the knowledge of people and communities on how these plants interact. Agroecology is a resource of both new and old examples of successful agro-ecological adaptation, and describes the enormous potential of agroecology: It can contribute to directly increasing yields, protecting resources, reviving the local economy and improving health, prosperity and resilience. In this context it is fundamental to promote sustainable agriculture and rural development through policy and technical assistance, networking, research, training and education for safeguarding and dynamic conservation of the world's agricultural heritage systems and sites.



Functional impact of transposable elements on the diversity of plant genomes

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Transposable elements (TEs) are DNA segments capable of changing their localization within the genome. They constitute a significant ‘dispersed repetitive DNA’ portion in virtually all plant genomes. Despite the fact that they were initially described ‘selfish DNA’ or ‘ultimate parasites’, at present the view of them being one of the major factors responsible for de novo variability and genome plasticity is more and more prevailing. On the basis of their mechanism of transposition, TEs are divided into two classes. Retrotransposons (class I elements), transpose via an RNA intermediate in a replicative manner, which possibly results in the accumulation of numerous copies of elements from a single family, while DNA transposons (class II elements) do not require the RNA intermediate for transposition and most of them is mobilized upon physical excision of the element and reintegration elsewhere in the genome (a ‘cut-and-paste’ mechanism).

The most straightforward effect of TE mobilization is a knock-out mutation, which requires that the TE copy is inserted into the coding region, resulting in its disruption. In many cases, such mutations may frequently revert back to the wild type upon excision of the element, generating mosaic phenotypes, e.g. purple stripes on maize kernels. TEs have been widely used as insertional mutagens and large collections of gene knock-out lines resulting from TE insertions are available for model plants.

However, the impact of TEs on the host genome can be much more complex than that imposed by insertional gene silencing, and includes modifications of gene expression, introduction of new transcription start sites, splice sites, polyadenylation signals, exon shuffling and formation of novel hybrid transcripts and proteins, molecular domestication of TE-encoded genes, epigenetic regulation of gene expression and a range chromosomal rearrangements, including tandem duplications, inversions, deletions, translocations, spreading of satellite DNA, etc. Although most of these rearrangement are deleterious, some rare events may occur to be adaptive or, in case of crops, to provide a character that is desired from the agronomy or consumer standpoint.



New methods in the use of fertilizers in plant nutrition

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A good fertilization practice of contemporary agriculture is based on the use of mineral fertilizer rather than organic. The advantages of the use of mineral fertilizers should be focused on the nutrients direct and fast availability to the plants, their lower price and their quite rich nutrient content. However, the costs of fertilizer inputs still does not overburden plant production, so, the excess use of mineral fertilizer very often cause some environmental disadvantages (leaching, pollution of soil and water, micro-organisms destruction, soil acidification/alkalization or reduction in soil fertility). The sufficient and balanced nutrient plant supply, which could fulfill different quantity and quality yield criteria, could be solved through the modern concept of fertigation (technique of supplying dissolved liquid fertilizer (LQF) to crops through an irrigation system). Also, very useful type of fertilizer (slow release fertilizers – SRF), can score a same goal of precise fertilization, with small pollution effects and small nutrient losses. A special approach to the fertilization practice has been progressed by the use of full molecule in plant nutrition (amino acids, humate and humic acid type of fertilizers and other organo-mineral complexes). In the contest of organic agriculture, a considerable progress has been done in composting process, making organic materials more suitable for plant production. This paper presents the results of one such product, where the chicken manure were processed by flay worms by using special technological process, making this organic fertilizer more valuable.



Animal models for research in human and veterinary medicine

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The use of animals as models of human anatomy and physiology began in ancient Greece and still is essential to the development of new and more effective methods for diagnosis and treating diseases that affect humans and animals. Animals are biologically similar to humans, more than 99% of DNA is sharing with chimpanzees and almost 98% of DNA with mice. A variety of animals provide very useful models for the study of diseases afflicting both humans and animals. Almost 95% of these animals are rats, mice specifically bred for research, less than 5% of these animals include rabbits, guinea pigs, sheep, fish, frogs, insects. The lowest number (0.75%) of the animals in research are cats, dogs and primates.

Scientists interrogate animals at multiple levels: molecules, cells, organs and physiological functions in healthy and pathological conditions, what has allowed to discover innovative ways to prevent/cure illnesses such as cancer, asthma, hypertension. Some examples include production of vaccines (polio) and antibiotics (penicillin) or even organ transplants.

Biomedical research benefit not only humans, the same methods have improved the lives of animals, has helped develop many animal vaccines to fight rabies, leukemia, tetanus in dogs and cats. Animal research has helped preserve nearly extinct species due to new reproductive techniques being applied to endangered species.

As soon as the advances were made in the field of genetics, researchers have used similar technologies to generate transgenic mice, rats, cats, dogs, rabbits, pigs, sheep, goats, cattle, chickens, zebrafish, but the mouse continues to be powerhouse for biomedical research.

Additionally, new techniques led to develop specific “omic” technologies aimed at the universal detection of genes (genomic), mRNA (transcriptomic), protein (proteomics) and metabolites (metabolomics) in a specific, usually small biological sample which may help to discover new medicine. Undoubtedly, the recognition of the role of microbiota

(gastrointestinal and dermal) led to develop a new research era- gnotobiotics for control of microbial genetics known to impact the host physiology. In future the animal models probably will move towards models that most appropriately mimic human conditions, called “humanized models” such as transgenic animals expressing human genes.

The greatest challenges faced by modern biological research concern complex, multifactorial, diseases for which all experimental approaches-biochemistry, genomics, cell culture, biostatistic and animal models will be complementary. Nevertheless, animal models will play an important role in translational research and improvement of human and animal health.

Supporting: DS KFEZ/2016-2017



Molecular breeding of cereals with improved tolerance to selected abiotic stresses: from QTLs and associations to new candidate genes and selection tools

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The application of molecular breeding strategies may be a powerful tool for improving plant tolerance to abiotic stresses. In our studies performed in barley drought tolerance and tolerance to winter conditions were taken into consideration. The aim of the study was to develop effective molecular marker systems with application potential for the selection of spring barley genotypes of higher drought tolerance and winter barley genotypes with increased winter hardiness. The experiments were performed on 1214 spring and 142 winter barley genotypes delivered by two Polish breeding companies (DANKO and Strzelce Plant Breeding). The genotyping was performed in DArT/DArTseq system. For phenotyping the results of both multipoint field trials and controlled experiments were exploited. Two approaches: biparental mapping populations and association mapping were used in the case of drought tolerance, while winter hardiness were studied by marker-trait associations only. After selecting of QTLs or associated markers DArT/DArTseq markers were converted for PCR ready markers (SSR, STS) for practical use. Additionally the loci/markers were search for the genes of potential importance for both stress tolerance. In the case of both traits marker sets with high selection value were developed. The use of association mapping and phenotyping of drought response under controlled environment revealed additional DArTseq and SNP markers strongly associated with physiological parameters for drought tolerance. Eight out of 19 drought-tolerance markers and six out of 20 winterhardiness markers revealed sequence similarity with barley genes which may be connected with drought tolerance/winterhardiness, respectively. Additional analysis of gene expression during cold acclimation of genotypes different significantly in their winter hardiness allowed to select three genes as candidates for winterhardiness in barley.

The study was founded by The National Centre for Research and Development (Poland) project GENMARK (PBS1/A8/1/2012) and National Science Centre, Poland (UMO-2013/09/N/NZ9/01588).



The future belongs to the thoughtful and the nimble

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It is easy to see the problems facing agriculture in Europe and North America. Consumers are receiving and making decisions based on half-truths, misinformation, lies and opinion masquerading as facts. The sources of these are the following in alphabetical order: celebrities (e.g. chefs and prominent actors), governments, industry and non-government organizations. The following are examples of this misinformation:

- Use of words such sustainability and environmental stewardship without defining what is meant.
- Employing the term “natural” on food labels to indicate or imply safety and/or quality. The term represents wooly thinking with no clear definition for the term. Moreover, there multiple natural agents that are dangerous such as food borne pathogens together with plant and animal toxins.
- Branding specific brands of chickens as “hormone free” when hormones are not used in poultry production anywhere.
- Production practices such as cage free or free range where we anthropomorphize animals. Would we like to live in a cage? If not, animals should not.
- The term, genetically modified (GM). The term only includes insertion a genes from other organisms as opposed to “traditional breeding” or “classical breeding”. Again this represents a lack of clarity. Plant breeding employs approaches such as increasing the numbers of mutations by either radiation or chemical mutagens. It is not clear why mutating a plant is preferable to specially inserting a gene. Mutation is clearly not traditional. Moreover, classic refers to Roman and Ancient Greeks.

This absence of clarity is confounded by a general lack of scientific literacy and some distrust of scientists. I might be argued that organic agriculture is an example that challenges scientific literacy. It is not clear why it makes any difference that an ammonium ion comes from degradation of manure or from catalytic nitrogen fixation.

Science based decision making is not uniformly supported as seen from the following:

1. Policy making by determining what you want first and then looking for evidence for it. Regulations are often not based on sound science rather on the professional opinion of a group of scientists or, worse, the opinions of a small group of scientists chosen for their viewpoint, perhaps interspersed with policy professionals or advocates for specific positions.
2. Politics dictating position:
 - People on the political “Right” dismissing global warming/climate change and the unifying principle of biology – the theory of evolution.
 - People on the political “Left” opposing vaccination and GM crops.

Should we be optimistic? We would argue emphatically yes. Western society is based on science. Sound science is essential to human health, development and poverty alleviation. In addition, we consider that truth will triumph in the end and that good people will always do the right thing eventually!

WORKING GROUP 1: PLANT SCIENCES

Oral presentations



Genetic identification, physiological and biochemical particularities and effectiveness of soil cyanobacteria in plant-microbe interaction

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Data on the identification and phylogeny of heterocystic soil nitrogen-fixing cyanobacteria of the genera *Nostoc*, *Desmonostoc*, *Cylindrospermum*, *Nodularia* were obtained for 15 collection strains. Taxonomic cyanobacterial strains diagnoses based on the morphology and analysis of 16S rRNA, *rbcL* and *nifH* genes have been refined; the nucleotide sequences of the cyanobacterial strains are deposited in GenBank. On the website of the ACSSI Algology Collection (<http://acssi.org/>), information on this strains of cyanobacteria studied. The dynamics of cell biomass accumulation and pH variation during the annual cycle of cultivation under conditions of artificial media as an indicator of productivity and technology have been identified for strains. Strains 1 (ACSSI 091), 3 (ACSSI 149), 4 (ACSSI 150), 57 (ACSSI 57), 82 (ACSSI 82), 144 (ACSSI 271) were highly productive and accumulated 0,0010–0,0046 mg absolutely dry mass/ml of medium after nine months of cultivation. A Database of the physiological potential of cyanobacterial strains is created, the efficiency of photosynthesis, nitrogen fixation and enzymatic activity is established. The functional dependence of the physiological potential of strains with the productivity of test-plant of wheat is shown. Each of the studied strains is unique in its genetic and physiological nature. The metabolic potential of phototrophic strains is the decisive factor for bacterization of the plant-microbial interaction, providing stimulating and inhibiting effects, or their absence on wheat plants. This will allow to develop a new methodological approach to modelling the effectiveness of the agrocenosis. To develop microbial preparations of biofertilizing and stimulating action, strains 1, 3, 4, 57, 144 are recommended. In addition, the strain 4, 82, 144 is recommended for preparations with herbicidal action.

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Sugar profile in fruits of two apple cultivars grown in integrated and organic production systems in a northern climate

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Western Norway, which is under the influence of fjords and the Gulf Stream together with long summer days, is suitable area for both integrated (IPM) and organic fruit production (OFP). The aim of this study was to compare fruit sugar profile of two main commercial apple cultivars (Discovery and Red Aroma) from both organic and integrated production grown in a high density planting system under similar agro-climatic conditions. The most common sugars in the apple fruits were fructose, glucose, sucrose and galactose, which amounted around 43%, 33%, 11% and 4.5%, respectively. Fructose varied from 60.86 g/kg fresh weight (FW) (Discovery, OFP) up to 72.93 g/kg FW (Red Aroma, IPM). Glucose had a range between 45.59 g/kg FW (Red Aroma fruits, IPM) and 59.83 g/kg FW in fruits of cultivar Discovery, (OFP). Red Aroma, (IPM) showed the lowest sucrose content (14.97 g/kg FW), while cultivar Discovery, OFP, had the highest level of this sugar (19.56 g/kg FW). Galactose had the narrowest range, from 6.07 g/kg FW (Red Aroma, OFP) to 9.07 g/kg FW (Discovery apples, IPM). According to the levels of glucose, fructose and sucrose, the sugar index was calculated. It showed that Red Aroma fruits, IPM are significantly sweeter than those from organic production. On the contrary, Discovery fruits from organic production gave significantly sweeter fruits than those from IPM. Another 13 sugars (trehalose, arabinose, isomaltose, melesitose, gentobiose, turanose, raffinose, isomaltotriose, maltose, panose, maltotriose, ribose and xylose) and three sugar alcohols (sorbitol, galactitol and mannitol) were as well found in fruit of both cultivars grown in both production systems, but as minor constituents.



The improvement of genetic resistance to the *Plasmopara halstedii* pathogen and *Orobanche cumana* parasite, in sunflower genotypes, resistant to herbicides

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There is a high competition between sunflower crop and weeds, for water, nutrients, space and light. This competition is producing losses of 20–70% in sunflower seed yield.

The introducing in practice of the genotypes resistant to imidazolinone or sulfonylurea herbicides it is a revolution in sunflower crop.

Sunflower resistance to herbicides was identified in the wild sunflower *Helianthus annuus*, in 1998 year, in USA. Using the developed cultivated sunflower inbred lines, as sources for resistance, there have been obtained, by the genes transfer, the inbred lines with high agronomic traits, resistant to these types of herbicides. The sunflower genotypes resistant to herbicides can be used in practice, in the CLEARFIELD or Express-Sun systems.

The virulence of *Plasmopara halstedii*, which produces downy mildew in sunflower has increased in the last period and new virulent races of this fungus appear.

The parasitic plant *Orobanche cumana* (broomrape) is the most important biotic constraint to the production of sunflower, in all counties where this crop is grown, excepting North and South America.

There is a diversity of *O. cumana* races, identified worldwide. The appearance of new races of this parasite has reduced considerably the available sources of resistance, in cultivated sunflower. A high level of resistance, for both, *P. halstedii* pathogen and *O. cumana* parasite was found in wild *Helianthus* spp. Resistance to the most virulent races of these, pathogen and parasite has been transferred from wild *Helianthus* into cultivated sunflower, by interspecific hybridization.

Using the sources of resistance to this pathogen or the broomrape parasite, for the most virulent races which are now present in Europe, we have introduced genes of resistance, by using backcross or recurrent selection methods, in the best lines, which already are finished for the resistance genes transfer for herbicides or being still in process of releasing this work.

Key words: sunflower, resistance, herbicides, downy mildew, broomrape.



Plant biotechnology importance on solving environmental challenges in agriculture

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Agriculture is vitally important for the economies of Armenia. Our country faces serious challenges in their agriculture sector. Agriculture growth has been slowing down considerably during the last three decades, due mostly to problems of drought, land degradation, pests and diseases. New approaches needed to solve sufficient amount of tasks in agriculture. We need to establish policy environments conducive to agricultural development. Nowadays important paying attention not only in some aspects of agricultural and environmental problems, but also in specific tasks such as protecting agriculture production and environment.

In order to effectively develop economy Armenia puts the priorities of agriculture modernization by capacity building in food safety system and developing new approaches in agricultural research. Necessary knowledge and its application is very important in the rapidly developing field of agriculture to solve environmental tasks.

In recent years, in Armenia there has been growing dissatisfaction with chemical pesticides due to their damage to the environment, risk to the human health, and reduction in efficacy. Taking into consideration this situation when challenges in agriculture are various and contradictory new technologies will be needed to develop in order to address environmental challenges in agriculture.

Agriculture is at the forefront of socio-economic development in all developing countries to feed the millions of mouths and plant biotechnology offers major tools to enhance agricultural production. Plant biotechnology confers important agronomic traits such as insect and herbicidal resistance. Plant tissue culture, which is an important area of plant biotechnology also crucial for the development of highly adapted high-yielding stable varieties, food security and economic growth not only in Armenia, but also in the emerging economies of the world.



The overcoming of interspecies incompatibility by lectins is way for creating interspecific hybrids of flax

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Increasingly important in genetic and breeding research becomes donor search of new genes. Quite promising in this respect are wild species that are evolutionarily close to the crops. Quite convenient object for studying of incompatibility is a genus *Linum*, which includes more than 200 species, but is cultivated among them only one species – *Linum usitatissimum* L. At the same time various wild species are characterized by high resistance to stress factors, different fatty acid composition of seed oils, and the presence of substances of secondary metabolism – lignans, which have anticancer and cardiovascular pharmacological properties. However, to obtain hybrids between different species of flax standard methods of hybridization and selection is still almost failed.

Our previous data with *Linum perenne* L. has pointed out lectins can overcome the self-incompatibility in these species. Due to the fact that the mechanisms of self-incompatibility and interspecies incompatibility are very similar, we have hypothesized that these lectins can overcome the barrier of interspecific incompatibility within the genus *Linum*. Therefore, we have extracted and purified glucose and galactose-specific lectins from anthers and pistils of long-styled *L. perenne* flowers. The long-styled stigmas of *L. austriacum* L., *L. squamulosum* L. and *L. tenue* L. were treated by these solutions. Stigmas were pollinated with pollen from short-styled flowers of *L. perenne* L. Pollen tube growth was monitored using aniline blue. In a incompatible cross (intraspecific crosses) the pollen germinates but the pollen tube growth is inhibited in the stigma, whereas in a compatible crosses (treated by the both membrane and cell wall galactose-specific lectins from anthers) pollen tube reaches the ovary within two hours after pollination. Our results indicate that lectins might have a role in signalling pathways involved in pollen tube recognition and formed seeds in interspecific crosses between *Linum* species.



Determination of the most efficient pollenizer for the pear cultivar ‘Ingeborg’ (*Pyrus communis* L.) in a nordic climate

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Norwegian pear (*Pyrus communis* L.) production has been in decline for the last 25 years. This was mainly because of old cultivars, with low ability to compete in the market and strong competition from imported pears, mainly ‘Conference’, ‘Ingeborg’ (‘Conference’ × ‘Bonne Louise’), a triploid (3n), is currently the main commercial pear cultivar grown in Norway. However, fruit set and subsequent yields of this cultivar have proven to be variable and overall rather low. Pear seeds found in ‘Ingeborg’ fruits are often under-developed, suggesting pollen incompatibility and/or incomplete fertilization might be significant causes of poor fruit set. Different strategies are tested to improve the fruit set like applying bioregulators during flowering, parentage analysis using microsatellites or SSRs (simple sequence repeats) revealing the female and male parents of the plant and crosspollination with different fathers as potential pollinators. The aim is to determine the most efficient pollenizers for this cultivar. Fluorescent microscopy of the dynamics of pollen tube growth (third, sixth and ninth day after anthesis) in the style (upper, middle and lower third) and ovary (locule, micropyle and nucellus) in some crossing combination was done. Fluorescent microscopy showed that the highest percentage of pollen tubes that penetrated the locules of ovary in ‘Ingeborg’ occurred in combination ‘Ingeborg’ × ‘Fritjof’ (80%) followed by ‘Ingeborg’ × ‘Anna’ (70%). The experiments will be extended and repeated for another season. Main results from the different experiments will be reported.



Pioneering catalytic RNA-based strategy for mitochondrial gene regulation in plants

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Genetic processes in mitochondria are complex, diversified, and of primary importance. Their dysfunction causes incurable neurodegenerative diseases in humans, whereas in plants they contribute to key agronomical traits. For cellular homeostasis, the mitochondrial genetic system needs to cross talk with the other compartments. Up to now, transformation approaches were successful only in yeast and in *Chlamydomonas reinhardtii*.

The aim of the work was to develop a new and efficient tool to down regulate plant mitochondrial genes with specific catalytic ribonucleic acids (ribozymes). Based on that some of the tRNAs are imported from the cytosol to mitochondrion, we have developed the tRNA-like from the 3' end of the Turnip yellow mosaic virus (TYMV) genomic RNA (tRNA mimic, tRNA-like shuttle), which can drive a cargo RNA into mitochondria in plant. As a first step, a transcript with trans-cleaving hammerhead ribozyme attached to the tRNA-like shuttle was expressed in transformed plants, then imported into the organelle and finally, the directed knockdown of a mitochondrial mRNA in eukaryote was achieved (Methods Mol. Biol., 1265, 227–254, 2015).

A specificity of our pioneering ribozyme-based approach was proved on the well-known *atp9* gene in *Arabidopsis thaliana*, and then it was used as the tool in reverse genetics for studying function of the unknown gene of the mitochondrion-encoded maturase, *matR*. Ribozymes, designed to cleave inside both the *atp9* and *matR* reading frame, were driven into the organelle as “passenger” molecules with a tRNA-like shuttle (Plant Cell, 28, 2805–2829, 2016). For the first time, we generated the mitochondrial loss-of-function plant, followed by the functional characteristics of mitochondrial gene.



Contemporary methods of mutagenesis and their application in modern agricultural plant studies

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Recent decades after a long years of stagnation we observe renaissance of application of methods of mutagenesis i.e. nuclear and other techniques in agricultural, environmental and food sciences. On the basis of traditional mutagenic approaches, it was developed new effective methods of mutant production, identification, screening and phenotyping with application of new molecular genetic methods. New methods are overlapping the new knowledge from different branches of science and allow not only to produce effectively new mutants with new characteristics but to study the genes function (functional genomics), protein functions in organism of plants, microbes and animals (proteomics) and general metabolism characteristics i.e. in plants (metabolomics) etc. Such methods as TILLING, CRISPR (i.e. CRISPR-cas), VIGS etc. are developing in great scale and allowed right now to understand a lot of functions of plant genomes i.e. resistance to abiotic and biotic factors of environment, water regime and flowering etc. as well as to produce the new contemporary mutants with attractive traits i.e. with improved end-use quality which can be alternative to GMO. The characteristics of new mutagenic approaches will be given and recent achievements of their applications in plant science will be discussed related to agricultural crops. Possible international collaboration in mutant development and study under International Atomic Energy Agency (IAEA) will be presented.

WORKING GROUP 1: PLANT SCIENCES

Poster presentations



Methods to discriminate differences in pigmentation and photosynthetic activity in variegated leaves

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Photosynthetic activity in variegated leaves of the ornamental and medicinal plant, *Coleus × hybridus hort.*, was estimated by image analysis and point data measurements of major chlorophyll fluorescence parameters. Both methods revealed heterogeneity in capture, transfer, and dissipation of excitation energy but chlorophyll fluorescence imaging was more suitable in examining very narrow pigmented leaf areas.

Although higher concentration of chlorophyll was noticed in purple parts of the leaves compared to the green ones, the values of minimal and maximal fluorescence yield at the dark- and light-adapted state (F_0 , F_m , F_0' , F_m' , respectively) were a little lower than those in the green sectors, indicating photoprotective effects provided by anthocyanins and Car.

Some discrepancies in photometric and chemical assessments of pigments were noticed which can be explained by the nonuniform distribution of radiation within a leaf but also the interference from anthocyanins to the chlorophyll index measurements.

Borek M., Bączek-Kwinta R., Rapacz M. 2016. *Photosynthetica*, 54, 331.



Different impact of swailing on seeds of plants of European bio- and agricenosis

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Swailing is a part of agricultural practice, but is illegal in many European countries. As the indirect effect of swailing is the emission of volatiles, the goal was to identify their impact to seeds of different species of angiosperms occurring or grown in Europe. The response of seeds to swailing-generated volatiles was sometimes differentiated within a family, and even within the species. However, the stimulation of germination and/or increased seedling vigour was established in the cases of *Apium graveolens*, different forms of *Ocimum basilicum* and *Brassica oleracea* var. *capitata*, as well as *Plantago major*, *Trifolium repens* and *Thymus serpyllum*. The volatiles compensated for light in the case of positively photoblastic seeds of *Matricaria chamomilla*. The inhibition of seed germination and/or reduced seedling vigour was demonstrated in case of *Carum carvi*, *Anethum graveolens* and *Myosotis arvensis*. As similar results were obtained in the experiments carried out *in vitro* and in the soil, the indirect impact of swailing-generated volatiles on plant habitat composition is likely. The explanation of the obtained effects can be mainly the crosstalk with phytohormones.

Bączek-Kwinta R. 2017. Swailing affects seed germination of plants of European bio- and agricenosis in a different way. *Open Life Sciences*, 12(1), 62–75.



A comparison of sensitivity of SPR and ELISA for detection of potato virus Y in extracts of infected plants

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Potato virus Y (PVY) is the most economically important virus infecting potato and many other crops. Label-free surface plasmon resonance (SPR) detection of PVY and simultaneous enzyme linked immunosorbent assay (ELISA) testing for PVY in infected plants was investigated for evaluating their sensitivities.

The objective of this research was to use same commercial monoclonal antibodies (mAb) as the specific recognition element in a SPR-biosensor and in ELISA for rapid detection of PVY in positive (infected) and negative plant samples. A SPR-biosensor was fabricated using ELISA-mAbs that are immobilized on the gold surface functionalized with self-assembled monolayer (SAM). The SPR-immobilized antibodies captured PVY in all of eight serially diluted infective plant samples and caused increase in refractive index (RI). RI of the biosensor was changing from 1.33525 ($\pm 2.09\text{E-}06$) to 1.33642 ($\pm 4.16\text{E-}06$) at the working channel and linearly related to the concentration of PVY in infected plant samples. Whereas there was no distinctive RI increase for PVY-free samples at the SPR-reference channel (from 1.33522 $\pm 1.79\text{E-}06$ to 1.33524 $\pm 2.00\text{E-}06$). These eight serially diluted plant samples were assayed with ELISA system. However, detection level of this system was limited by a PVY-positive extract reconstituted at ratio of 1:50 and more clearly at ratio of 1:25, which could be estimated as plant samples of contained PVY less than 5 and 10 ng/ml, respectively. We can suppose than the application of label-free immunosensor's method can be more sensitivity with a specific antibodies to certain PVY isolates compare with labeled ELISA testing.

Key words: Surface plasmon resonance, PVY, ELISA, Monoclonal antibody, SPR-biosensor



Molecular (ISSR) and biochemical markers used for estimation of genetic variation in Polish cultivars of Italian ryegrass *Lolium multiflorum* (Lam.) and *L. multiflorum* var. *westerwoldicum* (Wittm.)

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Italian ryegrass *L. multiflorum* is the most economically important, annual forage grass, widely cultivated in Europe, North and South America and New Zealand. It is outcrossing species with chromosome constitution $2n = 2x = 14$.

The ISSR (Inter Simple Sequence Repeats) was used to evaluate the range of genetic variability between the eight tetraploid cultivars: five of *L. multiflorum* (Gaza, Atos, Gizel, Turtetra and line No. 604) as well as *L. multiflorum* var. *westerwoldicum* (Kaja, Telga, Koga). The method is based on highly polymorphic sequences of satellite DNA, consisting of nucleotide sequences tandemly repeated in thousands of copies. Amplifications were carried out with 6 ISSR primers. Genetic diversity within and among *L. multiflorum* cultivars was measured by the percentage of electrophoretically detected polymorphic bands.

Isozymes were assayed by horizontal starch gel electrophoresis. Five enzyme systems (phosphoglucose isomerase, superoxide dismutase, glutamate oxalacetate transaminase, diaphorase and peroxidase) were used to array allelic diversity at five polymorphic loci. Each cultivar was characterised by frequencies of different phenotypes. They were highly polymorphic and showed differences in the heterozygosity level. The most polymorphic turned out two enzyme systems: phosphoglucose isomerase (PGI) and peroxidase (PX). Both methods, i.e. ISSR as well as electrophoretically detected isozymes show differences and some degree of relationships between individual cultivars, however not very sharp among the two varieties in question.



Expression pattern analysis of ACC synthesis and EREBP genes in response to drought stress in chickpea different growth stages

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Chickpea (*Cicer arietinum* L.) one of the most important grain-legume crop, is grown in more than 45 countries, mostly in arid and semiarid zones. Plants respond and adapt to water deficit at both the cellular and molecular levels. A large number of genes have been described that respond to drought at the transcriptional level and the mechanisms of the molecular response to water stress in higher plants have been analyzed by studying the expression of genes responding to drought and other abiotic stresses. The expression pattern of ACC synthesis and EREBP genes in two chickpea genotypes MCC283 and MCC80 in the different growth stages under drought stress were investigated. For drought treatment, soil-grown 30 day-old (vegetative growth stage), 60 day-old (early pod visible) plants were subjected to progressive drought by withholding water for 2, 4, and 6 days and untreated plants were used as control. RNA was extracted from leaf and then cDNA was synthesized. RT-qPCR analysis of ACC synthesis and EREBP expression using specific primers showed different expression patterns in different stages of both chickpea genotypes. Differential expression of ACC was observed in both genotypes in various phonological stages and its timing, duration and intensity of drought treatments. The expression levels of EREBP in both genotypes were increased significantly from 2 to 6 days of water deficit in vegetative and early pod visible stages. The increase in ACC synthesis and EREBP expression in the drought treatment for both genotypes in the vegetative growth stage and early pod visible might be an adaptation to overcome the stress condition, supplying energy for growth and survival, thus helping the plant to survive.



Usefulness of measuring hydraulic conductivity in xylem in assessing the effects of plant growth regulators on plant water management

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The experiment investigated the effects of zearalenone, 5-aminolevulinic acid and triacontanol on water management in maize exposed to reduced watering. Water hydraulic conductivity was measured with a high pressure flow meter – HPFM (Dynamax Inc., Houston, USA). Plant water management was also assessed based on other parameters, such as relative water content, gas exchange, stomatal conductance, and leaf osmotic potential. Additional evaluation of plant condition involved photochemical activity of PSII. The results of hydraulic conductivity measurements provided new information on plant water management at a tissue level. The method might be useful in investigating the mechanisms that control the effects exerted on plants by various investigated compounds. However, it was hardly useful in evaluating plant condition and water management. Another disadvantage was the fact that it was highly time consuming and that utmost precision was necessary to obtain reliable results. Further limitations involved plant age, as the plant stem must be of specific thickness. Future hardware modifications of the analyzer might improve the method and expand its possible use. An indisputable advantage of the method was its low cost. Measurements of hydraulic conductivity were also less affected by atmospheric conditions than an assessment of gas exchange or photochemical activity.

In conclusion, evaluation of hydraulic conductivity in xylem might be useful in advanced research aimed at elucidating the mechanisms of action of various substances. However, it is not recommended for determining the impact of these substances on the overall water status of a plant during initial experiments when a number of combinations are investigated.



Economical production of biomass of aquatic plants *Lemnaceae* as an ecological source of energy

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Nowadays one of the most widely developed trends in the production of renewable energy is the use of biomass of energy crops, including aquatic plants (*Lemnaceae*). Research on the production of biofuels from plant biomass has become dynamic because it offers the possibility of becoming independent of fossil fuels, reducing CO₂ emissions and climate change, it also makes recycling of post-industrial waste after methane fermentation possible as in combination with the ozonation process it can be used for the production of water biomass.

The production of traditional energy crops (energy farming) can be related to the competitiveness of available soils and their intended use for food production, reduction of biodiversity of energy crops and the consumption of large quantities of water, which is a scarce and valuable natural resource. Another significant problem is the reduction of fertilizer and pesticide use in power plantations, which justifies the search for new and efficient sources of plant biomass whose production will be pro ecological.

The aim of the study was to perform analyzes of the production of *Lemnaceae* plants using liquid waste from the fermentation industry ozonated at different time intervals and varying ozone doses. Plants were cultured *in vitro*, at 25°C room temperature and 40 W light.

The obtained results showed that the studied waste from methane fermentation and ozonation could be used as a supplement of the medium in *Lemnaceae* biomass plant cultivation and they increased their fresh and dry biomass yield, as compared to the control.

There are possibilities of using multi-acquired plant biomass as biogas, biofuels, in bio-refineries, for phytoremediation and as a high protein animal feed.

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Phytotoxkit as a rapid test to evaluate the stimulating effect of ash from burned energy crops on germination and growth of sorghum plants

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The aim of research was to evaluate the laboratory Phytotoxkit test in rapid assessment of stimulatory impact of ashes on growth of sorghum plants and their suitability as ecological fertilizers in this crop production. In the experiments, the air dried plants of Jerusalem artichoke (*Helianthus tuberosus* L.) and sorghum (*Sorghum bicolor* L.) were used. Phytotoxkit plates were filled with the prepared mediums and then seeded with sorghum seeds. The number of germinated seeds was counted daily and then the plant growth rate was determined on the basis of their height measurements every few days. During plant growth, the index of chlorophyll content and gas exchange in leaves (net photosynthesis, transpiration, stomatal conductance and intercellular CO₂ concentration) were measured.

The obtained results show that the used Phytotoxkit can be very useful in evaluation of the stimulating effect of ash on sorghum growth and that it can be used as a preliminary test for plant production. The research show that the unit weight of Jerusalem artichoke ash was about twice higher than of sorghum one. Supplementation of the garden substrate with ashes improved seed germination and caused increased growth of sorghum seedlings. The improved growth of plants and yield of biomass was associated with the increased physiological activities in leaves, such as index of chlorophyll content, net photosynthesis, transpiration and stomatal conductance, coupled with decreased intercellular CO₂ concentration. However, these improvements depended on the kind of ash and its doses. The fastest plant growth and biomass production were observed in the horticultural substrate containing about 5% ash from burned sorghum plants and about 2% from Jerusalem artichoke.

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Association mapping of drought tolerance-related traits to complement a traditional biparental QTL mapping study

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We used 109 spring barley genotypes exhibiting high or low drought tolerance to elucidate the associations between Diversity Array Technology sequencing (DArTseq) and single nucleotide polymorphism (SNP) markers and various physiological parameters related to plant responses to drought conditions. We identified 67 associations including 34 new markers (i.e., 16 DArTseq and 18 SNP markers). The results for three markers were consistent with the data obtained in an earlier traditional biparental QTL mapping study. The regions neighboring markers on linkage group 2H contained the highest number of significant marker–trait associations. Five markers related to the photosynthetic activity of photosystem II were detected on chromosome 4H. The lowest association levels for the selected markers were observed for the sequences neighboring DArT markers on linkage group 6H. A chromosome 3H region related to water use efficiency and net photosynthesis rate in both biparental QTL, and association study, may be particularly valuable, as these parameters correspond to the ability of plants to remain highly productive under water deficit stress. Our findings confirm that association mapping can increase the density of existing QTL maps without recreated mapping populations.

WORKING GROUP 2: SOIL SCIENCES

Oral presentations



Studying of capability of soil fungi to utilize a grain shot waste from brewing industry

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It is known that soil fungi have a huge ecological potential to degrade cellulose materials and for the efficient bioconversion of cheap raw biomass. In this research, fungi were isolated from soils in Akmola region of Kazakhstan and their macro- and microscopic properties are studied. Fungal isolates were belonging to *Penicillium* spp., *Aspergillus* spp., *Cladosporium* spp. and others. Genus of fungi was determined by their morphology and is corresponded to results of the sequencing analysis of ITS region.

The cellulase activity of soil fungi were evaluated using 3,5-dinitrosalicylic acid assay and compared with the activities of six *Trichoderma* and other strains that are obtained from Republic collection of microorganisms. *Cladosporium cladosporioides* 9PF strain and deposited *Trichoderma* strains were not showing a big difference in decomposition of Na-carboxymethyl cellulose and estimated final concentrations of glucose are 0.38–0.54 mg · ml⁻¹. A grain shot from brewing industry was used to study a capability of soil fungi to utilize this cellulose-containing by-product. 5.8% of grain shot medium containing 0.2% urea or a yeast extract or NH₄H₂PO₄ as an additional source of nitrogen was inoculated by 2-days culture of *Cladosporium cladosporioides* 9PF. In 4 days, an amount of reducing sugars was 0.695 and 2.176 mg · ml⁻¹ under culturing *Cl. cladosporioides* 9PF on the grain shot medium with and without 0.2% of urea, respectively. However, sugar concentrations were corresponded to 1.283 and 0.407 mg · ml⁻¹ after 8 day's cultivation of 9PF strain on this medium with and without 0.2% of urea. All the conducted *Cl. cladosporioides* culture experiments were repeated with *Tr. hamatum* 67.

Periodical cultivation of *Cl. cladosporioides* 9PF on the grain shot medium was investigated for the first time. It was observed that a spore-formation of *Cl. cladosporioides* 9PF is depend on an urea adding to the medium as a source of nitrogen and before that there has been no any reports yet on influence of urea for the growth of the genus *Cladosporium*.

Key words: soil fungi, *Cladosporium cladosporioides*, sequencing, by-products, reducing sugars



Reclamation of ash damp by adding the river's mud

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In this paper, the ash damp reclamation was in a focus as a one of the most critical ecological black spot worldwide. In Serbia, it is located 90 km away from Belgrade near the Danube River at Kostolac's Thermal Power Plant (TPP). The ecological problems of disposed ash as a byproduct of coal combustion in TPPs are well known concerning its huge daily mass production and its potential pollution threat to the soil and groundwater in surrounding. Generally, this negative impact of such ash deposit derive from its bad physical structure, where its small particles (3–200 μm) could be spread by wind over the 10 km in surroundings and it's bad chemical composition, usually induced by the presence of increased level of some hazardous elements. This lignite ash damp has been formed on the open field dump near TPP, which sized approximately 70 ha. The disposed ash posses a high content of boron (B $1,65 \pm 1$ mg/kg) and heavy metals (Pb $3,36 \pm 2,8$ mg/kg; Ni $43,3 \pm 6,51$ mg/kg; Cr $26,58 \pm 3,89$ mg/kg; As $45,45 \pm 0,77$ mg/kg and Cd $< 0,05$ mg/kg). Therefore, reclamation measures need to prevent further contamination of surroundings by ash wind spreading and to keep as much as possible hazardous elements inert. This rehabilitation measure concerns covering ash deposit by 5–10 cm layer of river's mud, which has been taken out during the process of cleaning one of the Danube's TPP's streams. As an ash damp cover, the used river's mud is in charge to prevent the mobility of ash particle by coating a surface layer which start to be inert to the influence of the external factors. This is manage by the forming an incrustation of firmly tided small particles (sand 2%, silt 87% and clay 11%). This tend also to make conditions for cultivating of plants by improving the low nutrients status of the ash (N – $1,48 \pm 0,62$ mg/kg, P_2O_5 – $8,53 \pm 4,17$ mg/100g, K_2O $12,36 \pm 5,14$ mg/100g). This type of reclamation was successfully tested in pot experiment with barely and grass cultivation.

WORKING GROUP 2: SOIL SCIENCES

Poster presentation



Dissolved organic carbon and total nitrogen release from deadwood of different tree species in various stages of decomposition

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The aim of study was to estimate how the deadwood of different tree species in various stages of decomposition affected dissolved organic carbon and total nitrogen concentration. The deadwood of seven species (*Common alder*, *Common aspen*, *Common ash*, *Silver fir*, *Norway spruce*, *Common hornbeam* and *Silver birch*) were selected. Five logs from each species in the third, fourth and fifth decay classes were chosen for analysis. The investigation was carried out in Czarna Różga Reserve in Central Poland. The tension lysimeters were installed under each logs in the humus horizon. The suspension were collected between March and May (every two weeks). The concentration of total carbon (TC), inorganic carbon (IC), dissolved organic carbon (DOC) and total nitrogen (TN) were determined. The results obtained demonstrated that DOC and TN concentration were influenced by deadwood tree species and decay class. With the advancement of the deadwood decomposition the DOC and TN concentration increased slightly. Differences in the DOC and TN concentration between the logs of various species were noted.

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WORKING GROUP 3: ANIMAL & FOOD SCIENCES

Oral presentations



Possibility of using different forms of radiation to improve the safety and hygiene of meat

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The food safety at processing stage is primarily based on maintaining high hygiene standards and adequate food preservation methods. Currently hygiene aspects are taken into account at the design stage. Thanks to nanotechnology self-disinfecting surfaces can be designed. Ionizing and microwave radiation may be a sufficient methods for food preservation without involving antimicrobial additives. The aim of the study was to evaluate the antimicrobial activity of gamma radiation and microwave radiation against *C. jejuni* in meat, and to evaluate antimicrobial activity of UV-C radiation against *C. jejuni* on the surfaces of conventionally produced ceramic wall tiles and the same tiles coated with photocatalyst layer (nano-TiO₂). Ground beef and pork was subjected to gamma radiation (⁶⁰Co) treatment, with doses up to 1.0 kGy and microwave radiation (180 W) for up to 150 sec. To evaluate the UV-C (253.7 nm) disinfection efficiency in the absence or presence of TiO₂, conventionally produced tiles and tiles coated with TiO₂ were subjected to UV-C radiation for up to 3 min. The enumeration of bacterial cells that survived the radiation treatment was performed using the serial dilution technique. Each dilution were plated onto Karmali agar and incubated under a microaerobic atmosphere (42°C/48 h). Comparing both preservation methods, it was found the method that allows for the most precise bacterial reduction level determination is ionizing radiation. Uneven heat distribution in meat subjected to microwave radiation may result in the formation of cold and hot spots, which offered *C. jejuni* some protective effect. The bactericidal action of UV-C was stronger on the surfaces coated with TiO₂. The results showed, that the use of wall tiles coated with nano-TiO₂ may find application to improve the hygiene of non-food contact surfaces. Nevertheless, the sensitivity of *C. jejuni* to physical factors, often referred to as very high, was overestimated.



In vitro effect of 4-nitrophenol and 3-methyl-4-nitrophenol on STAR, HSD3B and CYP19A1 mRNA expression in the chicken preovulatory follicles

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The aim of this study was to assess the *in vitro* effect of 4-nitrophenol (PNP) and 3-methyl-4-nitrophenol (PNMC) on the main steroidogenic gene (STAR, HSD3B and CYP19A1) expression in chicken preovulatory follicles. Hy-Line hens (n = 6) were decapitated 2 h following ovulation, and 3 the largest preovulatory follicles (F3 < F2 < F1) were isolated from the ovary. The fragments of the granulosa (GL) and theca (TL) layers of these follicles were incubated for 6 h in medium supplemented with PNP or PNMC (10–6M and 10–5M in respect to the TL and GL, respectively). Progesterone, testosterone and estradiol concentrations in the medias were determined by RIA while mRNA expression of STAR, HSD3B and CYP19A1 were measured in the tissues by real-time qPCR. Results were statistically evaluated by one- or two-way analysis of variance followed by Duncan's multiple range test at $P < 0.05$. Both nitrophenols significantly decreased sex steroid secretion by all preovulatory follicles ($p < 0.05$ – 0.01). PNP and PNMC diminished STAR expression in the TLs of F3 and F1 follicles, and the TLs of F2 and F1 follicles, respectively ($P < 0.05$ – 0.01); however, they did not affect this gene expression in the GLs. PNP decreased HSD3B in the TLs of F3 and F1 follicles and in the GLs of F2 and F1 follicles ($P < 0.05$ – 0.01). The inhibitory effect of PNMC was found only in the TL of F2 follicle ($P < 0.01$). PNP inhibited CYP19A1 transcription in the TLs of F3 and F1 follicles and in the GLs of all preovulatory follicles ($P < 0.01$). PNMC did not influence CYP19A1 expression in the TLs; however, it diminished this gene expression in the GLs ($P < 0.01$). Results of this experiment reveal that PNP and PNMC are negative modulators of sex

steroid secretion in the chicken ovary; they affect steroidogenesis in the preovulatory follicles by inhibiting the mRNA expression of steroidogenic genes.

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Effect of storage temperature on survival of *Staphylococcus aureus* in yoghurt and kefir

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S. aureus is capable of producing thermo-resistant enterotoxins and is often the cause of food poisoning. Yoghurt and kefir are fermented milk products which constitute an important segment of the dairy production in Poland. The presence *S. aureus* in these products may be a result of bad quality of raw milk, inadequate heat treatment, re-contamination and poor hygiene during processing and packaging.

The aim of the study was to determine the behavior of *S. aureus* during storage of commercially produced yoghurt and kefir at different temperatures.

The samples of yoghurt and kefir were inoculated with the mixture of three *S. aureus* strains and stored at 5°C, 10°C, 15°C, 20°C and 25°C. Samples of yoghurt were stored for 48 h and tested every 4 hours for number of *S. aureus*. The samples of kefir were stored for 120 h and tested every 12 h. From each sample a series of decimal dilutions were prepared and plated onto Baird Parker Agar. Plates were incubated for 48 h at 37°C. The experiment was performed in five replicates. Bacterial counts, transformed into decimal logarithms, were used for calculations and mathematical modeling using the Microsoft® Office Excel 2007 and IBM SPSS Statistics 23.

The number of *S. aureus* decreased linearly with storage time in all samples. In samples of yoghurt stored at 25°C the number of *S. aureus* decreased at the highest rate (D-10 value 17.5 h), whereas at 15°C the reduction in numbers of bacteria was the least dynamic (D-10 value 38.7 h). In samples of kefir the numbers of *S. aureus* also decreased at the highest rate at 25°C (D-10 value 44.1 h) and at lowest rate at 15°C (D-10 value 234.1 h). The mean inactivation rates of *S. aureus* calculated from primary linear model was fitted to linear secondary and polynomial models. Equations obtained from secondary polynomial model can be applied as a tool for prediction of inactivation rate of *S. aureus* in yoghurt and kefir stored under temperature range from 5 to 25°C.



Influence of eggs disinfection with using DERGALL® water solution on the results of chicken hatch

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Maintaining high hygiene standards is a necessity in the poultry incubation process. A proper disinfectant should not only be of high efficiency (biobility) but also safe for animals, humans and the environment. Therefore, in disinfection of hatching eggs highly toxic formalin pairs are currently replaced by spraying eggs with aqueous solutions of compounds i.e.: peracetic acid, glutaraldehyde and/or hydrogen peroxide. Another innovative solution seems to use for the disinfection of hatching eggs preparations based on silicone compounds for forming the surface of the shell-dimensional polymer network, i.e. DERGALL®.

The study was carried out in production conditions (ZWD Wolbrom, Poland). Hatching eggs (4000 eggs, breed Ross 308, 34 weeks old,) were randomly divided into 8 experimental groups (10 repetition × 50 eggs = 500 eggs per group). Eggs from each group were sprayed 0, 1, 2, 3, 4.5 and 6% DERGALL® aqueous solution about 2 hours before start of incubation. The control group was not disinfected. Hatch results and also developmental stage at the time of death, possible infections, malformations and malpositions in died eggs were determined. The effect of concentration on the above parameters was analyzed by means of one-way ANOVA and differences between groups were tested by Tukey test.

It was found that the results of hatching from fertilized eggs were (mean ± SD) in control group 90.9 ± 4.39 or sprayed pure water respectively 90.6 ± 4.91 , while the use of DERGALL® solution at 3% and 4% raised hatching to 94.3 ± 3.00 and 92.6 ± 5.42 ($P \leq 0.05$) while solutions of 5% and 6% caused a fall in hatching to 89.3 ± 5.54 and $54.7 \pm 13.53\%$. The early cause of loss in hatching was the early mortality of embryos.

In conclusion, studies have shown that 3% DERGALL® solution can be safely used to disinfect chicken hatching eggs.

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Performance of *Trichogramma* (Hymenoptera: Trichogrammatidae) parasitoids feeding on honey sources

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Honey is the miraculous product of honeybees and a naturally delicious. Its flavor and texture vary with the type of flower nectar from which it was made. Apart from natural food source, the well-known functional properties of honey are its antioxidant and antimicrobial activities. The efficacy of natural enemies as biological control agents is sometimes limited by food sources and phenological asynchrony with their host or prey populations. Most parasitoids depend on carbohydrate-rich foods to maximize their longevity and reproduction potential. We conducted a preliminary study on the use of different honeys as food sources for *Trichogramma* (Hymenoptera: Trichogrammatidae) species. The worth of honey as a food source for *Trichogramma* was evaluated in the laboratory by testing the effect of different honey diets on the longevity, parasitism and other parameters. The most commonly available honeys are made from a variety of flowers, pine, citrus, chestnut, sunflower and cotton in Turkey. *Trichogramma* females fed on sunflower and citrus honeys lived significantly longer than females fed on cotton and flower honeys. The females fed on pine and chestnut honeys had the shortest longevity. *Trichogramma* females fed on chestnut and sunflower honeys parasitized significantly more than fed on flower honeys. Females fed on cotton honey were had the lowest parasitization. These results showed that providing *Trichogramma* with sunflower and chestnut honey resulted in greater longevity and fecundity, respectively.

Key words: *Trichogramma*, honey, parasitization, nutrition, longevity, fecundity

WORKING GROUP 3: ANIMAL & FOOD SCIENCES

Poster presentations



The using of adsorbent mikotoxin Funginorm to increase meat productivity of fattening pigs

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The most important problem of pig production in the field of feeding is the control of molds and mycotoxins, which they produce in the course of their vital activity. Mold infects the grain in the field, and then during storage and processing. This causes significant economic damage, since the basis of the animal's diet is the grain, which is the main source of mycotoxins. Contamination of grain with mycotoxins is possible at all stages of its production. Consequently, the problem affects a wide range of enterprises in both feed and food industries. At present, the use of fodder additives with mycotoxin-adsorbing properties is especially promising, and in most cases they are used for the purpose of detoxification.

To carry out the scientific and economic experience, according to the principle of analogs, 80 heads of pigs were selected. Pigs for fattening were divided into 4 groups of 20 heads each. The average live weight of the pigs was 54.1–54.7 kg. The control group used only the basic diet for feeding pigs on fattening. In the first test group 1.0 kg/ton of the new generation adsorbent Funginorm was added to the main diet, in the 2nd test group was added 2.0 kg/ton of adsorbent and in the 3rd test group was added 3.0 kg/ton of adsorbent. As the main ration for experimental pigs used mixed fodder SK-26.

Funginorm is a new generation adsorbent for birds and pigs, used to suppress the development of mold fungi and neutralize mycotoxins in feeds. As the controlled parameters for the fattening pigs of all the experimental groups were the live weight, average daily increments and feed conversion.

The results of the first month's studies indicate that the pigs of the first and second experimental groups had the most pronounced growth intensity advantage. The average daily increase in the pigs of the second test group was 627 g in a month of studies, which is significantly higher by 14.0% than in the control group. The average daily gain in the first and third test groups was 610 g and 577 g, which is higher than in the control for 60 g and 27 g, respectively.

During the second month of the experiment, the advantage remained was achieved in terms of the intensity of the growth in the live weight of the experimental pigs for fatten-

ing. In the second trial group, the mean daily gain was 703 g, which is significantly higher than in the control group by 98 g, respectively.

Preservation of pigs on the fattening of all experimental groups was 100%. In general, during the entire period of the experiment, the average daily gain in live weight in the second test group was 665 g, which is significantly higher by 15.1% than in the control group. In the first and third experimental groups, the average daily increase was 633 g and 615 g, which is 9.5% and 6.4% significantly more, in comparison with the control group. The inclusion of this adsorbent in the pig's fattening diet at doses of 1,0–3,0 g/kg of mixed fodder reduced the conversion of feed and increased the feed conversion ratio by 6.8–15.3% compared to the control group.

In order to prevent, in the feeding of pigs for fattening, we recommend the use of a new generation of adsorbent Funginorm in doses of 1,0–3,0 g/kg of mixed fodder to reduce the effect of mycotoxins in mixed fodders and to increase productive indicators.



Cadmium accumulation and depuration in muscles of Prussian carp (*Carassius gibelio* Bloch) after sub-chronic cadmium exposure: ameliorating effect of melatonin

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The aim of this study was to determine whether melatonin (Mel), which is a known antioxidant and free radical scavenger, is able to protect against the accumulation of cadmium in muscles of Prussian carp females exposed subchronically to this metal. These females received melatonin implants and were exposed to 0.4 mg/L or 4 mg/L Cd over either a 5-month period or a 3-month period, followed by a further 2 months of purification in clear water. Furthermore, this study was to evaluate the cadmium/melatonin relationship with essential bioelements like copper (Cu), zinc (Zn) and iron (Fe). Cd, Cu, Zn and Fe concentrations were determined using atomic absorption spectrometry method.

The rate of uptake of Cd increased with the time of exposure in the muscles, the highest cadmium concentration (0,61 mg/kg) was observed after 5 months in the group exposed to the highest dose of cadmium. The levels of muscle bioaccumulation of cadmium exceed the safe levels for human consumption in groups exposure to the highest doses of cadmium. Also water administration of Cd caused a significant increase of Cu, Zn and Fe in the muscles. Depuration of accumulated cadmium from the muscles depended mainly on duration of the elimination period. Cadmium level in the muscle was decreased after 2 months after the end of cadmium exposure.

Simultaneous administration of melatonin to cadmium challenged fish offset these detrimental changes. The results suggest that melatonin co-administration can effectively protect against the accumulation of cadmium in muscles and changes in trace metal concentrations.

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Detection of copy number variations in layer chickens and their association with production traits

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A total number of 2048 phenotyped hens from one brown and two white layer chickens pure lines were used to detect copy number variation (CNV) and perform association with economically important production traits. Individuals were genotyped within years 2011–2015 by the Hy-Line International research program for breeding management purposes using the standard Affymetrix 600k panel. Axiom® CNV Summary Tools and PennCNV were used to call CNVs, using the standard deviation (SD) of LRR ≤ 0.35 , BAF drift < 0.01 and waviness factor ≤ 0.04 as filtering criteria. Only CNVs consisting of 5 or more consecutive SNPs were used for analysis and individuals with more than 30 called CNVs were excluded. In total, 3350 CNVs merged into 1246 CNV regions (CNVRs) were detected. The mean number of CNVs per individual ranged from 1.98 to 4.87.

Analyzed phenotypes included 21 traits, some measured at multiple ages, giving in total 41 phenotypes per bird. Association analysis between CNVRs (present in at least 10 individuals) and phenotypes was carried out using a standard linear model in R, with hatch as the only fixed effect and one CNVR at a time (coded as number of copies 0,1,2 = normal, 3). Several associations of CNVRs with phenotype were detected ($p < 0.01$), including body weight, egg quality, egg weight, shell color and quality and number of sellable eggs. The most interesting associations were located on chromosomes 1, 2, 3, 5, 6, 10, 23 and 27. A number of CNVs that showed associations with production traits overlapped with previously detected QTLs and with protein coding genes with functions connected to immune response, membrane integrity or transcription factor activity.

In conclusion, use of the 600k chip enabled screening thousands of individuals for CNVs at a relatively low cost. Although the phenotypes evaluated are mostly polygenic, the analysis shown that CNVs can play a role in shaping the phenotypes and are an important source of variation within the genome.



The effect of combined calcium-cadmium *in ovo* injection on the activity of indicative hydrolases in tissues of newly hatched chicks

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Cadmium is one of the most dangerous occupational and environmental poisons. This heavy metal disturbs the calcium homeostasis by inhibiting calcium channels and/or related proteins. However, large dietary calcium intake can protect organism against the absorption, cummulation and toxicity of cadmium.

The aim of the study was to examine the effect of simultaneous calcium and cadmium ions administration *in ovo* on hatching results and hydrolytic enzymes activity in the tissues of newly hatched chicks. Egg albumen was injected on day 4 of incubation with cadmium alone (50 nmol per egg) or in combination with calcium (5 or 25 $\mu\text{mol Ca}$). Moreover, saline and saline supplemented with 5 or 25 $\mu\text{mol Ca}$ were used for control groups. Hatching results and embryopathological evaluation were performed. For both studied metals their distributions in tissues of 1-day old chicks were examined by the LA-ICP-OES method. The activities of N-acetyl- β -D-glucosaminidase, α - and β -galactosidase, α - and β -mannosidase, α -glucosidase, α -fucosidase and arylsulfatase in 1-day old chicks blood, kidney and liver were tested spectrophotometrically using synthetic substrates.

Cadmium-induced decrease of hatchability of eggs (35.7%) was improved by calcium co-administration (43.9 and 47.3%, for a dose of 5 and 25 $\mu\text{mol Ca/egg}$, respectively) as compared to the control group (52.7%). Similarly, the accumulation of cadmium in liver, kidneys and blood was limited by calcium ($P < 0.05$). The use of calcium (at higher used dose) as a protector during *in ovo* injection compensated the cadmium induced changes in tested enzyme activities to the level observed for the control group. The most evident tendency between calcium dose and activity of chicken blood enzymes were shown. Concluding, the appropriate supply of calcium seems to protect chick embryo against cadmium-induced disorders during the development.

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The effect of ultrasonic processing on honey quality

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Honey is a natural sweet substance used by people not only as a food product but also as a medicine. It is high viscosity product of characteristic flavor, aroma, color and texture. As it is a supersaturated sugar solution, with more than a 70% sugar content and relatively low water content of about 18%, honey naturally crystallizes. Among methods used for honey decrystallization ultrasonic processing is an effective treatment to destroy undesirable components, such as crystals and yeast cells responsible for honey crystallization. As a non-thermal processing technology, it causes lower HMF increase as well as better retention of biological activity of honey.

The aim of the study was to check the influence of ultrasonic processing on the selected honey quality parameters (enzymatic and antioxidant activities as well as antibacterial properties) with regard to the varietal differences. The possibility of re-crystallization of honey was also tested. The effectiveness of ultrasonic decrystallization was compared with standard method used by beekeepers (decrystallization in water bath).

The advantage of the proposed method of honey liquefaction is: significant time reduction (about 50 times), limited temperature increase (below 50°C), possibility of preserving the biological properties of honey (i.e. diastase number, antioxidant and antibacterial activity, no HMF formation) with simultaneous pasteurization of honey (ultrasound inhibits the development of fermenting yeast, which cause fermentation of honey during storage), delay recrystallization of honey (2–3 times in comparison to the conventional method). Honey sonication can be used as a cost effective way of facilitating the distribution of honey and meeting the needs of consumers preferring honey in liquid form (Patent application P. 420598).



Additional 15 STR panel for parentage verification in horses

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Parentage testing of horses and individual identification is based on seventeen microsatellites loci. Single-locus exclusions or other problematic cases of alleged parentage could be a sign of STR typing problems, typing closely related individuals (for example in small populations or endangered breeds) and indigenous breeds with reduced number of animals or reduced gene pool. Additional verification with alternative microsatellite set in these cases would provide a higher probability of exclusion and greater certainty of parentage testing.

A set of 15 STRs was used in one multiplex PCR reaction. Primers were labelled by four dyes. Two types of reaction mixtures were tested. Markers were identified with capillary electrophoresis on 3130xl and 3500 Genetic Analyzer. The study presents our preliminary results and the aim of our project is to characterize the microsatellite 15 TKY System for Polish indigenous horse breeds and to verify the single-locus exclusions in horse parentage. The preliminary tests indicate the successful amplification of microsatellites. A further research would include higher number of parentage testing cases, especially close related individuals and the validation. The additional panel of microsatellites would provide a higher reliability in performing parentage testing and individual identification. Moreover, set of 15 STRs could be a tool for monitoring a genetic biodiversity of indigenous breeds in population studies.



Effect of 4-nitrophenol (PNP) and 3-methyl-4-nitrophenol (PNMC) on mRNA expression and activity of selected caspases in white and yellowish ovarian follicles of the chicken

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4-nitrophenol (PNP) and 3-methyl-4-nitrophenol (PNMC) are major metabolites of several organophosphorus compounds. They are also considered to be nitrophenol derivatives of diesel exhaust particles. Nitrophenols, classified as endocrine disruptors, may induce testicular toxicity, ovarian and liver damage, nephrotoxicity and suppress adrenocortical function. In male rats PNP induced apoptosis in kidney and testes.

The aim of the study was to analyze the mRNA expression of apoptosis regulating enzymes: caspase-3 and caspase-8, and activity of caspases: -3, -8 and -9 in laying hen ovarian follicles following *in vitro* treatment with PNP or PNMC. Follicles: white (1–4 mm in diameter) and yellowish (4–8 mm) isolated from the ovary of laying Hy-Line hens were incubated for 6 h in Eagle's medium supplemented with PNP or PNMC (10⁻⁶ M). The mRNA expression of caspase-3 and -8 was analyzed with real-time qPCR and the caspase activity was measured with fluorometric methods.

The results showed, that PNP significantly decreased caspase-3 and -8 mRNA expression in white follicles, however, in yellowish follicles the effect of PNP was opposite ($P < 0.05$). The activity of caspase-3, -8 and -9 in white follicles significantly decreased in PNP-treated group, while the activity of caspases in yellowish follicles did not differ among control and PNP-treated group. PNMC decreased the mRNA expression of caspase-3 and -8 as well as activity of caspase-3, -8 and -9 in white follicles. The mRNA expression and activity of caspases in yellowish follicles did not differ between control and experimental group.

Results of this study reveal that PNP and PNMC may in a different way, affect the caspase mRNA expression and activity in the laying hen ovary. Their impact depends on the developmental stage of the ovarian follicle. The white follicles are more sensitive to nitrophenols than yellowish follicles.

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The influence of the new protein feed supplement on the productivity and natural resistance of broiler chickens

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The world agrarian science steadily develops in all important directions, creating innovations that are in demand, and promotes their wide promotion into practice. Among the most important problems that need to be addressed in the livestock sector in all countries of the world is the deficit of fodder protein.

One of the creative ways to solve this problem is a new protein fodder supplement grown on the basis of whey of fodder yeast *Debaryomyces hansenii* var. BIM Y-4. The additive contains 47.9% protein and is registered according to TU BY 100185198.183-2015.

The reconnaissance experiments conducted by us on the inclusion of this additive, as a component of import-substituting fishmeal, in the rations of repairing young hens showed encouraging results. We informed about this earlier, in particular in the published materials of the international scientific conference in Krakow.

In our next experiment on broiler chickens, replacing 5% of fishmeal with 5% of the new protein feed additive ensured an increase in the growth rate of the young at 3.7% while reducing feed costs per unit of growth of live weight by 4.5%.

The increase in the bioresource potential of broilers of the experimental group is due to the increase in the status of natural resistance of the organism through an increase in phagocytic activity of leukocytes by 6.2%, lysozyme activity of blood serum by 5.1% and bactericidal activity by 2.3%.

The economic feasibility of import substitution of 5% fish meal with the same amount of new protein feed supplement is confirmed by economic efficiency.

It was found that the additional profit for the cultivation of 50 broiler heads was USD 2.1. The debut versions of our research with the use of the innovative feed supplement

based on whey as an import-substituting protein component in rations of agricultural poultry create the prerequisites and the need for a comprehensive study of this bio-nutrient in the rations of various types of the agricultural animals.



Effect of silver nanoparticles on caspase-3 activity in chicken granulosa cells

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Silver nanoparticles (AgNPs) are nowadays widely common in many customer products and predicted to use in poultry production. Therefore, there is a growing need to determine the possible side effects of AgNPs on the environment and living organisms. The aim of this study was to evaluate the *in vitro* effect of AgNPs on caspase-3 activity in granulosa cells isolated from follicles of the chicken ovary. The experiment was performed on six Hy-Line Brown hens at the age of 25 weeks. Chickens were decapitated 2 h after ovulation and yellow preovulatory follicles (F3-F1) were dissected from the ovary. Fragments of granulosa layers of the individual follicles were dispersed by incubating with 0.3% collagenase (10 min, 37°C), filtered and washed in PBS. Cells were suspended in M199 medium supplemented with fetal bovine serum (10%) and antibiotics (0.2%) and seeded into 96-well plate at concentration of 5×10^4 viable cells/well. After 48 h the medium was changed to M199 with reduced concentration of FBS (5%) and cultured for the next 24 h with the addition of AgNPs 13 nm and 50 nm (0.1, 1, 5 µg/ml). Activity of caspase-3 was evaluated using Caspase-3 Assay Kit (BioVision). Statistical analysis was performed using analysis of variance and dose effects were interpreted based on planned polynomial contrasts. Results were considered to be significant at $P < 0.05$. Analysis revealed that 50 nm AgNPs do not influence caspase-3 activity, while 13 nm AgNPs increased it when used at higher doses (1 and 5 µg/ml). The obtained results suggest that the effect of AgNPs on caspase-3 activity in granulosa layer of preovulatory follicles is size and dose dependent and smaller nanoparticles are more likely to cause apoptosis than larger ones.

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Effect of PCB153 and 4-OH-PCB153 on iodothyronine secretion from the thyroid gland of the hen (*Gallus domesticus*) – *in vitro* study

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Polychlorinated biphenyls (PCBs) disturb function of endocrine system. Due to their chemical stability and strong lipophilic character these compounds have ability to bioaccumulate in fatty tissues of living organisms. Hydroxylated metabolites of PCBs (OH-PCBs) are formed in the liver and their existence has been confirmed in adipose tissue, and their biological effects in the endocrine system is obscure. The aim of this study, therefore, was to compare the *in vitro* effects of PCB153 with its metabolite 4-OH-PCB153 on iodothyronine secretion by the chicken thyroid gland. The experiment was carried out on 28-weeks old Hy-line Brown hens which were kept in lighting schedule of 14L : 10D and fed ad libitum. Explants of the thyroidal glands were isolated from hens (n = 6) 2 h after ovulation. They were weighed and incubated for 24 h at 39°C in Eagle's medium supplemented with PCB153 or 4-OH-PCB153 at concentrations of 0 (control group), 0.1 (ng/ml), 1 (ng/ml) and 10 (ng/ml). Following the incubation the medias were collected and kept at –20°C till determination of thyroxine (T4) and triiodothyronine (T3) concentrations by RIA method. The results obtained were statistically evaluated by means of one-way analysis of variance (ANOVA); differences between means were analyzed using Duncan's test (p < 0.05). The experiment revealed that PCB153 at concentration of 10 ng/ml reduced T4 and T3 levels in the medium by 30% and 35%, respectively (P < 0.05). 4-OH-PCB153 at concentrations of 0.1 ng/ml increased concentrations of T3 by 52% (P<0.05). In the other groups, there were no statistically significant changes in T4 and T3 levels. The results obtained suggest that PCBs and OH-PCBs affect T4 and T3 secretion from the chicken thyroid gland. PCB153 is a direct inhibitor of iodothyronine secretion from thyrocytes. Further studies are necessary to explain a mechanism of PCB and OH-PCB action in the avian thyroid gland.

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Straw wine as a noble Polish sweet wine. Production techniques and their impact on the bioactivity of product

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The aim of this study was to examine the effect of different techniques of straw wine production on quality of their final product as evaluated by phenolic compounds content and biological activity. One of the three tested methods (the one applied in 2015) allowed production of wine with higher bioactivity and thus considerably better overall quality.

Three straw wines were compared. They were produced from the 'Hibernal' grape variety at the University Vineyard in Garlica Murowana (southern Poland) employing three independent methods. First, all the grapes were dried for 5 weeks under controlled conditions. Then, in 2014 the fruit were pressed and the stum subjected to fermentation. In 2015, the grapes were grinded down and fermented; then, after a month the free-run juice was separated and the rest of pulp pressed. In 2016, the intact whole grapes were flooded with the young wine of the current generation of 'Hibernal' var. and then fermented. The rest of pulp was pressed followed by the separation of free-run juice.

The content of phenolic compounds was determined with the Folin-Ciocalteu method. Biological activities of the extracts were estimated based on their antioxidant and anti-radical capacity spectrophotometric assays, and then expressed as a Trolox (a synthetic antioxidant) equivalents (TE): (1) FRAP (the ability of the extracts to reduce ferric-tripiridyltriazine (Fe^{3+} -TPTZ to Fe^{2+}), (2) CUPRAC (utilization of copper (II)-neocuproine reagent as chromogenic oxidizer), (3) DPPH (bleaching of purple reagent solution).

The most promising technique of the straw wine production was the one applied in 2015. This was concluded judging by the polyphenolic content (3748,5 mg gallic acid eq.s/L), the antioxidant capacity (10,2 mmole TR/L for FRAP and 24,6 mmole TR/L for CUPRAC), and the antiradical capacity (5 DPPH mmole TR/L). All the above values were significantly greater than the respective ones obtained with the two remaining wine-making procedures.



Thermography as an welfare indicator of fur-chewing chinchillas

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Heat emission, reflected in a animal's body temperature is related to health and physiological processes associated with feed efficiency. Therefore, it seems interesting to examine how fur biting has reduced the welfare of chinchilla (*Chinchilla lanigera*) caused by the body heat lost increase.

Adult chinchillas were divided in two groups: control – normal behavior (n = 25) and experimental – fur-chewing behavior (n = 23). The environmental parameters as well as the food and water intake were monitored daily. Thermovision measurements were conducted with use of camera A20 (FLIR Systems Inc). Six thermograms were performed for each animal. The obtained data were used to calculate the heat loss (Q [W]) by the fur chewed and normal skin surface.

The food and water intake by control animals were 20.7±4.52 g and 15.9±3.45 ml while fur-chewing chinchillas were 25% and 33% higher, respectively ($P \leq 0.05$). The same relationship has been retained for relative food (control: 37.4±5.87 g/kg BW; fur-chewing: 43.8±6.90 g/kg BW) and water (control: 29.3±7.76 ml/kg BW; fur-chewing: 35.9±7.53 g/kg BW) intake ($P \leq 0.05$). Based on this results and a fodder energy can be calculated intake of metabolic energy on 2.2 W for control and 2.8 W for fur chewing animals. Calculated heat flux through bitten areas was 2.50 mW · cm⁻² i.e. 1.49 times more than through undamaged chinchilla fur ($P \leq 0.05$). This resulted 21% higher total heat loss of fur chewing animals from whole surface (1.37±0.17 W) in comparison to control (1.13±0.09 W) ($P \leq 0.05$).

In the conclusion, the thermographic measurements show clearly that the fur chewing increased a heat emission by the body, which may lead a decrease of a thermal comfort of the animal and thus its welfare. Further development of thermography, coupled with biological modeling and metabolic studies, may lead that this method will become a sensitive tool for animal welfare evaluating.

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Effect of *Staphylococcus aureus* infection on the heat stress protein 70 (HSP70) level in chicken embryo tissues

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Staphylococcus aureus bacteria are components of physiological biocenosis of skin or mucous membranes in some animals genres but also they are dangerous opportunistic pathogens responsible for infections of various localization, course or manifestations. Proteins produced by these bacteria destroy tissues, leukocytes and cause haemolysis of erythrocytes. Host organisms response by defence mechanisms. Production of heat stress proteins (HSPs) is one of defence responses of infected host organism. To evaluate infection and host defence mechanisms some animal models of experimental infection are reported. Use of chick embryo model allows to demonstrate of adequate differences in staphylococcal virulence depends to strain genotype. The aim of study was to examine the changes in heat shock protein HSP70 levels in chick embryo tissues after infection caused by *S. aureus* strains no. tu2, pa3, ch5, ch10, ch21 and ch24. The bacteria were injected directly into fluid of amnion cavity and incubated 10 days. The mortality of particular chick embryos was reported and the tissues for further analysis were taken every day from day 13 to day 19. The levels of heat stress protein HSP70 were determined by dot-blot method. Results showed that the strains no. ch5, ch21 and ch24 were the most virulent. HSP70 levels increased in all groups of injected embryos at the same time the hatching process was started. The presented study showed that the infected chick embryos were characterized by higher HSP level from 12.3% up to 19.7% compared to the control group. The morphological analysis

showed numerous erythrocytes with damaged cell membranes and morphological changes of erythrocytes. Changes in the level of HSP70 protein can be useful indicator of infection caused by *S. aureus* bacteria. Additionally, chicken embryo is helpful research model in studies of pathogenesis of diseases caused by bacteria.

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Effect of α -tocopherol *in ovo* supplementation on antioxidant enzyme activity in tissues of hatching chicks

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Process of hatching requires a great effort from the chick's organism and therefore can generate increased free radical formation. Free radicals are neutralized i.a. by antioxidant enzymes (i.e. superoxy dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)) as well as egg-containing antioxidants i.e. carotenoids or tocopherols. Therefore, seems intriguing to investigate the effect of α -tocopherol (vitamin E) *in ovo* supplementation on the activity of the chicks' antioxidant system.

At 17. days of incubation (E17) the embryoned eggs (120 eggs, Ross 308 line) were randomly divided on three equinumerous groups and next α -tocopherol (Sigma-Aldrich) at dose 0.0, 0.5 and 5.0 mg/egg dissolved in 0.1 ml peanut oil (SIGMA Aldrich, P2144) was *in ovo* injected. At E18, E19 and E20 the 12 eggs of each group were sampled, the embryo was euthanized by decapitation and next the liver was collected. The sampled tissues were homogenized in phosphate buffer (pH 7.0; 100 mM), centrifuged and collected supernatant. The activity of SOD and CAT and concentration of glutathione (GSH) were spectrophotometrically analyzed in supernatant.

There was occurred that SOD activity in chick livers is similar during whole hatch process and was not changed by α -tocopherol *in ovo* supplementation. The activity of CAT was the highest at E18 (before start the internal pipping) and decreases gradually at the follow stages of hatch. Supplementation of 0.5 mg α -tocopherol per egg caused reduction of activity CAT c.a. 20%. Similar glutathione concentration was the highest before start the internal pipping and decreases gradually at the follow stages of hatch. However

supplementation of 0.5 mg α -tocopherol per egg decreased of GSH concentration c.a. 25% but only at E18.

Concluded, the results indicate that α -tocopherol supplied *in ovo* can change of antioxidant enzymes activity in tissues of hatching chick. Therefore seems reasonable to continue research into the effect of supplementation in antioxidant substances on the welfare, health status and quality of chicks.

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Fish exposition to low concentration of mancozeb as a reason of ultrastructural and physiological parameters changes

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The aim of the study was to evaluate the effects of fungicide mancozeb ($1.0 \text{ mg} \cdot \text{l}^{-1}$) on carp. The investigations were performed on carp under aquarium conditions. Fish were divided into 2 groups: control and exposed to mancozeb for 14 days. After the exposure, the fish were subjected to purification (30 days).

In the first some pieces of organs: gill, anterior and posterior kidneys and spleen were taken for ultrastructural analyses. Next, plasma cortisol, catecholamines, protein and triglyceride concentration, and blood glucose were studied. The AST and ALT activity in plasma and gills ATPase were also studied.

In gills destruction of lamellae, hypertrophy and hyperplasia of respiratory epithelium were observed. The structure of hepatocytes mitochondria was damaged. Vacuoles with different content and increase the quantity of RER were observed. In the epithelium cells of renal tubules of posterior kidney, destruction of mitochondrial structure, increase of quantity of RER and its fragmentation, and different electron density of brush border were detected. In hematopoietic part of the kidney, its separation from the extractory part, loosening of the connective tissue stroma and hematopoietic cells were noticed. In the anterior kidney and spleen loosening of their structure were observed. Cortisol concentration was variable. The adrenaline concentration gradually increased to the highest value after fish exposure; after the purification period was close to the control. The concentration of noradrenaline was reduced. Glucose concentration, after initial increase,

gradually decreased to the lowest value after the purification. Triglyceride concentration was variable, reaching the highest value at the end of fish exposure; after purification was comparable to the control. The total protein concentration was variable – increased in the first hours of exposure and further close to the control. The activity of AST and ALT and ATPase was reduced.

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Early development of vimba (*Vimba vimba*) at different temperatures

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The aim of the present study was to describe the details of embryonic development of vimba and to evaluate the effect of thermal conditions on development success and hatch quality.

The eggs and sperm of vimba (*Vimba vimba*) were obtained during artificially stimulated spawning. The eggs were fertilized and incubated in tap water at 3 temperatures: 14°C, 18°C and 20°C.

The eggs swelled (up to 55%) during 20 minutes from fertilization regardless of temperature. At the beginning of development survival of embryos in all temperatures was very high (over 80% at the stage of body formation) indicating high fertilization rate. The highest decrease of survival occurred at the stage of body segmentation (at 14°C all embryos died). During entire development survival of the embryos gradually decreased, and at 18°C 66.6% of them hatched while at 20°C – 73.8%. Also the rate of development depended on temperature of incubation and was highest at 20°C.

During the embryonic development frequency of body malformation at 18°C was significantly lower than in 14 and 20°C. All larvae hatched with tail first. Newly hatched larvae at 18°C were bigger than those at 20°C. Percentage of deformed larvae among newly hatched was higher at 18°C than at 20°C. The most common morphological abnormalities were: spine curvature, heart edema, body shortening and yolk sac malformation.

The obtained results show that 20°C is the most appropriate temperature for embryonic development of vimba, while lower temperatures not only extend development rate but also disturb development resulting in reduced survival and hatch quality.



The effect of copper and cadmium on early development of ide (*Leuciscus idus* L.)

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Some heavy metals, including Cu, and Cd are detected at high concentrations in many aquatic environments and have a harmful effect to living organisms. The effects of many metals on early stages of some cyprinid fish species have been well recorded, but still scarce on ide. Due to that the aim of the study was to evaluate the effects of copper and cadmium on embryonic development of ide.

Embryos of ide after fertilization were incubated in $0,1 \text{ mg} \cdot \text{dm}^{-3}$ of Cu, $0,1 \text{ mg} \cdot \text{dm}^{-3}$ of Cd or in clean tap water as control. Both metals significantly decreased swelling of eggs. Metals delayed rate of embryonic development, especially at the stage of body movements, and rate of hatching (beginning and end) – Cu elongated the process and Cd shortened comparing to control.

Among newly hatched larvae 6 types of body malformations were distinguished: spine curvature, C-shaped body, head deformation, yolk sac deformation, heart edema, and body shortening. Only first two types of deformations were observed in control, whereas complex ones were more often in Cu and Cd. Present study showed that cadmium exposure was more toxic to early stages of ide than copper.



The effect of selected methods of the heat processing on the content of vitamin C in two varieties of the endive (*Cichorium endivia* L.) and the globe artichoke (*Cynara scolymus* L.)

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The aim of experiment was to assess the effect of selected methods of thermal treatment processes on the content of L-ascorbic acid in leaves of some endivia and globe artichoke-hearts varieties.

The experimental material were two of endivia varieties ('Cigal' and 'Marconi') and of artichoke ('Imperial F1' and 'Gross Camus de Bretagne'). The following technological processes were applied: cooking (traditional and steam cooking), blanching (traditional and steam), braising (stewing).

The assessment of L-ascorbic acid content was performed by spectrophotometric method at $\lambda = 578$ nm with Ascorbic Assay Kit (L-ascorbate, Megazyme). The obtained results were subjected to statistical analyses with licensed Statistica v. 12.0. The relevance of differences between means was estimated with post-hoc Duncan test ($\alpha = 0,05$).

On the base of obtained data, it can be stated, that irrespectively of the kind of the applied method of culinary treatment, significant lowering of the content of L-ascorbic acid was recorded in leaves of the endive and hearts of the globe artichoke in comparison to fresh raw material.

The process of traditional cooking caused the biggest decrease of the content of the vitamin C in hearts of examined varieties of the globe artichoke ('Imperial' – $0.72 \text{ mg} \cdot 100^{-1} \text{ f.m. g}$; 'Gross Camus de Bretagne' – $0.9 \text{ mg} \cdot 100^{-1} \text{ f.m. g}$). Also the steam cooking and braising (for variety 'Gross Camus de Bretagne') influenced the substantial reduction of the compound. The smallest losses of the value of the of L-ascorbic acid was stated (appropriately for 'Imperial' – 18.1%; and for 'Gross Camus de Bretagne' – 10.9%) as a result of traditional blanching.

Also leaves of two varieties of the endive were characterized by a reduced content of L-ascorbic acid, as a result of thermal of culinary processing, particularly for traditional blanching and the steam cooking. The smallest drop in the content of the compound were found for examined varieties of the endive during steaming.



The preferences of taste and the frequency of consumption of fruit juices among nursery children

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Juices (especially fruit) are ones of most willingly drunk drinks by children. It is possible to count them as one of elements of the meal (the equivalent for the fruit or vegetables in the diet of the child attending to the nursery). However, one should remember that they can not be the basic liquid to quench thirst.

The aim of this work was to evaluate the taste preferences and frequency of consumption of fruit juices by children aged three to six.

The questionnaire survey was conducted among parents of children attending to nursery (4 institutions) in the municipality of Wieliczka. 200 persons took part in questionnaire survey. The questionnaire sheet contained 14 questions. Achieved results were subjected to statistical analysis with applying the Chi² Pearson test.

Analyses of achieved results confirmed that children irrespective of the sex willingly had drunk of fruit juices. Everyday consumption of fruit juices, was declared by the 30% of parents. The same percentage of respondents also informed that their kids consume of this type of products, 3–4 times during the week. 13% of children had drunk fruit juices less than 3–4 a week.

Parents (40% of the whole) served to their children juices bought in shops. Slightly few respondents (32%) declared that they were preparing juices at home. Unfortunately the prevailing majority of children nursery (70%) did not drink vegetable juices. It was also declared that boys had more often consumed vegetable juices than girls.

As for taste preferences for fruit juices the prevailing majority traded was: apple, and then orange and multifruit.

It deserves to underline the fact, that beside from juices, respondents declared the mineral water as the other most often consumed drink by nursery children.

The predominant majority of respondents declared that they had been guided by principles of health nutrition and had paid attention to that juices served to children had not contain the addition of sucrose.



The anomalies of the development of oven, irreditive ferel and koi coils in growing in the installation of closed water supply

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When inspecting the farms' basins, sturgeon was found to have no nasal septum. Individuals with such anomaly instead of a double nostril opening on each side of the head had a single aperture. Most likely, the main reason for the occurrence of these anomalies are fluctuations in water temperature during embryonic and postembryonic development. Often there were anomalies in the development of the organs of vision, expressed in the underdevelopment or absence of one or both eyes. Often the sign of the absence of the eyes was transmitted to the offspring, therefore, in order to avoid such problems, such individuals should be rejected at early stages. Another common anomaly in this enterprise is the underdevelopment of the pectoral fins. The underdeveloped pectoral fins (or their complete absence) on one or both sides are most often the result of traumatizing the larva by other fish when switching to active feeding, or a high density of planting, which causes the pectoral fins to twist. The truncated gill cover, which does not completely cover the gill cavity, leaving the gills open, is also a rather frequent anomaly in the household. The shortening and curvature of the vertebral column, often found in fish in this farm, is most likely the result of unfavorable environmental factors in embryogenesis or the result of close inbreeding.

Among the first occurring anomalies of sturgeon development in this farm are: a change in skin pigmentation, congenital absence of skin pigmentation, and bifurcation of the nasal stem. The description of such an anomaly in the literature sources we did not find and suggested that this is most likely due to violations in the work with the producers.

In the survey of basins for the cultivation of fish-planting material for trout at the fish farming complex of the BSAA, three types of abnormal development were found: bifurcation of the head (double larva), dropsy of the yolk sac and curvature of the spinal column. Larvae with a double head were found in the incubation shop (there were 20–30 larvae with double heads per 10 thousand larvae), they already hatch from eggs, lived no

more than 2–3 weeks and died. Larvae with dropsy of yolk sac lived no more than two weeks and died. For 10 thousand larvae and fry there are 5–10 individuals with a similar anomaly.

According to some scientists, such anomalies are the result of using for breeding unripe and overripe eggs, as well as caviar from too young or very old producers. Curvature of the spinal column is one of the most frequent deviations in the development of individuals, since from 100 thousand larvae of fish with curvature of the spinal column it is necessary from 3 to 10%. Some of them die, some with full-fledged individual development are realized as fish-planting material for fish-breeding complexes, where they grow to commercial fish and are used mainly for processing. It should be noted that this anomaly quite often occurs in different species of fish and according to the literature is a consequence of unfavorable growing conditions.

In koi carp producers in the farm, diseases and developmental anomalies are rare. For example, seven individuals of carp with a curvature of the vertebral column were found. This anomaly relates to functional diseases characteristic mainly of fish that are bred in captivity and is a consequence of unfavorable environmental factors in embryogenesis or the result of close inbreeding. In fry, exophthalmia with hemorrhagia of the cutaneous covering of the head was detected, which is most likely a consequence of mechanical injuries.



Qualitative analysis and quantification of basic poultry species based on 12S-rRNA analysis

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EU regulations require food manufacturers and animal feeds to match the reality of information on the quality of the product. For this reason, the development of ways to check the components declared with real has become an important research challenge in recent years. The aim of this paper is to develop a qualitative method for the identification of four basic species of poultry and to quantify them using Real-time PCR. For the study, a fragment of the mitochondrial DNA 12S-rRNA gene was delimited by primers: 5'taa-gccacacccccacgggta; 5'ctggcacaagattaccaaccctg to which designed Tamra probes with sequences: 5'aaacttgacttagccatagcaacc; 5'catagcaactttagggttggttaa; 5'agcaacagcctaactcaagta; 5'agccatagcaactttagggttggt respectively for gallus (*Gallus gallus*) and turkey (*Meleagris gallopavo*) ducks (*Anas platyrhynchos*) and geese (*Anser anser*).

Presented primers are biologically specific to all poultry species tested, while probe species homology shows that the proposed primer/probe kit will allow qualitative and quantitative distinction between the most important species of poultry. Based on the standard curve formed from the meat mixture of the above species, the percent DNA content of the species can be determined.

The implementation of the method developed in the laboratory will allow for verifying the compliance of the manufacturer's declaration with the actual composition of the food products.



Effect of the addition of corn DDGS and different sources of fat to the feedstuff of pigs on backfat transcriptome

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Addition of the cDDGS (corn Dried Distillers Grains with Solubles) to the feedstuff of pigs is profitable because of its relatively low price and high content of proteins. However, a large amounts of DDGS in the diet worsens the quality of backfat of pigs. In order to oppose this effect, fats with high content of saturated fatty acids are used as an addition to the feedstuff. The aim of our study was to compare transcriptomes of the backfat from pigs obtaining diet without DDGS with those obtaining DDGS in the feedstuff. Additionally, we have compared the transcriptomes of the backfat from pigs obtaining different sources of fat in the diet. Animals were divided into four feeding groups. The diet differed among groups in terms of the presence of corn DDGS (groups II, III, IV – 20%) as well as the type of fat used (rapeseed oil – groups I and II, beef tallow – group III, coconut oil – group IV). At the end of the fattening experiment, all pigs were slaughtered and samples of backfat for transcriptome analysis were collected from 6 animals in each group. Transcriptome analysis was performed by RNA-seq on Illumina HiScan instrument (Illumina). After bioinformatic analysis we have identified 93 Differentially Expressed Genes (DEGs) between group obtaining DDGS and rapeseed oil and group without DDGS. We have further evaluated that genes upregulated in the group of pigs without DDGS in the diet are mainly responsible for lipid metabolism. We have identified several reactome pathways affected by diet treatment, for instance: activation of gene expression by SREBF (SREBP) $p < 2,74E-06$, metabolism of lipids $p < 2,27E-04$, fatty acyl-CoA biosynthesis $p < 7,46E-04$. Our results shows that addition of the cDDGS to the feedstuff changes transcriptome of backfat of pigs.

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The effect of Cu and Zn nanoparticles on the ghrelin concentration in the turkey brain

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Copper and zinc are essential elements required by poultry. Copper is a component of various intracellular and extracellular enzymes such as cytochrome oxidase, ceruloplasmin and superoxide dismutase. Zinc is a component of metalloenzymes such as alcohol dehydrogenases, carboxypeptidase, alkaline phosphatase, thymidine kinase, RNA and DNA polymerase. Cu and Zn supplementation improved growth rate and feed conversion ratio in broilers. Results of experiments suggest involvement of Cu and Zn in the regulation of the nervous, endocrine and immune systems. However, the bioavailability of Cu and Zn is not efficient, and recently the form of nanoparticles are extensively tested.

Present experiment was conducted to determine effects of supplementary Cu and Zn nanoparticles on ghrelin changes in the brain of turkey.

One-day-old turkeys were divided into two groups: I. supplemented with Cu and Zn in traditional form (control) and II. in the form of nanoparticles. Each group was divided into subgroups with full supplementation (100%) or receiving only 10% of the dose.

Zinc and copper were supplemented by 98 days, then fragments of the hypothalamus, pituitary, striatum and cerebellum were taken out and directed to the estimation of ghrelin, concentration by RIA.

Supplementation with Cu and Zn nanoparticles significantly decreased the ghrelin concentration in the hypothalamus and cerebellum by 24 to 61% compared to control groups ($P < 0.01$). On the other hand, nanoparticles caused significant increase ($P < 0.01$) of ghrelin concentration in the pituitary and striatum (from 12 to 87%).

In summary, the results clearly showed the effect of both nanoparticles on the ghrelin, hormone regulating the activity of appetite center in the hypothalamus. It seems probable, that Zn and Cu affect the metabolism and probably had impact on the feeding through the ghrelin regulation.

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Automated DNA sizing technology for sex identification in birds

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To identify the sex of birds, techniques of molecular biology are used increasingly, because nearly 50% of birds are sexually monomorphic. The CHD gene has been widely used to determine the sex of birds, due to the high degree of conservatism and the presence of polymorphic variants of the Z and W chromosomes. PCR products are usually visualized after electrophoresis on agarose gel, where PCR amplification of the CHD gene produces a double (ZW) and single (ZZ) bands in females and males, respectively. In our laboratory we have implemented method of capillary electrophoresis for identifications the bands. 69 individuals were tested from genera Columba and 45 individuals from genera Aprosmictus, Cacatua, Myiopsitta, Platycercus, Polytelis and Psittacula. Genomic DNAs were extracted from feathers and buccal swabs. Sex-specific products of CHD gene was amplified using the QIAGEN Multiplex PCR and specific pair primer. One of pair primers were labelled with fluorescent dye 6-FAM, the amplified products were separated on 3100xl Genetic Analyzer and genotyped using GeneMapper software (Applied Biosystems). Variation in the intronic sizes of CHD-W and CHD-Z gene was detected by capillary electrophoresis. Two different picks of PCR products was produced from CHD-W and CHD-Z genes in females and one pick of PCR products from CHD-Z gene in males. The tested material had a high genetic diversity, were identified 5 gene variants in the range of 268–320 bp.



Involvement of metalloproteinases (MMP-2, -7 and -9) in the chicken postovulatory follicle regression

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Chicken postovulatory follicles (POFs) are an excellent model for examination of reproductive tissue regression. Unlike mammals, the follicle in birds after ovulation rapidly terminates the metabolic activity and fails to form a corpus luteum. POFs are regressed via the process of apoptosis within several days (approximately 6). However, the signals and molecular mechanisms regulating this process are not fully elucidated. In this study engagement of the matrix metalloproteinase-2, -7 and -9, crucial proteolytic enzymes regulating tissue remodeling, and their tissue inhibitors (TIMPs) in POF regression was investigated in the chicken ovary. Additionally, number of apoptotic cells and key markers of apoptosis (initiator caspases 2, 8 and 9 and executioner caspase-3) were examined as well.

The experiment was performed on laying Hy-Line hens ($n = 12$) at the age of 25 weeks, which were sacrificed 2 h after oviposition and 5 postovulatory follicles (POF1-5) were isolated from the ovary. Tissue samples were subjected to measurement of MMP activity with Biotrak Activity Assay, caspase activity by Fluorometric Assay Kit, and MMP and TIMP expression by qReal-Time PCR. The other POFs were embedded in paraffin for MMP localization by immunohistochemistry and apoptotic cell examination by TUNEL method.

It was found that the weight of POFs decreased from 440 ± 24 mg to 76 ± 9 mg whereas the number of apoptotic cell (TUNEL-positive) increased along with follicle regression. The activity of all examined caspases elevated gradually (approximately 80–150%) reaching the highest level in POF3, and next slowly decreased to the value noted in POF1. Relative expression (RQ) of MMP-2 mRNA increased gradually reaching the highest value in POF5. MMP-7 mRNA expression was slightly, but not significantly elevated, and MMP-9 transcript decreased after transition from POF3 to POF4. Ratio of relative expression of MMP-2:TIMP-2 was higher in POF2 to POF5 than in POF1, and

ratio of MMP-9:TIMP-3 was elevated in POF2 to POF4 compared to POF1 and POF5. Activity of MMP-2 and MMP-9 sharply increased in POF2 followed by decrease in POF3. Immunohistochemistry showed follicle- and tissue-dependent intensity of staining for MMP-2 and MMP-9 proteins.

The results obtained indicate that apoptosis in the POFs of chickens is the caspase-mediated, and the process might be initiated by the external or internal pathway. Moreover, participation of MMPs (especially MMP-2 and -9) and TIMPs in POFs regression is also strongly suggested.

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The influence of roundup on the embryonic development, survival and hatching of Prussian carp (*Carassius gibelio* Bloch)

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The aim of this study was to examine the effect of Roundup on embryonic development and hatching of Prussian carp (*Carassius gibelio* Bloch) larvae. Roundup, very popular herbicide is widely used in the agriculture even to control aquatic vegetation in ponds and lakes. So Roundup present in the aquatic environment may have an influence on the developing embryos. The experiment began by carrying out artificial spawning. The samples of eggs were obtained from 5 sexually matured Prussian carp females, stimulated previously with sGnRH analogue at the dose of $10 \text{ mg} \cdot \text{kg}^{-1}$ body weight. The samples of eggs obtained from each female were divided into two Petri dishes (about 150 eggs in each dish) and incubated separately after activation with common carp (*Cyprinus carpio* L.) sperm. After 1 minute water was replaced with 1 or $10 \text{ ng} \cdot \text{mL}^{-1}$ of Roundup – experimental groups or clean water – control group. Fungi Stop Konzentrat (TETRA, Germany) was used to prevent fungal diseases of incubated eggs. Water was changed twice a day in each dish. The incubation lasted for about 4 days. The percentage of living eggs after the first day of incubation, hatching rate and percentage of deformed larvae were observed. After analyzing the results with the Mann-Whitney test it was found no statistically important effect of Roundup on percentage of living eggs (after 24 h of incubation). There were no significant differences in the hatching rate at 69 to 90 hours of exposure. The number of deformed larvae of Prussian carp was similar in the experimental groups as well as in the control one. Summing up the results there is no adverse effect of tested concentrations of Roundup on the developing embryos and hatching of Prussian carp larvae. It would be interesting to evaluate the effects of this herbicide on the embryonic development of more sensitive fish species.

This research was financed by the Ministry of Science and Higher Educations of the Republic of Poland



Effect of magnetic field deprivation on embryonic development and hatching dynamics in Prussian carp (*Carassius gibelio*)

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Geomagnetic field (GMF) is an important component of Earth electromagnetic environment, necessary to stimulate and control the processes of metabolism in living organisms. Therefore, both natural and artificial disturbances of GMF cause disorders in the proper functioning of these organisms.

The aim of the study was to examine the impact of hypogeomagnetic conditions on embryogenesis and hatching dynamics of Prussian carp (*Carassius gibelio*). Research material was a roe collected from six females of Prussian carp, activated with sperm of two male common carps (*Cyprinus carpio*) and incubated on Petri dishes. The incubation of the experimental group was carried out under the geomagnetic deprivation conditions, obtained by placing the testing material in the geomagnetic shielding cages made of S235JRG2 steel which resulted in the reduction of the GMF to below 20 nT. The GMF was measured using BPM 2001 Geo-Magnetometer (Bio-Physik Mersmann, Germany). The control group was incubated in the natural geomagnetic field.

The obtained results indicated a highly significant impact of GMF deprivation on embryos death rate and hatchability. The greatest, accounting for 8%, prevalence of embryo mortality in the experimental group over the control group, was observed in the first 24-hour incubation period. Moreover, in the hypogeomagnetic conditions larvae hatching started 6 hours earlier than in the natural GMF. The deprivation of GMF did

not affect significantly the number of deformities in Prussian carp larvae. The performed study proved that at the early stages of development, such as embryogenesis and early ontogenesis, the organism of Prussian carp is very sensitive to the changes in the geomagnetic field.

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Effect of glutamic acid on catecholamines *in vitro* release from selected rabbit brain structures

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One of the major roles of glutamic acid (Glu) is as an excitatory neurotransmitter within the central nervous system. As a neurotransmitter, this amino acid influences several areas of the brain including the thalamus, brain stem, spinal cord, basal ganglia and pons. Catecholamines are synthesized in the brain, in the adrenal medulla, and by some sympathetic nerve fibers. Catecholamines, including dopamine and norepinephrine, are the principal neurotransmitters that mediate a variety of the central nervous system functions, such as motor control, cognition, emotion, memory processing, and endocrine modulation. The aim of the study was to establish the direct effect of three doses of Glu on noradrenaline and dopamine release from hypothalamus, hippocampus, amygdala and prefrontal cortex of rabbit. Brain structures were obtained from decapitated female 12 week old rabbit. After decapitation brain of animal obtained was placed in 0.9% NaCl. Then, each brain structures from each rabbit (approx. 50 mg) were placed in incubation wells (cell culture Sigma) containing 1 ml of incubation medium (Krebs phosphate buffer containing glucose 0.3% bovine serum albumin and 0.1% BSA) without (control) or three doses of Glu in a concentration of: I – 5 μ M, II – 50 μ M and III – 200 μ M. Each slice of tissue postponed scraps of each brain structure every 30 minutes to the next holes. Medium collected after 30, 60 and 90 minutes of experiment were used for dopamine and noradrenaline RIA measurement. The obtained results show that Glu influences directly but differential effect on catecholamines release from selected brain structures of rabbit. Generally, there was an inhibitory effect on catecholamines release, while supporting

and/or stimulating effect was visible in the case of prefrontal cortex. Our findings show that Glu more influences the brain's motivational structures, which may indicate its contribution to the stress response by modulating the amount of catecholamine released.

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The effect of the hypothalamo-pituitary-adrenal axis on adipocyte resistin and visfatin gene expression during inflammation

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The white adipose tissue undergoes molecular and cellular alterations, affecting systemic metabolism and inflammatory states. It is recognized that the adipose tissue produces multiple peptides, called adipokines, which play a significant role in the pathogenesis of a low-grade inflammation associated with obesity and metabolic syndrome, and in chronic inflammatory and autoimmune diseases. Among variety of adipokines, resistin and visfatin are proposed as important pro-inflammatory mediators, which also interfere with the central regulation of insulin sensitivity. Chronic stress, combined with positive energy balance, may be a contributor to the increased risk for obesity, especially upper body obesity, and other metabolic diseases. This association may be mediated by alterations in the hypothalamio-pituitary-adrenal (HPA) axis.

Thus, the present study aimed to examine the influence of HPA hormone axis supplementation on the expression of adipokines (resistin and visfatin) by adipocytes during inflammation.

Adipocytes, differentiated in culture, were treated with CRH, ACTH and glucocorticoids alone or together with TNF-alpha for 24 h, then the mRNA was quantitated by real-time polymerase chain reaction (real time PCR).

TNF-alpha alone increased visfatin mRNA levels after 24 h, but resistin mRNA did not change significantly ($P < 0.01$). CRH had no significant effect on adipokine gene expression, in contrast to ACTH (increased approximately 6-fold both of adipokines). After 24 hours in the presence of glucocorticoid an increased expression of visfatin and resistin was observed ($P < 0.01$). We found a synergistic effect of both hormones (ACTH, glucocorticoid) and TNF-alpha. Obtained results indicate strong stimulatory effects of stress hormones on the synthesis of inflammation-related adipokines in adipocytes.

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The effect of prolactin on selected hematological parameters in chicken blood

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A modulatory effect of prolactin (PRL) on the immune system has been shown in mammals and birds and it appears to be highly complex. In vivo the immunoregulatory role of PRL has been the subject of investigation, especially in the conditions of stress and in relation to reproduction. Hematological values (hemoglobin, hematocrit, number of erythrocytes, number and proportions of different forms of leukocytes, including the heterophil : lymphocyte ratio (H : L ratio) are commonly used in birds as the physiological indicators of health/well-being or stress caused by various factors. The present study aimed to examine the influence of the exogenous PRL treatment on the selected hematological parameters in the laying hen (*Gallus domesticus*).

The experiment was performed on Hy-Line hens (n = 16) at the age of 28 weeks, housed individually under a photoperiod of 14L:10D with free access to food and water. Animals were divided into control (injected with saline) and experimental (treated with recombinant chicken PRL at a dose of 500 µg/kg of b.w./0.2 ml) groups. Injection was performed daily for 8 successive days. Just before decapitation, on day 8 of the experiment, the blood was collected from the wing vein into heparinized tube and the values of hematocrit, hemoglobin, total red and white blood cells were estimated. Blood smears were made to calculate the H : L ratio.

It was found that the weight of the spleen in PRL-treated chickens decreased by 25% ($P < 0.05$) compared to the control hens. Lowered values of the hematocrit (by 17%), hemoglobin (by 26%) and total number of red blood cells (by 22%) were also observed in birds injected with PRL. In addition, PRL strongly inhibited ($P < 0.01$) the total number of white blood cells (by 53%) and changed the H : L ratio.

The results obtained indicate that PRL affects the hematological parameters in blood of chickens, which may have a significant impact on health and consequently productivity of birds.

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Behind pea axillary bud outgrowth there is an auxin-mediated competition

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Apical dominance and shoot branching regulation are one of the fundamental developmental processes based on auxin and its interaction with other hormones. Here we provide by auxin efflux inhibitor triiodobenzoic acid, proteosynthesis inhibitor cycloheximide as well as cytokinin and strigolactone treatment on a two-nodal-bud pea model system more insights into this phytohormone crosstalk during axillary bud outgrowth. Auxin canalization and its export into the main stem is crucial for regulation of bud outgrowth. Further, we show that cytokinins and strigolactone influence properties of auxin transport network.

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