Copper and zinc in dogs: impact of sex, age, and diet on serum levels

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The objective of this contribution was to evaluate the impact of sex, age and diet on serum levels of copper and zinc in dogs. Samples were obtained by private veterinarians. Owners of dogs provided information about sex, age and diet, and signed informed consent. Serum was frozen until photometric analysis of copper and zinc. Eighty-one samples were analyzed, 36 from male dogs and 45 from females. Animals were of various ages (0.5–16 years) and breeds. For statistical analysis, normality was tested by Kolmogorov–Smirnov test. Depending on the outcome, either t-test or one-way ANOVA with Student-Newman-Keuls test, or Mann-Whitney U test and Kruskal–Wallis test were used. Serum concentrations of Cu ranged from 1.8 to 8.7 µmol/l (median 4.4 µmol/l). Zinc concentrations were from 4.6 to 22.3 µmol/l (median 9.7 µmol/l). Impact of sex was significant for zinc. Males had median serum level 8.6 µmol/l while females 10.6 µmol/l. Copper was affected by neuter status. Neutered male dogs had lower median (3.6 µmol/l) than intact males (5.4 µmol/l), and both neutered and intact females (4.3 and 4.3 µmol/l, respectively). Age had no impact on Zn. For Cu, young dogs (<2 years) had lower concentrations (median 4.1 µmol/l) than old dogs (>11 years, median 4.8 µmol/l). Diet did not influenced copper, but there was a numerical difference in zinc according to feeding habits. Dogs fed more than 75% of home-made food had median of serum concentrations 8.9 µmol/l, dogs fed combination of home-made and commercial food 9.5 µmol/l and animals fed more than 75% commercial diet 10.3 µmol/l. According to our findings, serum zinc is affected by sex, and there is an ascending trend with larger proportion of commercial food in diet. Copper is affected by age. Also, neuter status has impact on Cu levels in male dogs.

Keywords: **home-made food, commercial food, nutrition, neuter status**

Exploring the pH-triggerable structure of siRNA-carrying liposomal nanoparticles as tools for treatment of hepatitis B

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Gene therapy using small interfering RNA (siRNA) molecules provides silencing of undesirable genes on mRNA translational level, which eliminates potential risks related to interactions with genomic DNA of the cell. However, due to their instability, these siRNA molecules must be delivered to the site of action *via* sophisticated delivery system. In this research work, we opted for liposomal vectors modified with polyethylene glycol chains attached by a pH-sensitive linkage. These liposomal nanoparticles were monitored in different buffer and pH conditions to verify some of their physicochemical properties required for an efficient siRNA delivery. Results from light scattering methods and cryo-transmission electron microscopy revealed that higher ionic strength environment reduced the diameter and zeta potential of the nanoparticles and that mildly acidic pH (6.0) caused structural changes leading to aggregation of the nanoparticles. These findings predetermine their favourable behaviour in biological systems.

Keywords: siRNA therapeutics, lipid nanoparticles, PEGylation, oxime linkage, pH-triggerability

Stallion semen cooling using different types of extenders

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Due to the advantages it offers, artificial insemination is an important part of current horse breeding. One of the limiting factors influencing the use of cooled insemination doses is the decrease in sperm survival over time, which can be influenced by selected extender. In our work, we focused on samples of insemination doses of Warmblood stallions diluted with a skimmed milk-based extender, INRA 96 and BotuSemen Gold extender stored at a temperature of 4 °C for up to 72 hours. Of the selected diluents, the skimmed milk-based extender reached the lowest values of total and progressive motility for all stallions and the lowest values of morphology for two of them ($p < 0.05$). Differences between INRA 96 and BotuSemen Gold were in sperm motility for one of the stallions, with INRA 96 reaching total motility of 53.66% and progressive motility of 17.13%, and BotuSemen Gold reaching 63.71% and 23.01% ($p < 0.05$). For two of the stallions, INRA 96 reached the highest values of sperm viability ($p < 0.05$). The skimmed milk-based extender, the advantages of which lie in its practicality and affordability, achieved lower values of the quality parameters of equine ejaculate than diluents with a chemically defined composition INRA 96 and BotuSemen Gold. There were differences between stallions in used extender. To maintain the best semen parameters, this should be taken into consideration and extenders should be tested for each stallion before the actual distribution of insemination doses to breeders.

Keywords: **stallion, sperm, ejaculate, extender, insemination dose**

Cross-linked-Pd0 polyethyleneimine catalyst for bioorthogonal chemistry

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Bioorthogonal therapy represents a promising tool for controlled pro-drug activation. However, efficient catalysis in the intracellular environment mediated by organometallic compounds still poses a great challenge. Herein, we describe the preparation of a palladium-trapped cross-linked catalyst. Polycationic polymer polyethyleneimine (PEI), an efficient transfection agent, was used as a carrier for palladium catalyst. To prevent palladium leaching and undesired palladium inactivation, PEI polymer with nested palladium catalyst was cross-linked with phosphine compound tetrakis(hydroxymethyl)phosphine chloride (THPC). The catalytic activity of cross-linked-Pd-PEI catalyst was assessed by the activation of a precursor fluorescent probe. The prepared catalyst exhibited catalytic activity in cell-free conditions in a PBS environment, as well as in a cell culture medium supplemented with FBS, which simulated the cellular environment.

Keywords: bioorthogonal chemistry, palladium-mediated catalysis, polyethyleneimine, tetrakis(hydroxymethyl) phosphonium chloride, nanomedicine

Molecularly imprinted polymers as a recognition element for the determination of disease markers

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Molecular imprinting is a very popular technique that allows preparation of molecularly imprinted polymers (MIPs) which have the ability to recognize imprinted molecules (analytes). MIPs are highly selective for the imprinted template and are chemically very stable. In our case, pepsin was used as a template/analyte molecule as it is a marker of gastroesophageal reflux. MIPs were prepared by suspension polymerization using a mixture of functional methacrylate-based monomers. Furthermore, in this work, MIPs were used as recognition entities in the sensor using quartz crystal microbalance.

Keywords: **molecularly imprinted polymers, methacrylic acid, functional monomers, quartz crystal microbalance**

Identification of volatile compounds produced by *Laetiporus sulphureus* using OSMAC cultivation strategy

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Basidiomycete fungus, *Laetiporus sulphureus* was analyzed for production of volatile secondary metabolites under different fermentation conditions, using the OSMAC (One Strain Many Compounds) cultivation strategy. In this study, the OSMAC strategy was based on application of three different types of media and two additional compounds - sawdust and ammonium chloride - that were added individually in the growing media prior the fermentation process. For the determination of volatile organic compounds (VOCs), two dimensional gas chromatography (GC×GC) with mass spectrometry (MS) was used with solid phase micro extraction (SPME) sample preparation (SMPE−GC×GC−MS). After the optimization process, PDMS/DVB fiber (blue) was applied for VOC preconcentration with following conditions: samples conditioning time in vials and fiber exposure to the headspace were set 30 min and 10 min, respectively. We confirmed that one fungal strain can produce a different VOCs profile depending on fermentation environment and we detected unique VOCs produced only in specific fermentation set-ups.

Keywords: *Laetiporus sulphureus*, OSAMC, GCxGC-TOFMS, volatile metabolites, SPME

Associations of *SOST* and *TNFSF11* genes polymorphisms with bone parameters in broilers

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In modern broiler lines, increasing demands are placed on skeletal integrity, where these lines are often characterized by poor calcification and high bone porosity. Bone abnormalities, as disruption of the normal skeletal growth process and homeostasis, are usually complex and lead to increased affinity for bone damage and many bone diseases. Bone quality traits can be improved by genetic tools, given the additive genetic background of these traits. In this study, attention was focused mainly on the search for polymorphisms in selected candidate genes that play an important role in bone metabolism. We studied the associations of single nucleotide polymorphisms in several regions of two genes (*SOST* and TNFSF11) with selected bone parameters (bone breaking strength, length, width, bone mass) in fast (Ross 308) and slow (Hubbard M22BxJA87A) growing broilers, whereas the individual hybrid lines were also compared on the basis of the monitored bone parameters. A total of 48 animals were tested. PCR and sequencing were used to determine the polymorphisms. In selected regions of studied genes, thirteen polymorphisms have been discovered. Five of these polymorphisms were in the introns and three were synonymous. Only one polymorphism was found in selected gene regions in the experimental group of animals, which showed associations with bone breaking strength, but this polymorphism was found in the intron. Other polymorphisms, especially those found in the exon, did not show associations with the observed bone parameters.

Keywords: **polymorphism, broiler, bone, *SOST*, TNFSF11**

Pesticides and long-term denitrification conditions

Kristina Panikova, Zuzana Bilkova, Jitka Mala

Groundwater is an important source of drinking water in many countries. Over the last decade, pesticides have been found at higher concentrations in these waters. This is potentially dangerous for people and nature. For the future, it is very important to understand the behaviour of pesticides under denitrifying conditions, which are typical for groundwaters. The aim of this study was to investigate the behaviour of atrazine, terbuthylazine and tebuconazole under denitrifying conditions during 28 days. These conditions were simulated in a semi-continuous long-term laboratory test with a single dose of the test substance at the beginning of the test. After 28 days, none of the tested pesticides showed an inhibitory effect on denitrification; on the contrary, they had a stimulating effect of up to 10% (terbuthylazine). Biotic loss was only measured in the test with atrazine (9.8%). The dominant mechanism of the loss of all tested pesticides was adsorption (terbuthylazine 68.4%, tebuconazole 82.7%, and atrazine 30.6%). The lowest residual amount of pesticide in water was measured with tebuconazole (17.3%), followed by terbuthylazine (31.6%) and atrazine (59.6%).

Keywords: **groundwater, biodegradation, adsorption, triazine pesticides**

DNA barcoding and metabarcoding in forensic entomology: casuistic and future challenges

Tereza Oleksakova, Vanda Klimesova, Hana Sulakova

Forensic entomology is based on knowledge of species involved in forensic cases. If neither the rearing of early developmental stages nor the determination of species by traditional methods is possible, genetic identification is the only option. The paper describes the case of a dead young child found in a lake with dead insect egg on its body. DNA barcoding was chosen for eggs species identification. The method is based on a segment of approximately 650 bp of a mitochondrial gene encoding cytochrome oxidase subunit I. Sequences were compared to the GenBank BLASTn and BOLD (Barcoding of Life Database) module. Based on the knowledge of the insect species, and the fact that these were only unhatched eggs, it was possible to estimate the colonization interval. The period of colonisation of the dead body by insects was determined to be approximately 8–14 hours. However, if it was a mixture of eggs of several species, DNA barcoding would not have given the necessary results. For this type of sample, DNA metabarcoding and massive parallel sequencing (MPS) can be used. This method would capture the full range of species present in the sample. For example, cases involving illegitimate Traditional Chinese Medicine (TCM) products, containing mixtures and powders of various protected species, could be analysed by this method.

Keywords: DNA Barcoding, DNA Metabarcoding, forensic entomology, species identification, massive parallel sequencing