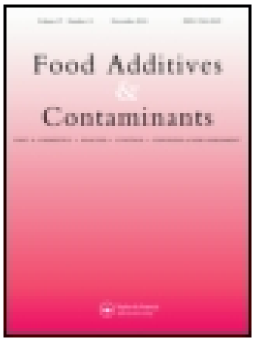

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Mineral composition and toxic element levels of muscle, liver and kidney of intensive (Swedish Landrace) and extensive (Mangulica) pigs from Serbia

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Abstract

Mineral composition (Fe, Zn, Cu, Mn, Se, Cr, Co, Ni, Na, K, Mg, Ca) and toxic element levels (Cd, Pb, Hg, As) of soil, feed and tissue (muscle, liver and kidney) from intensive (Swedish Landrace, housed indoors, fed a known diet, 4 years) and extensive (Mangulica, free-roaming, non-specified diet, 7-8 months) pigs was determined by inductively coupled plasma mass spectrometry (ICP-MS). Controlled nutrition produced pigs with higher concentrations of most minerals (muscle: Mn, Se, K, Mg; liver: Zn, Cu, Mn, Se, Cr, Ca; kidney: Zn, Cu, Mn, Se, K, Mg), but for Fe, the opposite trend was found. Long-term free-ranging pigs have higher risk of contamination by toxic elements (Cd exceeded the maximum residue level in kidney). Principal Component Analysis and Cluster Analysis were used to assess the effect of different pig breed/lifestyle (pig type) on element composition of muscle, liver and kidney of pigs. Multivariate data analysis showed good discriminating capabilities.

Keywords: mineral composition; toxic elements; muscle; liver; kidney; pig

Introduction

Meat and meat products are an important part of the human diet. Meat is a very rich and convenient source of high value proteins, essential vitamins and important minerals needed for normal growth and good health throughout life (Higgs, 2000; Lombardi-Boccia et al., 2005). It is well known that micro- (Fe, Zn, Cu, Mn, Se, Cr, Co, Ni) and macro- (Na, K, Mg, Ca) elements are vital for the normal functioning of almost all biochemical and enzymatic processes in the body (Goyer, 1997). Beside these elements, meat contains elements with toxic properties (Falandsyz, 1993; Adei and Forson-Adaboh, 2008), such as Cd, Pb, Hg and As, which do not have essential biological function but are transferred through the food chain. While toxic element contents in muscle are generally low, liver and kidney accumulate higher concentrations of these elements and may pose health risks to humans (Potthast, 1993; Jokanovic et al., 2012).

Pork is consumed worldwide in progressively increasing amounts (Williamson et al., 2005). In Serbia in 2013, average annual consumption of meat was 60.8 kg/inhabitant kg/year (EU average is 78 kg/inhabitant), of which pork was the most commonly consumed meat (27.3 kg), followed by poultry (17.2 kg) and beef (14.4 kg) (USDA Foreign Agricultural Service, 2013). One of the most widespread and fertile breeds of pig in Serbia is the Swedish Landrace, produced by intensive indoor industrial farming. The pigs are kept in controlled conditions and generally raised on concentrated feed with incorporated mineral supplements, in order to avoid mineral deficiency (Suttle, 2010). However, in recent years, pork consumers have become more interested in the breeding and production system of pigs. Production of so-called ‘natural’, ‘bio’ or ‘organic’ meat has become more popular. These production systems with pastures are becoming attractive mostly due to their environmental sustainability as well as the initial low cost of the production system.

For that reason, the production of free-range reared pigs, fed on natural feeds without growth promoters and antibiotics, is significantly increasing (Cava et al., 2003). In these extensive production systems, most of the animal feeds are locally produced, and animals' exposure to minerals directly depended to the environmental state in the area (Kumaresan et al., 2009). Extensive production in Serbia is largely focused on the breeding of one of the last local pig breeds, the Mangulica pig (Petrovic et al., 2010).

The aim of this study was to examine and compare element composition of muscle, liver and kidney of intensively (Swedish Landrace) and extensively (Mangulica) grown pigs from Serbia. Principal Component Analysis (PCA) and Cluster Analysis (CA) were used to assess the effect of different breed/lifestyle on mineral composition and toxic element levels of muscle, liver and kidney of the pigs.

Materials and Methods

Sample collection

For this study, tissue samples were collected from two representative intensive and extensive pig farms in Serbia. Both intensive pig farms were at the Institute for Animal Husbandry near Belgrade. Pigs (Swedish Landrace) were fed with concentrate consisting of dry corn (64.1%), livestock-grade wheat flour (10%), soybean meal (17.8%), sunflower meal (5%), lime (1.3%), monocalcium phosphate (0.7%), salt (0.4%) and the premix (0.5%; which contains the following elements: Fe-2.01%, Cu-0.408%, Mn-0.816%; Zn-2.52%, Co-0.01%, Se-0.002%). The extensive farms were in Bojcin Wood, near Belgrade, and Silver Lake Wood, near Veliko Gradiste. Pigs (Mangulica) were maintained on native extensive silvopastoral systems and were outdoors and feed was based on the farms' own resources (pasture, stubble, acorn and other forestry products) with addition of corn and wheat in winter, when the seasonal feed availability was low. Swedish Landrace (n=32) were slaughtered approximately after 7-8 months, with live weights of around 110 kg, while Mangulicas (n=32) were slaughtered after 4 years, with live weights of around 75 kg. All samples (n=192) of muscle (longissimus thoracis et lumborum (LTL), 200-300 g), liver (200-300 g) and kidney (both kidneys) were taken after slaughter, packed individually in plastic bags and transported to the laboratory where they were stored at -18ºC until analysis. Samples of soil and forage were collected only in the extensive farms. The first 15 cm depth of topsoil was taken from multiple fields and mixed into one composite sample. Forage samples were taken at the same time in each field. Samples of complementary feed (corn and wheat) given to extensive pigs and samples of concentrate given to intensive pigs were directly collected in each farm.

Sample preparation and reagents

Frozen meat samples were thawed at +4 ºC for a day before analysis and then homogenized. Samples of soil, forage and feed for each farm were oven-dried (60 ºC), mixed and sieved (0.5 mm diameter).

An amount, approximately 0.5 g, of each thawed, homogenized tissue, forage or feed, was transferred into a teflon vessel with 5 mL nitric acid (67% Trace Metal Grade, Fisher Scientific, Bishop, UK) and 1.5 mL hydrogen peroxide (30% analytical grade, Sigma-Aldrich, St. Louis, MA, USA) for microwave digestion. Approximately 0.5 g subsamples of soil were digested in 3 mL 67% nitric acid and 6 mL of hydrochloric acid (37% for trace analysis, Sigma-Aldrich, St. Louis, MA, USA) in a microwave digestion system.

The microwave (Start D, Milestone, Sorisole, Italy) program consisted of three steps: 5 min from room temperature to 180°C, 10 min hold at 180°C, 20 min vent. After cooling, the digested sample solutions were quantitatively transferred into disposable flasks and diluted to 100 mL with deionized water produced by a water purification system (Purelab DV35, ELGA, Buckinghamshire, UK).

Analysis of the following sixteen elements: Fe, Zn, Cu, Mn, Se, Cr, Co, Ni, Na, K, Mg, Ca, Cd, Pb, Hg and As, was performed by inductively coupled plasma mass spectrometry (ICP-MS), (iCap Q mass spectrometer, Thermo Scientific, Bremen, Germany). The most abundant isotopes were used for quantification.

Torch position, ion optics and detector settings were re-adjusted daily using tuning solution (Tune B, Thermo Scientific), in order to optimize mechanical and electrical parameters and minimize possible interference. Basic operating conditions of the instrument were: RF power (1550 W); cooling gas flow (14 L/min); nebulizer flow (1 L/min); collision gas flow (1 mL/min); operating mode (Kinetic Energy Discrimination-KED); dwell time (10 ms).

Standard stock solutions containing 1000 mg/L of each element (Fe, Zn, Cu, Mn, Se, Cr, Co, Ni, Na, K, Mg, Ca, Cd, Pb, Hg and As) were purchased from Reagecon (Shannon, Co. Clare, Ireland). These solutions were used to prepare standards for five-point calibration curves (including zero). Multielement internal standard (^{6}Li, ^{45}Sc, ^{71}Ga, ^{89}Y, ^{209}Bi) was introduced online by an additional line through the peristaltic pump, and covered a wide mass range. All solutions (standards, internal standards and samples) were prepared in 2% nitric acid.

Quality assurance

The analytical method was validated by Guidelines for Single Laboratory Validation (SLV) of Chemical Methods for Metals in Food (AOAC) and accredited according to ISO 17025. The quality of the analytical process was verified by analysis of the certified reference materials NIST 1577c (bovine liver, Gaithersburg, MD, USA) and ERM – CC135a (contaminated brickworks soil, Teddington, UK). Reference materials were prepared as samples using microwave digestion. Measured concentrations were corrected for response factors of internal standards using the interpolation method and were within the range of the certified values for all isotopes (Table 1). No information was given regarding Hg in the reference material NIST 1577c and, therefore, analytical recoveries of 97-105% were determined using spiked samples (c=10 μg kg^{-1}, n=10). Also, spiked samples were used for determination of Cd and As in soil (for Cd c=0.250 mg kg^{-1}, n=10; for As c=2 mg kg^{-1}, n=10), and recoveries were in the range 86-95% and 89-99%, respectively.

Table 1

Statistical analyses

The data were processed statistically using the software package STATISTICA 10.0 (StatSoft Inc., Tulsa, OK, USA). The ICP-MS determinations for each element in each tissue of the 64 pigs were averaged, and are expressed as means ± SE. The "skewness" and the "kurtosis" parameters were used to test the assumptions of normal distribution of the observed data. The skewness parameters showed minimal deviations from normal distribution, while the kurtosis parameter showed almost neglecting difference in "peakedness" compared to normal distribution, indicating that the experimental results showed a good approximation to a normal distribution. Analysis of variance (ANOVA) and Tukey’s HSD test for comparison of means were used to analyse variations of the elements levels in muscle, liver and kidney of different pig breeds/lifestyles (pig types).

Pattern recognition techniques (Principal Component Analysis, PCA, and Cluster Analysis, CA) were applied to the experimental data (used as descriptors) to characterize and differentiate the measured levels of elements in the pig tissues. PCA was used to discover any possible correlations among the measured results, while CA was used to classify samples of meat, liver and kidneys of different pig breeds/lifestyles into groups.

Results and discussion

The mineral composition of tissues (muscle, liver and kidney) of intensively and extensively bred pigs in Serbia is shown in Table 2. Also, levels of analysed elements in feeds and soil for both pig types are shown in Table 3.

Table 2

Table 3

Micro- and macro-elements

It is well known that diet is the main source of macro-, micro- and toxic elements. However, many factors, like chemical form and bioavailability of elements, interaction between elements as well as animals’ homeostatic mechanisms can have a strong impact on diet-element content relationship. Also, in extensive production systems, soil ingestion has an important role in mineral exposure (Suttle, 2010).

Concentrations of Fe were statistically significantly higher in all tissues of extensive pigs compared with intensive pigs (Table 2). Also, Fe level in forage was 2-fold higher than in concentrate feed, while soils contained a much higher Fe level than feed (Table 3), but were within the expected range according to the literature (Cabata-Pendias, 2011). Higher muscular metabolic activity related to grazing, soil consumption by the extensively reared animals (regardless of the fact that we did not measure the degree of soil ingestion), and age of breeding could be the reasons for higher Fe levels in tissues of extensive pigs. Among the few available studies of extensive breeding are those by López-Alonso et al. (2007, 2012). Those studies examined the trace element status and toxic metal accumulation in intensive and extensive pigs. The authors determined higher Fe levels in muscle of both pig types than was measured in the current study but found a similar concentration ratio between intensive and extensive pigs. To the best of our knowledge, most of the available literature data shows levels of elements in meat from intensive pigs. The mean Fe level in our intensive pigs was close to data reported by Lombardi-Boccia et al. (2005) and Bilandzic et al. (2013). Higher levels of Fe were found in studies of Marchello et al. (1985) and Adei and Forson-Abadoh (2008). However, Tomovic et al. (2011a) as well as data of Danish Food Composition database (2015) showed lower concentrations of Fe than in this study.

The mean Zn concentration in intensive pig muscle (17.00 mg kg^{-1}) was similar to levels reported by the USDA (2016), Lombardi-Boccia (2005) and Health Canada (2016), while the Zn level in extensive pig muscle (22.07 mg kg^{-1}) was in line with data for pig muscle (without specifying of pig type) from Croatian markets (Bilandzic et al. 2013) (22.20 mg kg^{-1}). The mean level of Cu in intensive pig muscle (0.46 mg kg^{-1}) was similar to that reported by Lombardi-Boccia (2005), while the level in extensive pig muscle (0.70 mg kg^{-1}) was close to data obtained by Health Canada (2016). The concentrations of Zn and Cu in liver and kidney of intensive pigs in the current study were statistically higher than levels measured in extensive pigs; this was also previously reported by López-Alonso et al. (2007, 2012). This could be explained by the fact that Cu and Zn levels were 3 to 7-fold higher in the concentrate feed given to the intensive pigs compared to those found in the forage and complementary feed of extensive pigs. However, statistically higher Zn and Cu concentrations occurred in the muscle of extensive pigs compared to intensive animals. Some studies established that there is no effect of dose and chemical form of Zn and Cu in pig diets on the muscle content of these metals (De Smet and Vossen, 2016), which could be reason for the opposite results we measured in muscle compared to liver and kidney.

The mean Mn levels in our extensive pig muscle, liver and kidney and intensive pig muscle were comparable to most available literature data (Health Canada, 2016; Danish Food Composition, 2015; López-Alonso et al., 2007, 2012). However, the Mn levels in intensive pig livers and kidney were higher than levels in the mentioned literature data. In the current study, muscle of both pig types had higher Se levels than was reported by the Danish Food Composition Databank (2015), but our levels were lower than those reported by López-Alonso et al. (2007, 2012) and Health Canada (2016). Se levels determined in liver and kidneys of intensive pigs were higher than in extensive pigs, as in López-Alonso et al. (2007, 2012); nonetheless, their results showed almost Se levels two-fold higher in liver than the results of the current study. Statistically significantly higher concentrations of Mn and Se were established in all tissues of intensive pigs than of extensive animals. In the case of Se, higher levels in intensive pigs were expected and could be explained by the differences of Se levels in feed. Similar results were found by Zhao, Wang and Yang (2016). Considering that Mn levels in concentrate and complementary feed were similar and much higher than in the soil and forage, an unexpected result was the higher Mn levels in intensive pigs.

The mean Cr and Co levels detected in intensive and extensive pig tissue were very low in the current study. Statistical analysis showed that there were significant differences in Cr levels in muscle and liver between two pig types as well as in Co levels in liver and kidney. Co was not detected (LOD=0.004 mg kg^{-1}) in muscle of either intensive or extensive pigs (Table 2).

Ni was not detected (LOD=0.050 mg kg^{-1}) in any of the pig tissues analysed. To the best of our knowledge, there is no available literature data about Ni accumulation in animals as related to soil exposure.

Concentrations of macro elements between tissues of intensive and extensive pigs were significantly different. Levels of Na and Ca were higher in extensive pigs, except for the Ca level in liver. Concentrations of K and Mg were notably higher in muscle and kidney of intensive pigs than in extensive pigs (Table 2). Generally, the levels of Na and K can differ due to variables other than feeding regime (Jukna et al., 2013; Wang, Huo, and Ren, 2008). Higher levels of Ca in extensive pigs could be explained by their free access to soil and tiny stones as abundant reserves of Ca.

Na and K levels in all tissues of intensive pigs were generally lower than in the literature data, while those of extensive pigs were higher (Danish Food Composition Databank, 2015; Health Canada, 2016; Marchello et al., 1985; Tomovic et al., 2011a; USDA, 2016). Levels of Mg in both pig types were comparable to data from databases of some countries (Danish Food Composition Databank,2015; Health Canada, 2016; USDA, 2016), while in liver, Mg levels were higher compared to the data of the same databases. The mean Mg level in intensive pig kidney was in agreement with data reported by the Danish Food Composition Databank (2015) and slightly higher than levels determined by Health Canada (2016), USDA (2016) and Bilandzic et al. (2013), while the Mg level in extensive pig kidney was slightly lower than data reported in those studies. Levels of Ca in muscle of both pig types were lower than levels published by the Danish Food Composition Databank (2015) and Health Canada (2016) (70 and 240 mg kg^{-1}, respectively). Mean Ca levels in liver of our pigs were lower compared to data from Health Canada (2016), USDA (2016) and Tomovic et al. (2011a). The Danish Food Composition Databank (2015), Health Canada (2016) and the USDA (2016) reported higher Ca levels in kidney than was measured in intensive pig kidney in the current study, but those levels were lower than Ca levels in our extensive pigs.

Toxic elements

The present study indicates the differences between toxic elements identified in pig muscle from those found in the liver and kidney. In muscle, toxic elements were found only at very low levels, often close to the limits of detection (Table 2). However, Cd primarily accumulates in the liver and kidney because it binds to metallothionein in these tissues (Bremner, 1979; García-Fernández et al., 1996). In humans, Cd exposure is known to cause kidney and/or skeletal damage, problems with vitamin D metabolism, anaemia and nervous system damage (WHO, 1992; Järup et al., 1998). The European Commission (2006) has established maximum residue levels (MRL) for Cd in muscle, liver and kidney intended for human consumption of 0.050, 0.5 and 1.0 mg kg^{-1} fresh weight, respectively.

Cd levels in liver and kidney were significantly higher in extensive than in intensive pigs (Table 2). Liver of intensive pigs contained Cd levels (0.051 mg kg^{-1}) lower than those established by Tomovic et al. (2011b) (412 mg kg^{-1}) and López-Alonso et al. (2007) (73 mg kg^{-1}). Gyori et al. (2005) examined Cd levels in liver of a fattened sow of the same local rare breed (Mangulica) in Hungary and determined lower levels than the mean Cd level in the extensive pigs in the current study. The levels of Cd in intensive pig kidney were in the range of those in many reports (López-Alonso et al., 2007; Bilandzic et al., 2010; Korsrud et al., 1985; Gyori et al., 2005). However, the mean Cd level measured in extensive pig kidney (1.612 mg kg^{-1}) far exceeded the MRL and was higher than levels established in many previous investigations (López-Alonso et al., 2007, 2012; Bilandzic et al., 2010; Korsrud et al., 1985, Gyori et al., 2005). The higher Cd levels in liver and kidney of our extensive pigs compared to intensive pigs were opposite to levels in the two pig types reported by López-Alonso et al. (2012) in Spain. Those authors explained their results in relation to the effect of Cu and Zn supplementation on Cd deposition in tissue. According to some studies (Grawe et al., 1997; Sapunar-Postruznik et al., 2001; Andree et al., 2010), Cd levels in feedstuffs strongly influence the Cd levels in liver and kidney of animals. Strong association between tissue Cd levels and the degree of grazing (and therefore soil ingestion) in cattle related to soil exposure has been established by Blanco-Penedo (2009). In our study, Cd levels in concentrate feed for intensive pigs were higher than levels in complementary feed but much lower than in forage and soil. So, the higher Cd levels in extensive pigs could be attributed to exposure to soil through grubbing in polluted areas, as Cd is easily transported from soils to edible parts of agricultural crops (Jalil et al., 1994; McLaughlin et al., 1996). Linden (2002) established that pigs raised organically outdoors had higher Cd levels in kidneys and faeces than indoor pigs, even with a lower Cd level in the organic feed. Therefore, an additional likely source of Cd exposure for outdoor pigs was soil, via rooting. Pompe-Gotal and Crnic (2002) reported the presence of free protein-thiol groups leads to strong fixation of heavy metals and this is the reason for Cd accumulation in liver and kidney of animals exposed to Cd. However, another cause of the higher Cd levels we observed in extensively reared pigs could be the age of the pigs. This is in line with Linden (2002), where a significant linear relationship between Cd levels in kidney of pigs and age at slaughter was established (Cd levels in kidney increased 2.8 μg kg^{-1} wet weight for each additional week of survival).

All Pb levels in pig tissues in the current study were below the MRLs (The European Commission, 2006). In muscle, Pb was not detected. Statistical analysis showed significantly higher Pb levels in liver and kidney of extensive pigs, which coincides with data published by López-Alonso et al. (2007, 2012). Levels of Pb in both pig types were lower than previously reported (López-Alonso et al., 2007, 2012; Bilandzic et al., 2010; Gyori et al., 2005). According to some studies (Fick et al., 1976), Pb does not accumulate in muscle, while other studies showed age-related differences in kidney and liver (MacLachlan et al., 2016). Also, differences in Pb accumulation could be associated with differences in Pb concentrations in feed. Our results showed that Pb levels were generally higher in forage and soil than in concentrate feed for intensive pigs.

In liver of intensive pigs and in muscle of both pig types, Hg was not detected. Hg levels in liver of intensive and extensive pigs were below or close to the limits of detection and were similar to the low Hg levels reported by López-Alonso et al. (2007, 2012). However, kidney of both pig types in the current study contained higher Hg levels than was reported by López-Alonso (2007, 2012). Hg content was significantly higher in kidney of extensive pigs, and reasons for that could be age of the pigs and Hg levels in forage and soil.

No MRL for As has been established by the European Commission (2006). As levels in our study were low, < 0.010 $mgkg^{-1}$, except in kidney of extensive pigs (Table 2). Arsenic has a short half-life in the body and does not accumulate, so the results obtained reflect recent exposure from soil and forages (NRC, 2005). Levels of As in liver and kidney were lower than levels published by López-Alonso et al. (2007, 2012).

Cluster analysis of mineral composition and toxic element levels

A dendrogram of mineral levels in the pig tissues using complete linkage as an amalgamation rule and the City block (Manhattan) distance as a measure of the proximity between samples (in a sixteen variable factor space) is shown in Figure 1. The measurement of this distance yields results similar to the Euclidean distance, but in this measuring technique, the effect of single large differences (outliers) is dampened (since they are not squared). The dendrogram based on ICP-MS data showed a clear distinction existed between the two pig types (Figure 1). The variability among two different types of pigs may be due to genetic composition, diet and environmental factors. As shown in Figure 1, there was more similarity in the mineral content of kidney for the two pig types, while muscle and liver from the two pig types was more different in mineral content. The linkage distance (shown on the ordinate axis) between the two main clusters was evident (nearly 2800).

Fig. 1.

The principal component analyses - PCA

Principal component analysis (PCA) was also applied as an alternative to CA to classify the samples. The algorithm of PCA can be found in standard chemometric material (Kaiser and Rice, 1974; Otto, 1999). In summary, PCA decomposes the original matrix into several products of multiplication into loading (in this case, the two pig types) and score matrices (in this case, levels of the 16 elements in muscle, liver and kidney). Pig types were taken as variables (column of the input matrix) and the measured levels of elements as mathematical-statistical cases (rows of the matrix).

PCA allows a considerable reduction in a number of variables and the detection of structure in the relationships between, in this case, the measured levels of the 16 elements and different pig types and tissues (muscle, liver and kidney) that give complementary information (Kaiser and Rice, 1974; Otto, 1999). The full auto-scaled data matrix consisting of 6 different samples was submitted to PCA. The number of factors retained in the model for proper classification of levels of the elements as found by ICP-MS in the original matrix, and transferred into loading (different samples) and score (mineral composition and toxic element levels) matrices were determined by application of Kaiser and Rice's rule. This criterion retains only principal components with Eigenvalues >1.

Fig. 2.

For visualizing the data trends and for the discriminating efficiency of the used descriptors, a scatter plot of pig types and tissues using the first two principal components (PCs) from PCA of the data matrix was obtained (Figure 2). As can be seen, there was a clear separation of the six inputs, two pig types and three tissue types (muscle, liver and kidney), according to the 16 measured elements. The quality results showed that first two principal components explained 80.91% of the total variance, which, therefore, could be considered as sufficient to describe the whole set of experimental data.

The levels of: Na (which contributed 11.33% of the total variance, calculated based on the correlation), Co (10.11%), As (9.17%), Cd (9.51%), Hg (9.56%) and Pb (8.28%) were the most negatively influential factors for the first principal component evaluation, while the levels of: Mg (which contributed 11.95% of the total variance, calculated based on the correlation) and K (10.55%) showed the strongest positive influence on the first principal component calculation. The most negatively influential parameters for second principal component were the content of: Cr (17.98% of the total variance), Mn (17.32%), Cu (15.15%) and Zn (20.27%).

The influence of different parameters that describes the observed samples could be evaluated from the scatter plot (Figure 2), in which the samples of kidney, with higher Se, As, Na, Ca, Hg and Cd content are located at the left side of the graph, while Swedish Landrace's liver samples are located at the bottom side of the graphic (samples with increased Zn, Cu, Pb, Fe and Cr). Mangulica liver samples are located in the central part of the graphic. The samples of muscle are located at the right side of the graphic, showing the highest K and Mg concentration.

Conclusion

From the obtained data, it could be concluded that different pig types can influence the levels of elements in tissues. Well-controlled nutrition could be a reason for the higher concentrations of most microelements in intensive pigs in the current study, while higher muscular metabolic activity and soil ingestion could be a reason for higher Fe levels in tissues of extensive pigs, while long-term free-range roaming may expose them to a higher risk of contamination with toxic elements. Liver and kidney of extensive pigs had high levels of Cd, exceeding the MRL in kidney. Therefore, further control of Cd in extensive pigs is needed to avoid intake of Cd levels that may pose risk to human health.

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Table 1. Results of analytical quality control (n=10) for determination of mineral composition and toxic element levels in tissues (muscle, liver and kidney) of intensive and extensive pigs.

Table 1

				CRM NIST 1577c			CRM ERM - CC135a		
Elements	Limit of detection	Limit of quantification	Method repeatability/precision as RSD	Certified value*	Analysed value**	Recovery	Certified value*	Analysed value**	Recovery
	(µg kg^{-1})	(µg kg^{-1})	(%)	(µg kg^{-1})	(µg kg^{-1})	(%)	(mg kg^{-1})	(mg kg^{-1})	(%)
As	1.2	4	3.57	19.3±1.4	20.5±1.1	106.2	-	-	-
Cd	0.4	1	8.99	97±1.4	97.9±2.6	100.9	-	-	-
Pb	2	3.8	3.65	62.8±1.0	63.3±2.6	100.8	411±26	417±17	101.4
Hg	0.3	0.9	6.90	-	-	-	2.9±0.6	2.8±0.5	96.6
	(mg kg^{-1})	(mg kg^{-1})	(%)	(mg kg^{-1})	(mg /kg^{-1})	(%)	(mg kg^{-1})	(mg kg^{-1})	(%)
Cu	0.022	0.066	6.26	275.2±4.6	271.9±5.7	98.8	107±5	111±4	103.7
Fe	0.08	0.23	4.71	197.94±0.65	197.43±5.21	99.7	47500±4600	45700±3950	96.2
Zn	0.124	0.372	10.52	181.1±1.0	180.9±1.8	99.9	345±49	330±25	95.9
Mn	0.004	0.011	4.47	10.46±0.47	10.55±0.25	100.9	390±40	398±21	102.0
Cr	0.002	0.007	4.47	53±14	51±2.8	96.2	455±59	429±29	94.3
Co	0.004	0.013	4.24	0.3±0.018	0.31±0.016	103.3	20±4	21±3	105.0
Ni	0.050	0.145	9.19	44.5±9.2	52.7±4.3	118.4	291±22	298±14	102.4
Se	0.011	0.032	10.94	2.031±0.045	2.055±0.066	101.2	0.9±0.3	1.0±0.3	111.1
K	2.21	6.63	2.85	10230±640	10540±300	103.0	16300±2600	15970±1450	98.0
Na	5.58	16.76	7.02	2033±64	2011±140	98.9	1700±270	1620±140	95.3
Ca	3.08	9.24	3.64	131±10	125±4	95.4	23400±2900	22150±1960	94.7
Mg	0.13	0.40	3.03	620±42	631±19	101.8	9400±1200	9250±750	98.4

[*] Certified value as given by the manufacturer.

[**] The data are presented as means ± standard deviation.

Table 2. Mineral composition and toxic element levels of tissues (muscle, liver and kidney) of intensive (I) and extensive (E) pigs.

Table 2

		Muscle		Liver		Kidney	
[mg kg^{-1}]		I	E	I	E	I	E
Microelement	Fe	4.80±0.16^{a}	10.05±0.88^{b}	289.74±6.39^{a}	450.68±28.40^{b}	60.16±2.13^{a}	92.60±5.70^{b}
	Zn	17.00±0.37^{a}	22.07±0.74^{b}	103.44±5.15^{b}	54.27±3.34^{a}	33.66±1.17^{b}	27.36±0.76^{a}
	Cu	0.46±0.01^{a}	0.70±0.04^{b}	37.73±2.04^{b}	4.21±0.21^{a}	9.70±0.34^{b}	5.68±0.55^{a}
	Mn	0.12±0.01^{b}	0.08±0.01^{a}	5.12±0.11^{b}	3.28±0.13^{a}	2.38±0.07^{b}	1.48±0.06^{a}
	Se	0.15±0.01^{b}	0.12±0.01^{a}	0.63±0.02^{b}	0.38±0.01^{a}	2.12±0.08^{b}	1.63±0.05^{a}
	Cr	0.050±0.003^{a}	0.075±0.010^{b}	0.115±0.015^{b}	0.080±0.006^{a}	0.086±0.010^{a}	0.083±0.006^{a}
	Co	n.d.	n.d.	0.025±0.001^{a}	0.042±0.002^{b}	0.031±0.002^{a}	0.053±0.004^{b}
	Ni	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Macroelement	Na	382.56±12.88^{a}	580.44±18.36^{b}	579.14±8.94^{a}	956.18±25.77^{b}	1085.84±5.28^{a}	1636.44±38.53^{b}
	K	2812.51±35.28^{b}	2365.99±32.47^{a}	2129.07±17.42^{a}	2256.54±20.29^{b}	1758.55±28.07^{b}	1554.63±40.33^{a}
	Mg	262.05±2.89^{b}	227.43±4.64^{a}	200.35±1.80^{a}	207.59±3.62^{b}	179.38±2.74^{b}	164.30±3.37^{a}
	Ca	47.40±3.28^{a}	65.07±2.57^{b}	68.34±2.84^{b}	59.05±4.94^{a}	45.27±1.37^{a}	164.79±12.26^{b}
Toxic element	Cd	n.d.	n.d.	0.051±002^{a}	0.225±0.030^{b}	0.220±0.008^{a}	1.612±0.229^{b}
	Pb	n.d.	n.d.	0.008±0.001^{a}	0.019±0.001^{b}	0.006±0.001^{a}	0.016±0.001^{b}
	Hg	n.d.	n.d.	n.d.	0.003±0.001	0.009±0.001^{a}	0.018±0.002^{b}
	As	0.0058±0.0007^{a}	0.0050±0.0003^{a}	0.008±0.001^{a}	0.009±0.001^{a}	0.006±0.001^{a}	0.019±0.001^{b}

*The values are presented as mean ± SE, $^{a-b}$ different superscripts within the same row for the same tissue (found in I and E), indicate significant differences of means, according to Tukey's HSD test ($p<0.05$); n.d.- not detected

Table 3. Mineral composition and toxic element levels in feed and soil (mean ± SE) in intensive (I) and extensive (E) pig farms.

Table 3

		I	E			
		Concentrate	Soil	Forage[a]	Complementary feed[b]	
[mg kg^{-1}]					Corn	Wheat
Microelement	Fe	109.67±11.10	17463.19±1941	192.78±28.40	40.45±2.10	35.48±2.00
	Zn	127.47±10.10	57.25±8.30	18.75±3.10	21.86±1.05	27.29±1.40
	Cu	21.21±1.05	18.60±3.60	6.91±0.95	3.75±0.35	5.62±0.60
	Mn	26.12±2.40	702.27±69.40	264.88±30.25	23.31±1.75	41.70±2.35
	Se	0.135±0.03	21.90±4.20	0.097±0.010	0.045±0.005	0.008±0.002
	Cr	0.198±0.02	38.04±8.15	7.82±1.00	0.150±0.010	0.082±0.010
	Co	0.061±0.005	9.32±1.10	0.13±0.002	0.014±0.004	0.014±0.003
	Ni	2.04±0.150	32.95±7.95	0.68±0.05	0.55±0.060	0.42±0.050
Macroelement	Na	1550.25±127.60	311.15±37.10	174.69±25.70	30.38±3.20	40.24±3.70
	K	6881.36±405.10	3903.57±350.20	3519.19±394.10	3641.41±308.10	4368.92±356.40
	Mg	1618.38±105.25	3818.64±410.60	1260.16±158.20	915.11±85.60	1042.64±75.20
	Ca	5066.77±310.20	5695.39±610.20	4592.37±510.30	281.20±25.40	428.30±31.30
Toxic element	Cd	0.034±0.009	0.247±0.035	0.131±0.040	0.014±0.003	0.023±0.006
	Pb	0.120±0.010	16.588±1.58	0.602±0.094	0.046±0.017	0.042±0.015
	Hg	0.012±0.003	0.041±0.010	0.018±0.004	0.009±0.003	0.007±0.002
	As	0.039±0.005	4.425±0.95	0.204±0.032	0.008±0.003	0.005±0.001

[a] Forage samples consisted of a pooled sample of acorn, pasture, stubble and other forestry products.

[b] Complementary feed consisted of a pooled sample of cereal mixture (corn and wheat).

Figure 1. Dendrogram of mineral composition and toxic element levels in muscle, liver and kidney for two pig types.

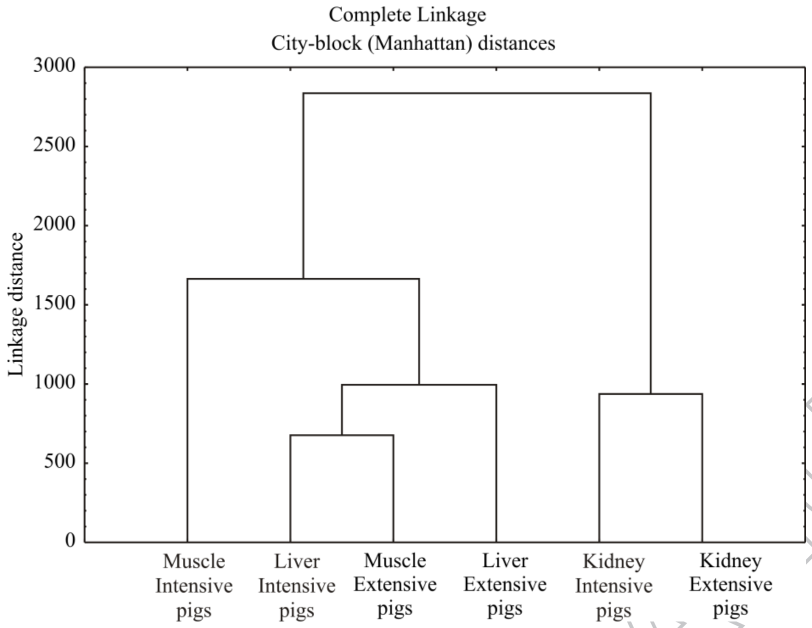

Figure 2. Biplot of mineral composition and toxic element levels in muscle, liver and kidney for two pig types.