

SESSION 4
TRACE ELEMENTS AND NUTRIGENOMICS

THE WAY AHEAD — FUTURE TRENDS IN TRACE ELEMENT NUTRITION

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During the last fifty years, much new information has accumulated on the functions and requirements of minerals and trace elements in man and animals. Although starvation and under-nutrition are restricted to certain poverty-stricken areas in Africa, Asia and South America, it has become increasingly evident that a marginal deficiency of trace elements such as zinc and selenium is fairly common even in affluent countries. According to recent estimates by the world health organization (WHO) and the food and agriculture organization (FAO), nearly 1/3 of the world's population suffer from symptoms of iron and iodine deficiency. Zinc deficiency is probably of the same magnitude, but unrecognized. Recent supplementation studies in Africa indicate the implication of zinc in human health and disease. Selenium like gold is distributed unevenly throughout the globe and only few human studies have shown the association between selenium status and its relation to specific human diseases. A lack of characteristic symptoms and appropriate diagnostic techniques are the reasons that a marginal deficiency of trace elements is not detected at an early stage. Even when the dietary intake is restricted, body functions are maintained for certain interval by homeostatic mechanisms and by making use of the body reserves. An ideal approach to study the long-term effect of sub clinical deficiency of trace elements such as zinc is to follow vulnerable groups in the general population over extended periods for signs and symptoms of deficiency. Another possibility rests in therapeutic trials. The response to iron supplementation to eliminate iron-deficiency anaemia is a classic example illustrating the impact of trace element supplementation in affected populations. Supplementation of zinc in children with growth retardation in China and elsewhere has recently provided exciting results. Until very sensitive, inexpensive and specific diagnostic tests are readily available for routine use to detect early signs of deficiencies in individuals, prevention must be attempted at the population levels. More needs to be done to supplement and fortify staple foods

with minerals and trace elements where a population is vulnerable. The expense of conducting supplementation studies in developing countries can be relatively low, in line with cheap labour and material costs. Moreover, it is both fairly simple and cost-effective to conduct follow-up studies after supplementation trials for extended periods. Much is known about the action required to assure a food-secure world. A great deal of thought and effort have been made in the past to identify priority action at the individual, household, community, national, regional, and global levels. During the World Food Summit meeting in Rome in 1966, the FAO reaffirmed the following: «It is the right of every person to have access to safe and nutritious food, consistent with the right to adequate food and the fundamental right of every one to be free from hunger». Like the earlier slogan by the WHO «health for all by 2000», nothing has been properly dealt with when it comes to trace element problems in developing countries. Apart from the population explosion and poverty, the fast growing countries such as India and China are forced to cope with serious environmental pollution. With rapid industrial growth, many harmful substances including toxic heavy metals are dumped either on land or in the sea. Acid rainfalls from polluted atmosphere will affect the soil composition which in turn will limit the availability of trace elements to plants and animals. This will ultimately decrease the trace element content in food. Most developing countries are mimicking the industrial revolution of the now affluent countries in a very short time and this is bound to result in the depletion of non-renewable resources, the degradation of the soil and water resources, the emission of gases that threaten climatic stability and the stratospheric ozone layer. These trends ultimately are bound to affect the very survival of Homo sapiens. It is no time for any rhetoric and we need to act, right now! As scientists, it is our duty to advise the international agencies and donor organizations to orchestrate the action plan to get the best results utilizing the available resources.

CHANGES IN MINERAL HOMEOSTASIS UNDER EXCESS PHYSICAL LOAD

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Adaptation to excess physical load was experimentally investigated in male Wistar rats, consumed balanced (Group 1) and mineral-deficient (Group 2) diet. Animals were subjected to physical exercise on treadmill during 8 weeks with gradual intensification of the load. Control group was not subjected to physical load and consumed balanced diet. After the experiment, samples of hair was collected and subjected to multielement ICP-AES/ICP-MS analysis in ANO CBM, Moscow, Russia. It was found that in Group 1 hair content of Ca, Mg became lower by 36.7% and 42.7% respectively while content of Na, P increased 8.4 and 3.6 times respectively. In Group 2 hair Ca became lower by 8.1%; P became increased 3.6 times. Among trace elements, Group 1 was characterized by rise in Cr (2.6 times), Fe (2.5 times), I

(10%) hair content and by decrease in Cu (51.6%), Co (39.5%), Zn (11.6%), Mn (65.4%), Ni (80%), Se (41.7%) content. In Group 2 Cr content rose 2 times, Fe — 4.5 times, Zn — by 17.9%; Cu content decreased by 48.2%, I — by 38.3%, Co — by 43%, Mn — by 56%, Ni — 2 times, Se — by 23%. Among toxic elements, in Group 1 a decrease in Cd (80.4%), Sr (3.2%), Sn (42.6%) levels was detected while content of Pb, Al, Hg showed a tendency to rise. In Group 2 content of Cd, Sr became lower by 78.3% and 84.8% respectively; content of Pb, Al rose 4.8 and 5.4 times respectively. Thus, the obtained results indicate development of diselementosis in rats on the background of high physical load. The most significant changes were observed in Group 1, which consumed deficient diet.

CORRECTION OF ECOLOGY-DEPENDENT DISTURBANCES IN MINERAL METABOLISM BY MINERAL FOOD SUPPLEMENTS

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The aim of the study was to investigate efficiency of correction of the disturbed mineral homeostasis by use of various mineral-containing biologically active food supplements. 84 workers of an industrial plant were divided in 5 groups depending on their diseases, occupational health effects, diet peculiarities and general mineral status. Each group was administrated with biologically active food supplements in ordinary dosage during 3 months. The supplements were: Group 1 — Bio-I (100 µg of I daily as iodine caseinate), Group 2 — Bio-Mg (60 mg of Mg daily as magnesium aspartate with 0,2 g of *Spirulina platensis*), Group 3 — Bio-I + Bio-Zn (15 mg of Zn daily as zinc aspartate with 0,2 g of *Spirulina platensis*), Group 4 — Bio-Zn + Bio-Mg, Group 5 — Bio-I + Bio-Zn + Bio-Mg. After the course of supplementation, 25 chemical elements were determined in

hair analysis of the patients by ICP-AES/ICP-MS methods. A positive effect of the correction was found in all groups, which was reflected in a decrease of toxic elements (Pb, Cd, Hg, As) in the organism and improvement in ratio of essential trace elements to toxic ones. The maximum positive effect was observed in Group 1, where total coefficient of toxic load decreased from 10.0 ± 2.5 to 2.8 ± 0.4 ($p < 0.05$). The only negative effect was a decrease in selenium status in Group 4, which was further diminished by intake of selenium-containing supplements. Also, questionnaires of the patients indicated an improvement of their feeling after correction in 72.5% cases, which reflected in good spirits and increase of working capacity. 70% of the patients noted a decrease in number of acute respiratory diseases per year, 64% noted improvement in condition of skin, hair, nails.

THE INFLUENCE OF MODERNIZATION OF COOKING TECHNOLOGY ON MICRONUTRIENTS PROVISION OF SCHOOLCHILDREN

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BACKGROUND: The micronutrient deficiency is widespread in Russian schoolchildren. This is why in 2008 the federal pilot project on improvement of the nutrition in schools in 20 regions of Russia started. **AIM:** To assess the effect of changes in technology of cooking on elemental and vitamin status of schoolchildren in Orenburg region. **METHODS:** Totally the nutritional status of 4000 schoolchildren by routine methods, hair and urine multi-element content (100 cases) by ICP-AES in ICP-MS and urinary vitamins concentration (50 cases) by HPLC in Centre of Biotic Medicine, Moscow before and 1 year after start of project were provided. **RESULTS:** Preliminary investigation of nutritional status the significant number of undernourished children with reduced functions of CNS and cardiovascular systems with simultaneously determination of suboptimal consuming of Ca, I, Zn,

Se, Co, B on daily food. In hair of school the elevated Mg, Fe, Si, Pb, Al and liveliest and decreased Se were found. For schoolgirls the elevated hair Fe, Na and decreased Ca, Se were typical. The profound B₁, C and moderate B₂, B₆, PP vitamins urinary excretion was detected. After 1 year of experiment the nutritional status and physical conditions of children were moderately improved. The hair data on Mg, Na, K have a tendency to normalization and elevation of Zn and Cu hair levels the improvement of provision reflected. Also, the urinary excretion of B₆, PP and C vitamins was increased, especially in schoolgirls. **CONCLUSIONS:** Modernization of cooking technologies and significantly improves the micronutrient status in schoolchildren.

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TOXIC AND NUTRITIONAL ELEMENTS IN RICE AND BEANS PRODUCED IN BRAZIL

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Rice and beans are an inseparable pair of staple foods for millions of people in Latin America and Caribbean. They form the core of the Brazilian diet eaten by all social classes in all regions of the nation supplying most of the essential nutritional factors. Rice and beans are protein-rich and contain vitamin B and a good amount of necessary minerals, and the consumption of the two together provides all the essential amino acids. Despite the importance of these nutritional properties, few data exist on chemical elements in rice and beans produced in the country, mainly concerning trace elements. In this context, here the chemical composition was evaluated by instrumental neutron activation analysis (INAA). A total of 39 samples of different varieties of rice and beans were collected in the local market of Piracicaba, state of São Paulo. The samples were oven-dried at 60°C to constant mass and ground in a titanium rotor mill. Portions of 300 mg were weighed into high purity poly-

ethylene vials and irradiated during 8 h at a thermal neutron flux of $5 \times 10^{12} \text{ cm}^{-2} \text{ s}^{-1}$ in the nuclear research reactor of IPEN/CNEN. For quality control, certified reference materials SRM 1573 Tomato Leaves and SRM 1515 Apple Leaves were included in the analytical series. The induced radioactivity was measured by high resolution gamma-ray spectrometry. Concentrations and respective uncertainties were assessed by the k_0 -method for fifteen chemical elements, i.e. As, Br, Ca, Cd, Co, Cr, Fe, Hg, K, La, Na, Rb, Sc, Se and Zn. High contents of Fe and Zn were found in both grains. Beans presented high contents of Se especially in the white varieties. The contents of As in the rice samples were below the maximum allowed limit of 1 mg/kg established by FAO. Mercury was below the detection limit in all samples analyzed. Therefore, the pair rice and beans consumed on a daily basis by the Brazilian population is definitely an important safe source of nutritional elements in the diet.

EFFECTS OF BRAZIL NUT INTAKE ON SELENIUM STATUS, GPx ACTIVITY AND DNA DAMAGE — RELATIONS WITH THE GPx1 PRO198/LEU POLYMORPHISM

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Alterations in nucleotides sequence may promote changes in codified amino acids and may affect the protein structure and functions. Some studies show that the GPx1P198L polymorphism is associated to lower blood Se levels and GPx activity. However, there are no reports on the effects of the Se supplementation regarding those biomarkers. Brazil nuts are recognized as a bioavailable Se food source. The aims were to determine the Se status and the levels of total erythrocyte GPx activity, and to evaluate the associations of the polymorphism with Se status and DNA damage in morbid obese women, before and after Brazil nuts intake. 37 women without known diseases have participated in the study. The Se levels were evaluated through HGQTAAS; GPx activity, through a commercial kit, and DNA strand breakage, through the comet assay. Fragments produced with flanking primers were sequenced. One Brazil nut provided ~290 µg of Se. 100% of the individuals presented Se deficiency in T0. The maximum GPx activity level was not reached by 97.3% of them. The intake of one

Brazil nut for 2 months did increase the plasma ($152 \pm 92\%$) and erythrocyte ($278 \pm 175\%$) Se concentrations, as well as the GPx activity ($66 \pm 75\%$). There were no differences among those biomarkers regarding the genotypes in both phases. However, there was a correlation between erythrocyte Se levels and GPx activity only in the P/P group. Individuals with the wild genotype presented a reduction in DNA damage levels in T1, which did not happen in those variant alleles carriers. Those who were L/L presented damage levels significantly higher than those P/P. In conclusion, Brazil nuts efficiently improve Se status and GPx activity regardless of genotype. Nevertheless, individuals carrying variant alleles seem to have an altered response to supplementation. Moreover, the higher DNA damage levels observed in those L/L subjects after supplementation may influence the risks for non-transmissible chronic diseases.

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THE EFFECTS OF DI-(2-ETHYLHEXYL) PHTHALATE ON THYROIDAL OXIDANT/ANTIOXIDANT STATUS IN SELENIUM-DEFICIENT AND SELENIUM-SUPPLEMENTED RATS

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Di (2-ethylhexyl) phthalate (DEHP) is the common plasticizer of the PVC plastics that are widely used in consumer products, food packaging and medical devices. Studies investigating the association between exposure to phthalates and thyroid function are limited. In animal studies, diets contaminated with DEHP were reported to cause thyroid alterations and low plasma thyroxin (T_4) concentrations compared to controls. The mechanism(s) underlying these effects are not fully elucidated. Taking into account the frequency of inadequate selenium (Se) intakes, its essentiality and antioxidant role in thyroid structure and functions, this study was designed to evaluate the effects of DEHP on oxidant/antioxidant status of thyroid in Se-deficient and Se-supplemented rats. Se-deficiency was produced by feeding 3-week old Sprague-Dawley rats with ≤ 0.05 mg Se/kg containing diet for 5 weeks, and the supplementation group was on 1mg Se/kg diet. DEHP treated groups received the com-

ound at 1g/kg dose by gavage during the last 10 days of feeding period. DEHP exposure increased significantly thyroidal superoxide dismutase (SOD) activity and level of lipid peroxidation (LP) measured as thiobarbituric acid reactive substances (TBARS); but did not change the glutathione peroxidase (GPx1), thioredoxin reductase (TrxR) and catalase (CAT) activities. With Se-supplementation none of the parameters changed, except for a significant increase in GPx1 activity. Se-deficiency decreased the GPx1, CAT and TrxR activities and increased LP. In Se-supplemented rats DEHP exposure did not change any of the parameters measured. However, DEHP exposure in Se-deficient rats increased SOD activity and LP level further. These results suggest that DEHP may affect the oxidant/antioxidant balance within the thyroid particularly in Se-deficiency, but other mechanisms may also have roles in the alterations of thyroid functions with phthalate exposure.

PECULIARITIES OF MINERALS IN IRANIAN DAILY DIETS]

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In this article, some of the main aspects of a comprehensive study on trace elemental content of a few typical daily diets of Iranians, along with the same study in a few more countries under conduction of International Atomic Energy (IAEA) are going to be presented. The actual purpose was to work out the intakes of important nutritionally trace elements via daily diets by certain study groups and/or by a reference man. This work was completed internationally till 1999, but it could be only accomplished locally very recently. The objective of this recent study was not only a review over the detailed work for 24 nutritionally important minerals measurement, but to look at the peculiarities such as interrelation, bioavailability, interaction between and among trace elements (essential, toxic, radioactive ones) plus carrying out some sort of crosschecking. Also energy intakes in mg(s) of trace elements per MJ per head are presented. In this recent work a few further study groups from Iranian were added to previous groups with the same objectives and to increase/verify the reliability of applied methodologies. The diets prepared by recording die-

tary regimes (RD), duplicate diets (DD) and market basketing (MB). Methods for elemental measurement were mostly NNA, ICP, AAS and for the measurement of some other parameters such as phytate, fibre and energy, the appropriate proven chemical methods were applied. Since the results are very plenty, therefore apart from briefing them in a few tables, the attempt is presenting them in forms of various and appropriate figures individually and all together where it is needed to do so. The results in compare with other countries and Recommended Daily Allowances (RDA) generally are fair but no doubt there are some deficiencies in I, Se, Fe, Zn in a few Iranian study groups and also being high or in rather borderline exposure of a few toxic trace elements in other study groups. There should be very much discussion about the bioavailability, interrelation among the most of trace elements such as Zn, Fe in presence excess amount of phytate and Ca, meantime from other hand some interaction and imbalances among essential, toxic and counterpart radio elements are observed which all are taken into consideration.

ZINC ABSORBANCE INHIBITOR IN IRANIAN STUDY GROUPS

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Bioavailability and/or homeostasis of some essential trace elements such as zinc, iron etc., in the presence of phytate, fibre and calcium are subject to alteration. These factors were measured in this study for Iranian diets in a frame of a Coordinated Research Project (CRP) for a few study groups. However, the most prominent dietary factor as an inhibitor in this regard is phytate. The phytate effect on zinc homeostasis is a chemical phenomenon dependent physiologically on pH in the gastrointestinal tract at or near the sites of absorption. Calcium is a synergistic co-precipitating factor in the complexation of zinc by phytate. Fibre has also a tendency to absorb insoluble compounds in gastrointestinal tract including zinc, iron and many other trace elements. One of the most known clinical observations regarding zinc deficiency was found in the rural area of the Fars province of Iran in the late 1950s at Shiraz University. The phytate con-

tent of the study groups diet was sufficient to affect the zinc absorption/reabsorption adversely. In hotter climates, sweat losses may also be significant and must be replaced via dietary intake. Also when calcium content of dietary intake is increasing, then, the effect of phytate is more severe toward affecting zinc homeostasis. These effects are not necessarily parallel but are synergistic in which case, the condition is more severe for zinc homeostasis. Though there is no certain mathematical relation between fibre content and other essential trace elements e.g. iron, zinc as yet, but the most experimental work shows that fibre may have an adverse effect on the absorption of essential elements. However the molar ratio of [Pa]:[Zn] and [Ca] x [Pa]:[Zn] in Iranian Diets in recent study are from 7–17 and 150–800 respectively. The critical ratios of [Pa]:[Zn] of 10 or less will provide adequate zinc to sustain homeostasis.

THE PROPOSED LEVEL OF FOODSTUFFS ENRICHMENT WITH MINERALS AND VITAMINS

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Additional enrichment of mass foodstuffs with vitamins and minerals is one of the ways of micronutrients deficiency liquidation. The fortified products must be simultaneously effective for compensation of existing micronutrient deficiency and thus remain safe for the health (have no risk to public health). On the basis of the analysis of the available in world practice documents regulating the process of foodstuffs enrichment with micronutrients, existing assortment of enriched foodstuffs and models of an estimation of maximal possible micronutrients intake from such products the following levels of minerals and vitamins fortification have been offered to establish. Vitamins and minerals must be present at a level that would contribute at least 15% of the Recommended Dietary Intake (RDI) and would not exceed 50% of the RDI per serving portion. The proposed size of the portion is 100 g of the wheat flour, 150 g of bread or bakery products (from wheat flour and rye-wheat flour), 200

ml of liquid dairy products or soya milk etc., 100 g solid and paste-like dairy products and protein products (tofu), 300 ml of soft drinks and juices from fruits (including berries) and (or) vegetables, 20 g of oils, spread or sauces, 50 g of cereal products (ready for eating breakfasts, extruded products, macaroni and cereal products for fast food). The setting content of vitamins and minerals in high energy enriched foods should be expressed per 100 kcal. The quantity of vitamins and minerals corresponding 15–50% of RDI should be contained in one portion of sugary or flour confectionery products (20–25 g), hard cheeses (30 g), canned foods and vegetable, fruit or berry concentrates (30 g) that corresponds to energy value of 100 kcal. Such degree of foods enrichment will allow to increase essentially diet micronutrient density, to reach RDI and to liquidate existing micronutrients deficiency at regular consumption of fortified products, remaining thus safe for public health.

BIOAVAILABILITY OF MACRO- AND TRACE ELEMENTS FROM DIETS WITH DIFFERENT LEVEL OF METABOLIC ENERGY

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INTRODUCTION: The optimal mineral balance in the body permits for organism to use energy effectively and do not reserve it as fat depositions. **AIM:** This study was dedicated to the investigation of metabolism of chemical elements in the poultry on the ground of enlargement of available energy content in the diet. **METHODS:** 90 of 12 days broilers (cross «Smena-7») Russia were investigated. In Gr. 1 broilers consumed diet with 11.0 MJ/kg, Gr. 2 and Gr. 3 were fed with energy 10% and 20% surplus, respectively. Routine physiological studies and ICP-AES/ICP-MS determination of 24 macro- and trace elements by Optima 2000 DV and ELAN 9000 in laboratory of Centre for Biotic Medicine, Mos-

cow, were provided. **RESULTS:** Balance investigation demonstrated, that the concentration of metabolic energy in Gr. 1 diet was 11.8 MJ/kg dry weight, in Gr. 2 and Gr. 3 it was 13.2 and 14.6 MJ/kg, respectively. We found, that the rate of accumulation of As, Cr, Ni was elevated and in case of Zn it was decreased, in dependence from the metabolic energy level increasing. The similar data concerning conversion of macro- and trace elements from diet to body of broiler were obtained. It was hypothesis that concentration of energy in diet correlated with metabolism of Cr and Ni, on glucose and insulin metabolism in the body. Role of Zn in energy metabolism also discussed.

VIEW ON SODIUM INTAKE FROM MEAT PRODUCTS IN ITALY

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Nutritional recommendations to lower sodium intake have been formulated in several industrialized countries. Outcome of surveys support the view that excessive sodium intake increased the risk of coronary heart diseases. The recommended sodium daily intake in Italy ranges from 0.57 to 3.5 g/day, with studies suggesting a dietary sodium restriction for most adult population. The majority of salt in the diet comes from processed foods. The main sources of not discretionary sodium in the Italian total diet are «Cereals» and «Meat and Meat Products» food-groups which contribute for 18 and 13%, respectively, to the total sodium intake. Meat products (salami, ham) consumption in Italy represents 20% of the total meat consumption (131 g/person/day). Today the reduction of salt to meat products has become a very relevant issue for meat industry. Meat products are typical items of Italian culinary tradition which show a great variety of products. Meat products greatly contribute to the total daily intakes of nutrients, especially micronutri-

ents including trace elements and B vitamins, however they are also a vehicle of additional sodium in the diet. This project aimed at a systematic study on sodium content in different type of salami (mortadella, salami, cotechino, zampone) and ham among the most consumed in Italy, some of these products have got the European PDO (Protected Denomination of Origin) certification. Meat products were analysed for their sodium content by atomic spectroscopy (ICP) to quantify the intrinsic sodium content and by the volumetric method of Volhard to quantify the sodium added to meat products as preservative. Our findings have allowed us to discriminate the salt coming from technological processing and pointed out a reduction in salt added more than 12% in all products analyzed. Data were compared to those coming from a survey carried out in 1993.

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INFLUENCE OF ENZYMATIC PREPARATIONS ON ELEMENTAL STATUS OF CARP AND HEN

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The using of enzymatic preparations (EP) on feeding of animals can change their elemental status and affects the quality of food. AIM: To investigate the effect of different enzymatic complexes in diet on the efficiency and content of macro- and trace elements in carps and hens. METHODS: In feeding of carps and hens the different EP were used: MEK-TSGAP-Russia, Avizym 1100, 1200, Celloviridin G 20x, Rovabio TM Exssel AP, Amylosubtillin G 3x. The complex of morphological and laboratory tests, including ICP-MS determination of multielement content of biological samples in CBM laboratory (Moscow) was provided. RESULTS: In general, the direct correlation between the elemental content of diet and animal body composition was evident. The diet with elevated starch and decreased protein content leads

to increasing of fat and decreasing of Zn content in muscles of experimental animals. The decreasing of toxic metals (Pb, Cd, Al) level in muscular tissue of animals was typical for groups, supplemented by all EP, especially, with glucanase-cellulase activity, in hens (Patent RF № 2270580, 2006). The feeding of hens by preparation with glucanase-cellulase activity leads to decreasing of Fe content in eggs (10–15%) and chicken (13–27%). The using of complex with amylolytic enzymes leads to decreased of Zn content. CONCLUSION: The content and quality of foods stuffs and using of EP in feeding of carps and hens influence on the elemental content of muscular tissues. In a result the ready products are more safe (with decreased Pb, Cd, Al levels), but also more poor in essential TE (Fe, Zn).

EFFECT OF THE ADDITIONAL FORTIFICATION OF BROILERS, DIET BY MO, CR, NI SALTS ON LIVER MORPHOLOGY

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The optimization of feeding of agricultural animals is an important task for food industry. The aim of this study was to investigate the influence of fortification of broilers by Mo, Cr and Ni inorganic salts on the morphological picture of liver as main metabolic organ of the body. Totally four groups of broilers (cross «Smena-7»; n = 30 in each) were involved in the experiment. Gr. 1 included control animals, Gr. 2 was fed by diet, enriched in MoO₃ (0.40 mg Mo per capita daily, depending on the amount of eaten food), Gr. 3 — Cr₂(SO₄)₃ (0.13 mg Cr per capita daily) and NiSO₄ (0.46 mg Ni per capita daily). In Gr. 4 the MoO₃ (0.39 mg Mo per capita daily), Cr₂(SO₄)₃ (0.14 mg Cr per capita daily) and NiSO₄ (0.47 mg Ni per capita daily) were added regularly to diet. Routine histological investigation and determination by

ICP-MS (laboratory of Centre for Biotic Medicine, Moscow) were provided. In results of our experimental work have shown the tendency to improvement of blood circulation in Gr. 2, but in Gr. 2 and especially in Gr. 3 the positive effects on morphofunctional status of liver were significant (enlargement of diameter and surface of main blood vessels, square of hepatocytes (1.7 as much as in controls, p ≤ 0.01) and its parameters (diameters of nuclei 2 times increased, p ≤ 0.01). In conclusion, our work demonstrated the beneficial effects on broilers liver morphofunctional activity of additional fortification of diet by Mo, Cr and Ni, and especially in case of its simultaneous administration. It intensified the utilization of food, increased the metabolic processes and improved the quality of chicken.

EFFECTS OF CHELATED TRACE ELEMENTS IN SWINE FEEDING

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The trials have been carried out in order to investigate the effects of chelated trace elements in swine feeding on growth and slaughtering parameters (first trial) and on minerals' digestibility (second trial). In both experiments the following dietary treatments were compared: a «Control» diet containing a conventional vitamin-trace element premix included at the recommended commercial dosage and resulting in an inorganic mineral supply exceeding the amounts suggested by NRC (1998) to fulfil the mineral requirements of swine (+50% for Fe and Zn and +300% for Cu and Mn, respectively); a «Chelated» group in which minerals were entirely administered under chelated form and according to NRC (1998) recommended dosages; and a third group in which minerals were administered according to NRC recommendations (1998) and supplied half in chelated form and half in inorganic form («Mix» group). The growth and slaughtering parameters (average daily

weight gain, feed conversion rate, dressing out percentage and lean meat yield), recorded on 54 pigs (3 groups each containing 3 replications of 6 pigs) in the body weight range from 30-to-160 kg, did not differ among the groups. The second experiment (Digestibility trial) was carried out by means of digestibility cages. Four pigs receiving the above described dietary treatments were allotted to a Latin square design. Outcomes of the digestibility trial indicated that the administration of minerals in the «mixed» form (half supplied in the chelated form and half in the inorganic form) resulted in a significantly (P < 0.05) higher digestibility of Copper and Zinc when compared to the Control diet. Tacking into account the high polluting power of Copper and Zinc, results from the present trials could be of great relevance in the framework of sustainable animal production with particular regard to the reduction of the environmental impact of swine manure.

TRACE ELEMENTS IN PEQUI (*CARYOCAR BRASILIENSE* CAMB.): A TROPICAL FRUIT OF NUTRACEUTICAL PROPERTIES

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Pequi (*Caryocar brasiliense* Camb.) is a native species from Brazilian Cerrado playing an important social and economic role as source of income and food for the population of this biome. The oil extracted from pequi is rich in vitamins A, B and C, with high concentrations of palmitic, oleic and linoleic acids, which attracts increasing interest from industries for production of cosmetics, biodiesel, drugs and foods. The pequi pulp has been patented as a new nutraceutical product for its antimutagenic, antioxidant and vitaminic properties [1]. Since the species is still untamed, the knowledge of its chemical composition and nutritional needs is a basic requirement for proper management in areas where it is at extinction risk. Therefore reliable information on trace elements is a valuable tool particularly for establishing soil-leaf-pulp ratios. This study focused at quantifying essential and toxic elements in these compartments by instrumental neutron activation analysis (INAA), recognized as a primary method by the

Consultative Committee for Amount of Substance (CCQM). Samples from 20 pequi trees and soil underneath were collected in Japonvar city, a traditional pequi producer in the Cerrado of Minas Gerais state. Ba, Br, Ca, Ce, Co, Cr, Cs, Fe, K, La, Na, Rb, Sc, Sm, Sr, Th and Zn were measured in the samples. The average amount of most trace elements was found to be comparable to those of other tropical fruits with high nutritional values [2,3].

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THE INVESTIGATION OF THE ACCUMULATION OF VANADIUM AND LITHIUM BY *SPIRULINA PLATENSIS* AND *SPIRULINA MAXIMA* CYANOBACTERIA'S CELLS

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Search of new compounds of vanadium possessing high hypoglycemic activity and low toxicity is carrying on in Canada, USA, Japan. Lithium compounds, as is well known, actively apply to treatment of psycho neurological diseases. The principal aim was to determine optimum concentrations of V and Li, added to nutrient medium. In that case contents of V and Li in cells are high and at the same time the rate of growth of cyanobacteria does not reduce.

Both cultures were cultivated in Zaruka medium, in constant lighting (1100–1300 lux) and temperature 26–28°C within 21 days. The biomass was defined by nephelometric method. The compounds of V and Li, in the form of $\text{VOSO}_4 \cdot 3\text{H}_2\text{O}$ and $\text{Li}_2\text{SO}_4 \cdot \text{H}_2\text{O}$, were added to medium in various concentrations on

the 7-th day of their growth. The contents of V in cells of *S. platensis* and *S. maxima* were defined by method of thermal-electric atomic absorptive spectroscopy. The determination of lithium was carried out by method of flame atomic absorptive spectroscopy. The analysis of macro- and trace-elemental composition of cells of cyanobacterium *S. platensis* and *S. maxima* (Na, K, Mg, Ca, Fe, Mn, P, Cu, Mo, Zn, B, Cr, V, Li) was fulfilled on atomic emissive spectrometer on polychromator JCAP-9000.

It were defined the optimum concentrations of V and Li added to nutrient medium, for *S. platensis* it came to 0.75 g/l vanadium in medium and for *S. maxima* it was 1.0 g/l (accumulation of V in cells was 600 ppm and 912 ppm correspondingly). In the increasing

of concentrations of vanadium in medium, its contents in cells are rising proportionally until cells died. The rate of vanadium accumulation was higher in *S. maxima*, than in *S. platensis*. For example, when concentration of vanadium in medium was 0.25g/l, accumulation of vanadium in *S. maxima* was 230 ppm, in *S. platensis* — 125 ppm. The noticeable reduction of biomass quantity for *Spirulina maxima* was in medium with 1.25 g/l of V, for *S. platensis* — 1.0 g/l. *S. maxima* didn't grow in medium with 3.5 g/l of V, destructive concentration of V was 3.0 g/l for *S. platensis*.

Optimum concentration of Li for *S. maxima* was 0.25g/l, for *S. platensis* -0.75g/l (accumulation of Li in cells was 900 ppm and 750 ppm correspondingly). Maximum accumulation of Li in cells of *S. maxima* was revealed when the concentration of Li in medium was 0.25 g/l; maximum accumulation for *S. platensis* was noticed at concentration 1.0 g/l of Li. In increasing of

lithium content in medium to 2.5g/l its concentration in cells didn't grow practically. It is obvious that *S. maxima* include Li sooner and in larger quantity than *S. platensis*.

Heightened concentration of V in medium caused the similar alterations in micro- and macroelemental composition of both cultures, the contents of Zn, Mn, Cr, B-were increasing, concentrations of Ca, Fe, Mg in cells — decreasing at the same time. As far as lithium is concerned, its presence caused the increasing of Ca, Fe, Mg, Mn contents, the concentration of B increased slightly.

In such a way, the accumulation of V and Li by *S. platensis* and *S. maxima* was investigated, the optimal concentrations of these elements in nutrient medium were determined. It was shown, that *S. maxima* more tolerant to addition of V and Li and it accumulate these elements better than *S. platensis*.

COMPARISON OF THE SELENIUM CONTENT OF ORGANIC AND NON-ORGANIC MILK

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BACKGROUND: Demand for organic milk and dairy products is growing in the UK owing to their perceived health benefits. Selenium status in the UK is considered to be marginal and milk represents a significant source of selenium in the UK diet providing some 12% of selenium intake. It is therefore important to have accurate data on the content of selenium in milks from different parts of the country and on any differences in concentration between organic and non-organic milks. This study therefore aimed to evaluate any differences in selenium concentrations in the two milk sources throughout the UK. **METHODS:** Samples of supermarket own-brand organic and own-brand non-organic semi-skimmed milk were collected between June and August 2009. Milk was purchased from 14 counties in the South of England, one in Wales and one in Northern Ireland.

In addition, three separate samples of each of the major brands of organic milk were collected. Samples 3.0 g were prepared for analysis by addition of 2.0 mL nitric acid and digestion with microwave heating. Reagent blank and certified reference materials were prepared in the same way. Solutions were made to 10.0 mL with deionised water. Concentrations of selenium were measured by ICP-QMS in collision cell mode, after 1:50 dilution with a solution containing 0.5% v/v nitric acid and 100 µg/L Ge internal standard. **RESULTS:** Preliminary results show little variation in the concentrations of selenium in milk collected from different parts of the country or from different supermarket chains. Some differences between organic semi-skimmed milk and the paired non-organic semi-skimmed milk were found but there was no consistent pattern.

EFFECT OF THE DIETARY OREGANO (*ORIGANUM VULGARE*) ON COPPER AND ZINC BALANCE IN WEANED PIGLETS

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A 4-week study conducted on 20 weaned piglets (average initial weight 15 kg) evaluated the effects of the dietary oregano (*Origanum vulgare*) used in the presence/absence of phytase on Cu and Zn balance, while reducing/eliminating their inclusion in the diet as inorganic salts. Oregano was harvested from the wild flora and the Cu and Zn concentrations that were taken into consideration (9.85 ppm and 53.31 ppm, respectively) were the consensus values obtained in an interlaboratory study. The piglets were assigned to 4 groups (C, E1, E2, E3), housed in individual metabolic cages and fed on corn-soybean meal-based diets. The diet of the control group (C) with addition of 1% inorganic mineral premix (MP), contained: 40.92 ppm Cu, 144.96 ppm Zn. The experimental diets differed from C diet as follows: E1 – 3% oregano, 0% phytase (5000 PU/g), 0% MP; E2 – 3% oregano, 0.01% phytase, 0% MP; E3 – 3% oregano, 0% phytase, 0.5% MP, E4 – 3% oregano, 0.01% phytase, 0.5% premix. Groups E1, E2, E3 and E4 in-

cluded in the diet 0.5% Zn of the MP, because the dietary oregano amount didn't meet the requirements (NRC) for piglets. The balance was performed for 5 days every week. The Cu and Zn were determined by FAAS in the samples (weekly samples/piglet) of ingesta, faeces and urine. It was noticed that although the dietary Cu ingested by the groups without MP was 75% (10.08 ppm) lower than C, the absorption coefficients were only 47% (28.83) lower than for group C (54.22%), while in the groups with 0.5% MP, the absorption was just 10% (48.86%) lower than for group C. For Zn, where the amount ingested by the experimental groups was 33% (97.62 ppm) lower than for group C, the absorption coefficients were just 20% (46.3%) lower than for group C (57.64%). No significant differences were noticed for Cu and Zn in terms of apparent absorption, between the groups with/without phytase. The deposits of Cu and Zn in the main organs and serum (from slaughtered piglets) have been also evaluated.

WATER AS A «COMPLEX MINERAL»: TRACE ELEMENTS, ISOTOPES AND THE PROBLEM OF INCOMING MINERAL ELEMENTS WITH DRINKING WATER

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It is well known the influence of trace elements of drinking water on health and functional reserves of the organism. For example, reports on the relationship between the elemental homeostasis and indicators of the immune system of the human body, the imbalance of mineral elements homeostasis in populations consuming water with elevated levels of total mineralization. However, the consumption of air-conditioned water or distilled water may lead to shortages of mineral elements. The main source of trace elements in natural waters is aluminosilicate nanoparticles, which can reach the tissues and organs (including brain) without the destruction. That is an example of a natural way directional transport of trace elements. The objects of study: bottled water total mineralization up to 1 per litre, deuterium depleted water. Methods: Low Angle Laser Light Scattering (LLALS), graphite Zeeman AAS, biological testing of

Spirostomum ambiguum. Studying the structure and properties of water shows that water does not consist only molecules of H₂O, but there is a «mineral», including dissolved gases, salts, humic acids, nanoparticles, containing occluded or adsorbed form of mineral nutrients, hydronium ions (H₃O⁺, H₂OD⁺), hydroxide ions (OH⁻, OD⁻), hydrated electron e_{aq}⁻, free radical compounds (OH⁻, O₂⁻, OH), etc. Charged particle and free radical particle, nanoparticles, constantly present in the water, can initiate the assembly of molecular clusters into the giant density heterogeneity of water. We have shown that the concentration of giant clusters of water depends on the concentration of deuterium into the water, which can not be viewed solely as an impurity isotope. Thus, the combination of mineral and isotopic elements in the water determines the quality and stabilizing the structure of water and is an essential factor in mineral nutrition.

SELENIUM IN SERUM, TOENAILS AND DIET IN AN ITALIAN POPULATION

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BACKGROUND: Selenium is a trace element of strong toxicological and nutritional interest, and its deficiency and excess have been involved in the aetiology of several human diseases. Uncertainties exist about current selenium status in the Italian population and the optimal methodologies to be used to assess intake of this metalloid in epidemiologic studies. **APPLIED TECHNIQUES:** We analyzed in 56 randomly selected residents in Modena, a municipality of northern Italy, the selenium concentrations in serum and in toenails and the dietary intake of the metalloid. We also investigated the correlations between these indicators of selenium exposure. Serum and toenail selenium levels were determined using atomic absorption spectrometry with Zeeman background correction and neutron activation analysis, respectively, and dietary intake was assessed using a semiquantitative food frequency questionnaire and a specifically developed database of selenium content in foods. Correla-

tion analysis was carried out by calculating Spearman correlation coefficient. **IMPORTANT RESULTS:** Mean serum selenium concentration was 85.9 $\mu\text{g/l}$, with a median value of 89.2 and a standard deviation of 21.7. Average levels were higher in females (89.7) than in males (81.2). Mean toenail selenium was 0.65 $\mu\text{g/g}$, with higher values in females (0.68) than in males (0.61). Dietary selenium intake averaged 59.4 $\mu\text{g/day}$ (standard deviation 19.7), with slightly higher figures for males (60.1) than for females (58.8). We found an association of serum selenium concentrations with toenail levels of the metalloid ($P = 0.045$) but not with dietary intake ($P = 0.285$). Despite the limitations of the study due to the small sample size, these observations clearly indicate the complexity of assessing selenium status, suggesting an association between biological indicators of exposure such as serum and toenail selenium content, and little relation between dietary intake and biomarkers.

ELEMENTAL STATUS OF MARROW IN BROILERS AT DIFFERENT FEEDING RATE

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Trace elements play an important role in animal diet. Trace element inadequacy of feeding decreases animal productivity, egg-laying quality of hens, and causes various diseases. In this connection, influence of diets with different content of trace elements on elemental status of broilers' marrow was investigated. There were composed 5 groups of broilers: a control (sufficient diet) and 4 experimental groups with diets differing in content of minerals, digestible protein and energy. Marrow samples were taken from broilers on the 10th, 20th, 30th, 40th and 50th day of the experiment. Content of chemical elements in marrow was deter-

mined by ICP-AES/ICP-MS method in ANO «Centre for Biotic Medicine». It was found that marrow elemental status of control broilers changed in the course of the experiment for Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Na, Ni, Pb, Sr, V, Zn ($p < 0,05$), while content of Mn, I, P, Se, Si, Sn did not change. On the 50th day of the experiment only content of Li, Sn in marrow of broilers from control and experimental groups did not statistically differ ($p > 0,1$), while for other investigated elements significant differences ($p < 0,05$) were observed. Thus, feeding conditions have an influence on elemental status of marrow in broilers.