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DETERMINATION OF NICKEL CONTENT IN THE SEMIMEMBRANOSUS MUSCLE OF PIGS PRODUCED IN VOJVODINA

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The content of nickel was investigated in the M. semimembranosus of sixty-nine pigs from ten different genetic lines produced in Vojvodina. Nickel was determined by flame atomic absorption spectrometry after mineralization by dry ashing. The difference in the nickel content in the analyzed muscle tissues among different genetic lines of pigs was not significant ($P > 0.05$). Nickel levels ranged from 12.93 to 80.18 μg/100 g, with a general average of 32.41 μg/100 g. The average level of nickel was found to be higher than the levels observed in pork in some developed countries.

KEYWORDS: Nickel, *M. semimembranosus*, Pigs

INTRODUCTION

Meat quality is the sum of all sensoric, nutritive, hygienic-toxicological and technological factors of meat. The nutritive factors of meat quality comprise proteins and their composition, fats and their composition, vitamins, minerals, utilization, digestibility and biological value. The hygienic-toxicological factors of meat quality comprise microorganisms (bacteria, spores, moulds), shelf life (pH value, redox potential, water activity - a_w value, temperature of storage), residues (antibiotics, hormones, other pharmaceuticals) and contaminants (pesticides, mycotoxins, heavy metals, nuclides) (1-3).

Red meat (beef, veal, pork and lamb) contains high biological value protein and important micronutrients including iron, zinc and vitamin B12, all of which are essential for good health throughout life (4-7). Also, meat contains useful amounts of copper, magnesium, cobalt, phosphorus, chromium and nickel (7).

Nickel is a silvery-white, hard metal. Although it forms compounds in several oxidation states, the divalent ion seems to be the most important for both organic and inorganic substances, but the trivalent form may be generated by redox reactions in the cell (8). Nickel is widely distributed in nature, forming about 0.008% of the earth's crust. The core of the earth contains 8.5% nickel, deep-sea nodules 1.5%; meteorites have been found to contain 5-50% nickel (9). Agricultural soils contain nickel at levels of 3-1000

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mg/kg; in 78 forest floor samples from the northeastern United States of America, concentrations of 8.5-15 mg/kg were reported (10). The nickel content is enriched in coal and crude oil. Nickel in coals ranges up to 300 mg/kg; most samples contain less than 100 mg/kg, but there is a large variation by region (11). The nickel content of crude oils is in the range <1–80 mg/kg (10, 12).

In most food products, the nickel content is less than 0.5 mg/kg fresh weight. Cacao products and nuts may, however, contain as much as 10 and 3 mg/kg, respectively (9). According to Solomons et al. (13), dry beans, cocoa products, baking soda, and some nuts contain high levels of nickel (>2.0 μg/g); wheat and wheat products, shellfish, processed meats and many vegetables contain intermediate levels (0.2-2.0 μg/g); and whole and dried milk, fresh fruits, meat and eggs contain low levels of nickel (<0.2 μg/g). Nickel concentrations in drinking-water in European countries of 2-13 μg/liter have been reported (14). Nickel contents of pork have been reported in several studies. Reported data showed the nickel concentrations in pork were 5.5 μg/100g in Canada (15), 2.5 μg/100g in Denmark (16) and less than 1.6 μg/100g in Sweden (17). The highest reported nickel concentration in pork was 35 μg/100g in Nigeria (18).

Nickel is present in a number of enzymes in plants and microorganisms. In humans, nickel influences iron absorption and metabolism, and may be an essential component of the haemopoietic process. COMA (Committee on Medical Aspects of Food and Nutrition Policy) and FDA (US Food and Drag Administration) were unable to set recommended amounts for nickel intake. Based on extrapolation from animal data, the hypothetical human requirement for nickel would be 16 to 25 μg/1000 kcal or about 75 μg of elemental nickel per day (13). Nickel deficiency has not been observed in humans (19).

On the other hand, acute nickel exposure is associated with a variety of clinical symptoms and signs which include gastrointestinal disturbances (nausea, vomiting, abdominal discomfort and diarrhoea), visual disturbance (temporary left homonymous hemianopia), headache, giddiness, wheezing and cough. Approximately 7-10% of the population (predominately women) are affected by nickel allergic dermatitis. There is evidence suggesting that nickel ingestion may contribute to the exacerbation of eczema in sensitised individuals (19).

The lowest reported oral dose associated with acute effects in humans was 0.05 mg/kg bw (1.2 mg in a 60 kg adult) (19). Total diet studies indicate a total average oral intake of 200-300 μg/day (10). Early estimates of daily nickel consumption in the USA ranged from 300 to 600 μg (20). Recovery studies indicate an absorption rate of less than 15% from the gastrointestinal tract (21). Dietary intake of nickel in food is not expected to result in harmful effects (19).

On the basis of the available data from the Statistical Office of the Republic of Serbia, the total consumption of meat in Serbia is 43 kg/p/yr. Pig meat is the most widely consumed meat in the EU, as well as in Serbia, and the consumption has been steadily increasing (4). The Autonomous Province of Vojvodina (the northern part of the Republic of Serbia) is a region where the number of animals of the porcine species and the production of pork meat are of high economic importance. Most studies have focused on the proximate compositions, vitamins and other essential nutrients. In this sense, the aims of this work were: (i) to determine the content of nickel in pork muscle tissues (*M. semimembranosus*); (ii) to investigate the potential difference in content of nickel between ten

different genetic lines of pigs (two pure and eight crossbred pigs) used nowadays in Vojvodina for commercial pork production; (iii) to compare the results of our study with results found in other studies, i.e. other countries, for *M. semimembranosus* (ham).

EXPERIMENTAL

Animals, sampling and preparing

The pigs used in the present study were produced in a pig (cross)breeding programme provided by nucleus and multiplication farms in Vojvodina (GGP-GP traditional pyramid structure of genetic programme) (22). In this breeding programme, five pig purebreds were used. The Large White (LW) and Landrace (L) were used as female lines and Duroc (D), Hampshire (H) and Pietrain (P), were used as male lines. The investigation was performed on sixty-nine pigs (castrates males and females) from ten different genetic lines (two purebred and eight crossbred pigs): [LW, n = 8; L, n = 7; LWxL, n = 7; LxLW, n = 6; Dx(LWxL), n = 7; Dx(LxLW), n = 6; (DxP)x(LWxL), n = 8; (DxP)x(LxLW), n = 7; (HxP)x(LWxL), n = 6; (HxP)x(LxLW), n = 7].

The pigs were fattened at the ten biggest production farms in Vojvodina. The pig fattening involved the following phases: starting period (from 15 to 25 kg), growing period (from 25 to 60 kg) and finishing period (from 60 to 110 kg). The diets were based on corn and soybean meals, and were formulated to meet the nutrient requirements (23) for the different growth phases. The finishers were housed in pens with fully slatted floor and 0.80 m^2 space allocation per pig. Each pen contained 10 animals. The environmental temperature in the building was 22°C. All pigs had ad libitum access to diet and water.

The pigs were randomly selected at an individual live weight between 95 and 110 kg and about six months old. One pig from each genetic line was taken at every six mounts from the same farm.

All the pigs were slaughtered in the two biggest Vojvodinian slaughterhouses according to routine procedure. Carcasses were conventionally chilled for 24 h in a chiller at 2-4°C. After chilling, *M. semimembranosus* (SM) was removed from the right hind leg of each carcass. The meat samples were trimmed of visible adipose and connective tissue. The samples for chemical analysis (approximately 250 g) taken after the homogenization of the whole SM muscle, were vacuum packaged in polyethylene bags and stored at -40°C until analysis.

Analytical methods and quality control

The total ash content was determined according to ISO method (24).

The nickel content of the meat was determined after dry ashing mineralization according to the following procedure (25): a twenty-gram sample was weighed into a porcelain crucible and dried in a laboratory oven at 105°C for 3 h. After drying the sample was charred on a hot plate and then incinerated in a muffle furnace at 450°C overnight (16 h). If necessary, the ash was bleached with nitric acid/deionized water (1:2, v/v), evaporated to dryness and heated in the muffle furnace for 1 h. When a suitable ash

was obtained it was moistened with a small amount of water, treated with 10 ml of hydrochloric acid/deionized water (1:1, v/v) and evaporated to dryness. Finally, the ash was redissolved in 10 ml of hydrochloric acid/deionized water (1:9, v/v), transferred into a 25 ml volumetric flask and diluted to volume with deionized water.

Nickel was measured in the ash solution by flame atomic absorption spectroscopy according to the manufacturer's instructions (26). Measurement was made under optimized parameters given in Table 1.

Table 1. Parameters for the elemental measurement by AAS

Element	Wavelength (nm)	Band width (nm)	Flame	Sensitivity
Ni	232.0 nm	0.2 nm	Air-acetylene	0.1 μg/ml

A strict analytical quality control programme was employed during the study. The quality control of the analytical measurements for ash was performed using the following standard reference material (SRM): SMRD 2000 (Matrix meat reference material, National Food Administration, Uppsala, Sweden). For the determination of the Ni content the SRM samples were spiked with three different concentrations of this element. The results of the analytical quality control programme are presented in Table 2. In every series of samples, 2 blanks and 2 samples of standard reference material were included.

Table 2. Results of the analytical quality control programme (n = 8) used in the determination of total ash and nickel in *M. semimembranosus*

	Total ash	**Ni**
Certified concentration (g/100g)	2.65 ± 0.10	
Recovery (%)	100	103.2 ± 5.21
Limit of detection (μg/100g)		12.5

All chemicals used in the sample treatments were of high purity grade (Suprapur, Merck GmbH, Darmstadt, Germany) and deionized Milli-Q water was used throughout. The porcelain crucible and glassware were cleaned prior to use by soaking overnight in 10% v/v HNO_3 and rinsed with deionized Milli-Q water. Standard stock solution of analyzed metal was prepared immediately before use by dilution (with deionized water) of a 1000 mg/l standard solution.

All analyses were performed in duplicate.

Statistical analysis

All data are presented as mean, standard deviation (SD) and range (minimum and maximum concentrations). The analysis of variance (one-way ANOVA) was used to test the hypothesis about differences between a number of mean values. The software package STATISTICA 8.0 was used (27) for the analysis.

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RESULTS AND DISCUSSION

The average content, standard deviation and range for the total ash and Ni in the investigated samples of the muscle tissues (SM) of ten different genetic lines of pigs are presented in Table 3.

Table 3. Total ash and nickel content in the *M. semimembranosus* from the pigs in Vojvodina

Genetic line of pigs		Total ash (g/100g)	Ni (μg/100g)
LW (*n* = 8)	Mean ± SD	1.06 ± 0.02	30.76 ± 14.91
	Range	(1.01–1.07)	(16.05–59.03)
L (*n* = 7)	Mean ± SD	1.05 ± 0.02	37.06 ± 23.00
	Range	(1.03–1.07)	(12.93–80.18)
LWxL (*n* = 7)	Mean ± SD	1.03 ± 0.03	23.26 ± 8.38
	Range	(1.00–1.07)	(15.09–39.33)
LxLW (*n* = 6)	Mean ± SD	1.03 ± 0.03	39.47 ± 8.35
	Range	(0.99–1.08)	(31.23–49.60)
Dx(LWxL) (*n* = 7)	Mean ± SD	1.06 ± 0.02	36.71 ± 12.48
	Range	(1.03–1.08)	(23.94–59.33)
Dx(LxLW) (*n* = 6)	Mean ± SD	1.03 ± 0.02	31.36 ± 9.35
	Range	(1.02–1.06)	(23.04–43.88)
(DxP)x(LWxL) (*n* = 8)	Mean ± SD	1.03 ± 0.03	26.92 ± 11.02
	Range	(0.99–1.06)	(14.03–41.84)
(DxP)x(LxLW) (*n* = 7)	Mean ± SD	1.05 ± 0.02	31.85 ± 18.56
	Range	(1.02–1.07)	(16.12–66.91)
(HxP)x(LWxL) (*n* = 6)	Mean ± SD	1.04 ± 0.04	29.05 ± 19.10
	Range	(0.98–1.08)	(13.12–58.41)
(HxP)x(LxLW) (*n* = 7)	Mean ± SD	1.06 ± 0.02	38.07 ± 28.29
	Range	(1.02–1.08)	(17.45–75.08)
All animals (*n* = 69)	Mean ± SD	1.04 ± 0.03	32.41 ± 16.23
	Range	(0.98–1.08)	(12.93–80.18)

The average total ash content in the SM muscles (Table 3) was 1.04 g/100 g [ranging from 0.98, genetic line of pigs: (HxP)x(LWxL), to 1.08 g/100 g, genetic line of pigs: LxLW, Dx(LWxL), (HxP)x(LWxL) and (HxP)x(LxLW)]. The content of the total ash found in the present study did not differ significantly ($F = 1.433$; $P = 0.202$) among the SM muscle tissue belonging to different genetic lines of pigs. The average total ash values for the SM muscle in the present study are in agreement with the reported values in the literature (28, 29).

The content of nickel found in this study accounted for only 0.003% of the total ash of the SM muscle (Table 3). The order of the genetic lines of pigs according to nickel content in the SM muscle samples (Table 3) in μg/100 g was: LWxL < (DxP)x(LWxL) < (HxP) x (LWxL) < LW < D x (LxLW) < (DxP) x (LxLW) < D x (LWxL) < L < (HxP) x (LxLW) < LxLW. The content of nickel found in the present study did not differ significantly ($F = 0.566$; $P = 0.816$) among the SM muscle tissue belonging to the different

genetic lines of pigs (Table 3). On the other hand, animals belonging to the same genetic line, from the same farm, raised under the same conditions, given the same feed, and slaughtered at the same age had Ni content in the SM muscle that could differ up to six times (Table 3). The lowest, average and highest nickel content in the SM muscle was 12.93 (genetic line of pigs: L), 32.41 and 80.18 μg/100 g (genetic line of pigs: L), respectively. According to Greenfield and Southgate (30), meat, as a biological material, exhibits natural variations in the amounts of nutrients contained and the limits of natural nutrient variation are not defined. The Serbian regulation (31), as well as EC regulation (32), did not set maximum levels for Ni in any meat type.

The nickel levels obtained in pork in this study were higher than in pork (*M. semimembranosus*) of Canada: 5.5 μg/100g (15), Denmark: 2.5 μg/100g (16) and Sweden: <1.6 μg/100g (17). Levels which are equal to those in Vojvodinian pork have been obtained in Nigerian pork (140 μg/100g dry weight, approximately 35 μg/100g wet weight) (18). The relatively higher and non-uniform nickel levels in Vojvodinian pork indicate nickel availability in local agricultural environment.

CONCLUSION

The results of the present investigation show that the content of nickel determined in the *M. semimembranosus* of pigs was not influenced by the genetic lines. Compared with developed countries, the nickel content in the SM muscle tissue of the pigs from Vojvodina is higher. In addition, the obtained nickel composition could be used to provide regular nutrient compositional data of the pork meat in Serbia.

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ОДРЕЂИВАЊЕ САДРЖАЈА НИКЛА У *M. semimembranosus* СВИЊА ПРОИЗВЕДЕНИХ У ВОЈВОДИНИ

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У овом раду одређен је садржај никла у *M. semimembranosus* (н = 69) десет различитих генотипова свиња одгајаних у Војводини. Садржај никла је одређен пламеном атомском апсорпционом спектрофотометријом након „сувог спаљивања“ узорака. Садржај никла у мишићном ткиву није се значајно разликовао ($P > 0,05$) између различитих генотипова свиња. Одређени садржај никла био је у границама од 12,93 до 80,18 μг/100 г, са просечним садржајем од 32,41 μг/100 г. Просечни садржај никла, одређен у овом испитивању, већи је од садржаја никла који је у свињском месу утврђен у неким развијеним земљама.

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