The concentration of global crop and food animal production in regions where plant selenium content is low has led to a decline in the amount of selenium in the human food supply.
Selenium cycles through food systems, first by being removed from soils by plants and microorganisms which can take up the element into their tissue proteins and convert some of it to volatile metabolites that enter the atmosphere ultimately to be brought down with precipitation and airborne particulates (Allaway et al., 1964). Mobilization of selenium from soils is influenced by soil pH: alkaline conditions favor the conversion of inorganic selenium to selenate (Se\textsuperscript{6+}) which is not fixed in the soils, thus being more available to plants. Acidic conditions favor selenite (Se\textsuperscript{4+}) which adsorbs to clays and is strongly fixed by iron hydroxides, in such way being unavailable to plants. Selenium content in agricultural soils in Serbia is average 200 μg/kg (Maksimović, 1990).

Selenium content in plants varies according to the amounts of the element available in the soils. Availability of soil selenium to crops can be affected by such soil management procedures as irrigation, aeration and fertilization (Gisself-Nielsen, 1974). Grains and forages grown on agricultural soils in Serbia have variable amounts of this essential microelement. Selenium content in cereals grown in central Serbia (Jovanović et al., 1998), determined in over one hundred samples, is 19.61 μg/kg. Of all examined samples 92% had a concentration below 50 μg/kg. Thus, in accordance with the classification given by Kubota (1967) defining Serbia as a selenium deficient area. Animals raised using low selenium feedstuffs deposit relatively low concentrations in their tissues, while animals raised on a relatively high selenium nutriture yield food products with greater selenium concentrations.

In most diets, the dominant food sources of selenium are cereals, meats and fish. An analysis of US diets (Schubert et al., 1987) revealed that 5 foods (white bread, beef, pork, chicken and eggs) contributed about 50% total Se in the typical diet.

Selenium deficiency is associated with impairments in antioxidant protection, redox regulation and energy production as consequences of suboptimal expression of one or more of the selenium containing enzymes such as glutathione peroxidase and deiodinase. These impairments not only can cause deficiency signs in the classical sense, but also can contribute to health problems caused by physiological and environmental oxidative stress (Combs, 2001). Because human selenium status is a public health concern, researchers are interested in studies about nutritional means of increasing the selenium content of meat, milk and eggs using selenium supplemented feedstuffs.

The purpose of this study was to investigate the effect of different forms and doses of supplemented dietary selenium on its contents in pig and chicken meat, as well as to screen for selenium status in the healthy human population in the Belgrade area.

MATERIAL AND METHODS

Experimental groups
The experiment was carried out on pigs and broilers fed a diet containing different doses of selenium as sodium selenite or selenized yeast (Sel-Plex-). The
diet in both sets of experimental groups (i.e. pigs and broilers) was adequate in energy, protein, carbohydrate, mineral and vitamin contents.

A total of 24 pigs (Landrace x Yorkshire x Duroc) was randomly assigned in 4 experimental groups and fed with a corn-based meal supplemented with sodium selenite (0.10 mg Se/kg and 0.30 mg Se/kg) or selenized yeast (0.30 mg Se/kg). One group was left unsupplemented and served as a control. At the end of the experiment (body mass 75 kg) the animals were sacrificed at an abattoir and muscle and liver tissue samples taken for subsequent selenium content analysis.

Tissue selenium determination was carried out on a total of 42 broilers (Arbo Acre) randomly assigned to 7 experimental groups. Broilers were fed throughout the experiment with a corn-based meal supplemented with 0.05, 0.10 and 0.30 mg Se/kg as sodium selenite or selenized yeast. One group was fed without additional selenium and served as a control. At 6 weeks the birds were sacrificed and liver, breast and thigh muscle tissue samples taken for selenium content determination.

Selenium serum levels were determined in a randomized sample of 54 healthy adults of both sexes: 13 from the rural area of Barajevo and 41 from the inner city center of Belgrade.

Tissue and blood samples
Samples of pig liver and m. longissimus dorsi and samples of broiler liver, breast and thigh muscle were collected from 6 animals from each experimental group after slaughter. Each sample was packed in an airtight plastic container and kept at -20°C for subsequent selenium analysis.

Blood samples of healthy adults were obtained by puncture of v. antebrachii. To obtain the serum part each sample was centrifuged at 1500 x g for 15 minutes, the serum was separated and kept at -20°C for subsequent Se analysis.

Selenium determination
Selenium concentrations in tissue and plasma samples were determined using hydride generation atomic absorption spectrophotometry (Welby et al., 1987).

Statistical analyses
The data are expressed as arithmetic mean ± standard deviation (X ± SD). The effect of supplementation on tissue selenium concentration is expressed by regression equations and correlation coefficients The significance of differences between groups was calculated using Student's t-test where p<0.05 was considered statistically significant (SPSS Inc. Sigma Plot for Windows v.8).

RESULTS

Tissue selenium concentration in selenium supplemented pigs
From the obtained results it is evident that the form and dosage of supplemented selenium have a significant effect on Se tissue concentration in
both liver (Figure 1) and muscle (Figure 2) samples. The highest concentration (1.34 ± 0.147 mgSe/kg), compared with the unsupplemented control (0.66 ± 0.325 mgSe/kg), was in liver samples from pigs supplemented with 0.30 mg Se/kg feed as selenized yeast (SY). Addition of 0.10 mg Se/kg as sodium selenite (SS) to the feed did not result in a significant (p>0.05) increase in liver selenium concentration.
Samples of *m. longissimus dorsi* from the control group (0.12 ± 0.010 mgSe/kg) had a significantly lower Se content compared to the supplemented groups. The highest muscle tissue Se content was obtained by adding 0.30 mg Se/kg as selenized yeast (0.26 ± 0.075 mgSe/kg), however the differences in muscle Se content between treated groups was not as impressive as in liver samples.

By increasing the concentration of supplemented sodium selenite from 0.10 mg Se/kg to 0.30 mg Se/kg we did not obtain a statistically significant increase in pig's muscle tissue selenium content.

**Tissue selenium concentration in selenium supplemented broilers**

Dietary supplementation with increasing doses of sodium selenite (0.05, 0.10 and 0.30 mg Se/kg) in broilers resulted in a significant linear increase of selenium content in liver samples \((R^2=0.7919, p<0.05)\). In breast and thigh muscle samples the increase in average selenium tissue concentration did not follow such a clear linear pattern (Figure 3).

![Figure 3. Tissue selenium concentration in broilers supplemented with sodium selenite (SS)](image-url)
Broilers supplemented with increasing doses of selenized yeast (0.05, 0.10 and 0.30 mg Se/kg) showed a similar pattern to sodium selenite supplementation with, however some distinct differences (Figure 4). It is interesting to note that the correlations determined for breast and thigh muscle were identical ($R^2 = 0.9818$, $p<0.01$) and the average selenium content in these samples was higher compared to those from groups supplemented with sodium selenite. Selenium content in liver tissue was higher compared with muscle samples irrelevant to the form (organic vs. inorganic) of supplemented selenium.

![Figure 4. Tissue selenium concentration in broilers supplemented with selenized yeast (SY)](image)

**Plasma selenium content in healthy adults**

In order to determine selenium status of a population living in a rural and in an urban community we determined the plasma selenium content in a total of 54 healthy adults of both sexes. The plasma selenium value ($n=13$) in the rural population (Barajevo) was $38.19\pm 12.78 \mu g/L$. Plasma selenium content in healthy adults of both sexes in the central city area of Belgrade was $66.85 \pm 22.31 \mu g/L$, which is significantly higher compared with the values obtained for subjects resident in the rural area of Barajevo ($p<0.01$).
DISCUSSION

The National Research Council (NRC, USA) established an estimated safe and adequate daily dietary intake for selenium in 1980. At that time the recommendation was set from 50 to 200 μg/day for both man and woman. Repletion studies found that approximately 40 μg/day of selenium maximized the activity of the selenoenzyme glutathione peroxidase which has a major role in the antioxidant defense mechanism of the cell. This was important as it led to the establishment of a recommended daily allowance (RDA) for the element in 1989. After some corrections for body weight and subject variability the RDA was set at 70 μg/day for man and 55 μg/day for women. (Levander 1991 and Burk 2002).

Because of the great variance in selenium soil and plant content, tables providing selenium content in foods are difficult to establish. However, some foods are known to be generally high in selenium content. In general, animal products, especially organ meats, are greater in selenium content than plant sources (Burk and Levander, 1999). Vegetables and fruits are uniformly low in selenium (when expressed on a fresh weight basis), and provide only small amounts (<8% intake) in most human diets (Schubert et al., 1987).

Within the accepted range of selenium supplementation of livestock diets (0.10-0.30 mg Se/kg) muscles from most species tend to contain 0.30-0.40 mg Se/kg. Organ meats, such as liver and kidney, accumulate greater concentrations of selenium. The livers of most species generally contain about four times as much selenium as skeletal muscle (Combs and Combs, 1986). With the exception of the lens of the eye, selenium levels found in the liver are the highest within the body. This is in accordance to the obtained results, as in all experimental groups, the liver selenium content was 4-5 times higher compared with the values determined for muscle selenium in both control and Se-supplemented groups. Such differences between liver and muscle samples were maintained irrespective of animal species (pig vs. broiler) or form (sodium selenite vs. selenized yeast) of dietary selenium supplementation.

Efforts to optimize selenium content in foods should consider both the amounts as well as chemical forms of the element to be supplemented. Inorganic forms, such as selenite or selenate, can be effective as feed supplements to prevent selenium deficiency in domestic animals but these forms have a quite limited impact on the selenium content in meat, milk or eggs because Se can be retained only by being incorporated into the SeCys-containing proteins (Schrauzer 2000).

Increased tissue selenium levels can be achieved by using a source of selenomethionine (SeMet) as a feed supplement, as the selenoaminoacid is readily incorporated into tissue proteins (Shan and Davis, 1994). Selenized yeast has a high content of SeMet (min 50% of total selenium in selenized yeast is in the form of SeMet). As animals cannot synthesize SeMet they have to rely on dietary sources of plant origin (Schrauzer, 2000). The non selective incorporation of SeMet into body proteins explains the higher selenium content in liver and muscle tissue samples in both broilers and pigs. Our results are in accordance to the findings of Jacques and Kanyon (2002) and Kuricova et al. (2003) who determined
that at equal dietary doses of selenium (0.30 mg Se/kg) breast muscle tissue Se concentration was higher in samples from the selenized yeast group (0.37 mg Se/kg tissue) compared to sodium selenite group (0.16 mg Se/kg tissue). Kuricova et al. (2003) compared the effects of increasing doses of dietary selenium on the content of the microelement in broiler muscle tissue samples. Only if dietary selenium was in the form of selenized yeast there was a linear correlation between the two parameters, which is in accordance to our findings Bobcek et al., (2004) after supplementing finishing pigs with 0.30 mg Se/kg as organic selenized yeast had similar results where the level of muscle tissue selenium in Se-supplemented pigs was more than double compared with muscles of pigs supplemented with dietary selenium (0.377 vs. 0.922 mg/kg).

Not all species accumulate tissue selenium at equal rates. Results show that pork liver and muscle contained more than double the concentration of selenium in comparison with broiler tissues in both control and Se supplemented groups. Daun and Akesson (2004) compared selenium content in muscles from chicken, turkey, duck, ostrich and lamb. The study showed considerable variations among species which may be important for the oxidative stability and nutritional value of different meat products.

The most common causes of selenium deficiency in man, beside the existence of Se deficient areas (like Keshan in China), are: inadequate and non variate meals with low Se content, specific diets (like Vegan diet) and total parenteral nutrition. Insufficient intestinal Se absorption is common during gastrointestinal disorders. Increased expense and excretion are common during strenuous physical exercise, hyperthyroidism, fever and exposure to radiation, toxic substances or cytostatics (Larsson and Johansson, 2002).

In Serbia the estimated average daily Se intake in man is 27 μg (which is below daily recommendations). The greatest part is provided by meat and fish (41.92%), dairy products (27.16%) and cereals (24.43%) (Maksimovic et al., 1992). Research carried out by Backovic et al. (1999) on 125 residents in the Belgrade area has determined a low serum Se concentration. By comparing healthy subjects with those suffering from progressive malignant tumors (n=55) no significant differences in serum Se were established, probably due to extremely low levels in both healthy and diseased subjects.

The significantly lower serum Se in subjects from the rural area of Barajevo can be explained by the use of home-grown foods, while residents in the central city area have a more varied diet, thus increasing (but not sufficiently) the overall Se intake.

Applied to conditions in Serbia the most efficient way to incorporate Se in the food chain would be the to increase, by dietary supplementation of domestic farm animals (pigs and poultry), the average Se content in foodstuffs of animal origin. This method of Se fortification would be significant especially in rural areas where, for the preparation of daily meals, meat from homegrown and bred animals is used.

Due to high cholesterol content in foodstuffs of animal origin there is a tendency among health conscious consumers to look for high quality lean meat. The use of Se-fortified poultry and pork meat in daily recommended quantities
(expressed as 10% of daily energy requirements or 0.8 mg proteins/kg body mass) is a safe natural way to increase the daily intake of Se-methionine. This method of Se fortification is highly accessible and has definite positive health effects (prevention of disorders linked to Se deficiency) on the human population, at the same time avoiding the possibility of accidental selenium poisoning (Goldhaber, 2002).

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REFERENCES
SUPLEMENTACIJA U ISHRANI SVINJA U BROJLERA KAO NAČIN PROIZVODNJE MESA OBOGAĆENOG SELENOM

SADRŽAJ

PEŠUT OLIVERA, BACKOVIĆ D I ŠOBAJIĆ SLADANA

Teritorija Srbije je region sa deficitom selena (Se). U stanovništvu su nivoi Se u serumu niski. Posmatrano kroz lanac ishrane, životinje akumuliraju Se u tkivima, tako da meso u ishrani predstavlja značajan izvor ovog mikroelementa.

Cilj istraživanja je ispitivanje efekta različitih oblika i količina Se u ishrani svinja i brojlera na koncentraciju selena u jestivom mesu i jetri, kao i procena mogućnosti upotrebe takvog mesa u ishrani na teritoriji Beograda.

Uzorak od 24 svinja je podeljen u 4 grupe: kontrolnu i grupe kojima je dodavan Na-selenit u količini od 0,1 i 0,3 mg Se/kg hrane, i 0,3 mg Se/kg hrane u obliku Se-kvasca. Ukupno 42 jednodnevnih pilića podeljeni su u 7 grupa: kontrolnu i grupe kojima je dodavano 0,05, 0,10 i 0,30 mg Se/kg Na-selenita ili Se-kvasca, tokom tova. Iz uzoraka mišica i jetre određivan je Se metodom atomske apsorpcije spektrofotometrije (AAS). Nivo Se u serumu odeđivan je AAS iz uzoraka krvi 54 zdravih odraslih ispitanika: 13 iz Barajeva i 41 iz centra Beograda.

Se-kvasac je u svim dozama efikasniji u odnosu na Na-selenit u podizanju nivoa Se u mišićnom tkivu brojlera. Takođe se najefikasnije podizanje sadržaja Se u jetri i mišićima svinja postiže Se-kvascem u količini 0,3 mg Se/kg hrane. Nivo selena u plazmi ispitanika su niski i značajno su niži kod ispitanika iz Barajeva u odnosu na ispitanike iz centra grada (p<0,01).

Korišćenje svinjskog i pilećeg mesa obogaćenog selenom, u količinama uobičajenim domaćim dijetetskim navikama, omogućila bi pozitivne zdravstvene efekte u populaciji, značajne za ruralne regije sa deficitem Se gde se koriste pretežno prehrambeni proizvodi iz sopstvene proizvodnje.