МІНІСТЕРСТВО ОСВІТИ І НАУКИ УКРАЇНИ
ЛЬВІВСЬКИЙ НАЦІОНАЛЬНИЙ УНІВЕРСИТЕТ ІМЕНІ ІВАНА ФРАНКА
ІНСТИТУТ БІОЛОГІЇ КЛІТИНИ НАЦІОНАЛЬНОЇ АКАДЕМІЇ НАУК УКРАЇНИ
ЗАХІДНИЙ НАУКОВИЙ ЦЕНТР НАН УКРАЇНИ ТА МОН УКРАЇНИ
МІНІСТЕРСТВО НАУКИ І ВИЩОЇ ОСВІТИ ПОЛЬЩІ
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Apparently, this is due to the fact that V and Cr act as insulin-mimics and may increase the sensitivity to the hormone. Insulin, in turn, activates the antioxidant system of the body by increasing the enzymatic molecules synthesis. The obtained results are consistent with other data indicating the normalization of the prooxidant and antioxidant defense system by supplementing the administration of compounds V and Cr to the animals' diet with hyperglycemia (Suzan, 2013; Iskra, 2018). The introduction of V and Cr citrates into the animals' diet may be effective in preventing the violations of the antioxidant system for diabetes.

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PREPARATION OF LYOPHILIZED AMINORIBOFLAVIN USING THE HETEROLOGOUS EXPRESSION OF THE KEY GENE OF AMINORIBOFLAVIN BIOSYNTHESIS *ROS B STREPTOMYCES DAVAWENSIS* IN THE YEAST *KOMAGATAELLA PHAFFII.*

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Roseoflavin and its biosynthetic precursor aminoriboflavin (ARF) are produced by gram-positive bacteria *Streptomyces davawensis* and *Streptomyces cinnabarinus*. ARF shows the strong inhibitory effect against gram-positive bacteria such as *Staphyloccus aureus*, *Bacillus subtilis*, *Bacillus cereus* and *Micrococcus luteus* being non-toxic to mammalian cells. ARF is synthesized from flavin mononucleotide (FMN), which in turn is formed from riboflavin.

The aim of this work was to obtain the preparations of ARF to achieve the heterologous expression of the key gene of ARF biosynthesis *rosB S. davawensis*, encoding 8-dimethyl-8-aminoryboflavin-5'-phosphate synthetase in the yeast engineered strain of *Komagataella phaffii*. Early, in the department of Molecular Genetics and Biotechnology, Institute of Cell Biology, NAS of Ukraine the *rosB* and *FMN1* genes were introduced into the genomes of the yeast strain recipients. The transformed strain *K. phaffii* with pr*TEF1-FMN1*-pr*GAP-rosB* expression cassette resulted in accumulation of new yellow fluorescent compound present in relatively large amounts in the culture medium *K. phaffii* transformants.

Yeast cells of producer were grown in rich medium (YPD) in Erlenmeyer flasks on a gyro-shaker (220 rpm) at 30 °C during 44–48 h. Then, the cells were harvested, washed 3 times with sterile H_2O and incubated during 20h in cheap mineral medium (YNB). The cells were separated from the culture medium by centrifugation (4,000 rpm, 10 min, 4 °C). Total flavins from the culture media were analyzed by paper chromatography in 0.5 % Na_2HPO_4 (pH 8.0) according to retardation value (*Rf*: FMN – 0.50; RF (riboflavin) – 0.37; ARF – 0.10 (Juri N, et al. 1987). Flavin bands were eluted with boiling water and quantified by spectrophotometric analysis at appropriate wavelengths used molecular coefficients of extension (Chapman S, et al. 1999). Then, flavins of culture supernatants were adsorbed on Florisil (60–100 mach) (column 15 × 30 mm) and eluted with gradient of 50 % acetone–H_2O (1 : 1). Fractions containing flavin with Rf 0.10 were pooled and applied to cellulose column (100 × 25 mm) previosly washed with H_2O. Nonadsorbed flavins were eluted by H_2O. Obtained fractions were analyzed by paper chromatography in 0.5 % Na_2HPO_4. Fractions containing only flavin with Rf 0.10 were pooled and analyzed. Spectra analysis of obtained fractions flavins was performed. Spectra of flavins were recorded between 300 and 500 nm employing Hach Lange DR 6000 UV-VIS spectrophotometer. Absorbance peak at 478 nm corresponding to ARF was detected for researched preparation.

To obtain the solid samples of ARF, freeze dryer Alpha 1–2 ldplus A 16/50548, Martin Christ Gefriertrocknungsanlagen GmbH (Germany) was used. The optimal ARF lyophilization conditions, namely, the composition of mixture of stabilizing additives (buffering agents, bulking agents, protein stabilizers, and antimicrobial agents) as well as the parameters (temperature regime and duration) of freeze-drying procedure were found. As a result, the solid preparations of ARF were obtained and characterized.

The possible application of obtained ARF preparations will be discussed.

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BIOCHEMICAL AND MOLECULAR RESPONSES OF PULMONATE MOLLUSK *LYMNAEA STAGNALIS* IN EXPOSURES TO TRACE METALS AND THIOCARBAMATE FUNGICIDE

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The gastropode mollusks are sensitive to different kind of pollution. It was reflected by the impairment of the development, physiological activity and neurotoxicity. However, biochemical responses of snails to potentially toxic substances are studied scant. Pond snail *Lymnaea stagnalis* (Linné, 1758) a secondary-waterlungpond mollusk, is

known to inhabit most polluted biotopes and efficiently accumulate toxic substances from water and sediments. The goal of the study was to assess the biomarkers of effect in *L. stagnalis* exposed to common environmental pollutants.

The snails were exposed to copper (Cu^{2+}, 10 μg·L^{-1}), zinc (Zn^{2+}, 130 μg L^{-1}), cadmium (Cd^{2+}, 15 μg L^{-1}) or thiocarbamate fungicide Tattoo (mixture of propamocarb and mancozeb, 91 μg L^{-1}) during 14 days. The biomarkers of specific responses to certain substances as well as indices of stress and toxicity in digestive gland were analyzed. The results had shown common high vulnerability of responses: the depression of stress response due to the coordinated decrease of superoxide dismutase (Mn-SOD), catalase or glutathione-*S*-transferase and lactate dehydrohenase (LDH) activities, and decreased levels of glutathione (both GSH and GSSG). However, the levels of superoxide anion, products of lipid peroxidation (TBARS) and protein carbonyls were also decreased demonstrating the common oppression of antioxidant-prooxidant system and metabolic response activity. Total metallothionein protein concentration (MT-SH) increased in all exposures (by 1.4–3.6 times), attesting its involving more in stress response than in specific metal-related activities. These manifestations were accompanied by the increase of the caspase-3 activity (by 1.5–10 times) indicating up-regulation of caspase-mediated apoptosis.

Some responses were specific to the type of exposure. The exposures to Zn^{2+} and Cd^{2+} caused accumulation of the excess of these metals in tissue. Metal accumulation in metallothioneins (MT-Me) was increased by Cu^{2+}, Cd and Tattoo. The depletion of cholinesterase (ChE) was specific for Tattoo-exposed group, whereas Cu^{2+} and Cd^{2+} increased its activity. The exposures to Cu^{2+} and Tattoo induced cytochrome P450 dependent oxidation (ethoxiresoruphin*O*-deethylase, EROD), whereas the exposures to Zn^{2+} and Cd^{2+} decreased it. The most expected response of mollusks in exposures to metals Cd^{2+}, Zn^{2+}, Cu^{2+} is the accumulation of metal in tissue of digestive gland. This response was evident for Zn^{2+} and Cd^{2+}. The level of Zn^{2+} in digestive gland increased in all exposures to metals by 2−3 times in opposite to thiocarbamate action. Summarizing, MTs, ChE and EROD reflected the specific effects of contamination, whereas MT-SH, oxidative stress indices, LDH and apoptotic activity demonstrate general depression of defence mechanisms in exposed pond snail. When we compared responses of snail with studied previously responses of bivalve mollusk in the same exposures (Falfushynska et al., 2013), some opposite manifestations were found. It was the elevation of MT-Me, and also ChE and EROD responses. Importantly, the responses of snail were more sensitive and correspondent to the expectations for the biomarkers of exposure.

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THE INFLUENCE OF ESSENTIAL OILS ON THE MICROBIOLOGICAL QUALITY OF POULTRY MEAT

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The purpose of the work was to determine the effect of thyme and basil oils, demonstrating antimicrobial properties against microorganisms grown on microbiological media, on the stability of stored poultry meat. The raw material for research was poultry (breast) purchased in one of the Polish supermarkets. For the analysis media ENDO,TSA, VRBGA were prepared. 12 meat samples of 5 g were taken. Thyme oil was added to 4 samples, and basil oil was added to 4 more. 4 samples were a control sample. Serial dilutions from 5 g meat product were prepared in physiological solution after zero, first, fourth and sixth day of storage and transferred to sterile Petri dishes with selective media: ENDO, TSA, VRBGA. For growth media were incubated for 72 h at 37 ºC. After this time, the number of colonies was counted. In vitro studies confirm the bactericidal and bacteriostatic efficacy of basil and thyme oils. The addition of poultry oils limited the amount of bacteria grown on the dishes, in the case of thyme oil, a decrease in the number of bacteria from the *Enterobacteriaceae* family was also observed.

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CHANGES IN ANTIOXIDANT DEFENSE STATUS OF THE EQUINE BLOOD TREATED *IN VITRO* BY EXTRACT OBTAINED FROM *FICUS RELIGIOSA* L. LEAVES (MORACEAE)

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The interest has been *increased considerably* during *recent* years in finding natural occurring antioxidants for use in foods or medicinal products to replace synthetic antioxidants, which are being restricted due to their adverse reaction such as carcinogenicity (Sylvie et al., 2014). Plants, therefore, constitute the main source of natural antioxidant molecules which have the capacity to eliminate or neutralize the deleterious Reactive oxygen species (ROS)/free