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Original Scientific Paper

Levels of Cu, Zn, Fe and Mn in livers of different species of animals in Serbia

Jasna Đinović-Stojanović[1], Saša Janković[1], Ivana Branković Lazić[1], Danijela Vranić[1], Damjan Gavrilović[1] and Munevera Begić[2]*

[1] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040 Belgrade, Serbia*
[2] *University of Sarajevo, Faculty of Agriculture and Food Science, Zmaja od Bosne 8, 71 000 Sarajevo, Bosnia and Herzegovina*

A R T I C L E I N F O

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A B S T R A C T

Liver samples (n=67) of chicken, pork, beef and lamb were analyzed for levels of Cu, Zn, Fe and Mn. Livers were gathered from different meat processing facilities within the Serbian National Residue Monitoring Program in 2024. Analysis of micro elements was performed by inductively coupled plasma mass spectrometry (ICP-MS). Our data show that the mean level of Cu was significantly higher in lamb liver, while Zn and Fe were significantly higher in pork liver. The mean Mn levels determined in the liver samples were not statistically different between the animal species. The distribution of the elements in liver samples was studied by applying principal component analysis (PCA). The differing mineral status in livers of different species of animals implies differences in their nutritional benefits for the human diet.

1. Introduction

Animal liver is a major storage organ both for trace elements and heavy metals. It can accumulate higher concentrations of toxic elements than most other foods and may pose health risk to humans (*Potthast,* 1993). Some heavy metals and metalloids are always present in livers and are often essential to health, even though they are toxic at high concentrations (*Nriagu* et al., 2009; *Counotte at al.,* 2019). The levels of trace elements in animals' livers is of interest for the proper functioning of humans and animals (*Kicińska et al.,* 2019; *Klich et al.,* 2023). Inadequate intake of these elements may be harmful to animal health and meat production potential (*Suttle,* 2010). Micronutrients, such as Fe, Zn, Mn, B, Cu, Mo, Cl and Ni, play an important role in human body tissues. They are mostly involved in the functioning of enzymes, as cofactors or metal activators. Their deficiencies result in serious health and nutritional consequences in humans, causing dietary micronutrient malnutrition (*Guan et al.,* 2024; *Xu et al.,* 2025).

Scientific information about Cu, Zn, Fe and Mn levels in livers of different species of animals in Serbia is limited (*Tomovic et al.,* 2011; *Nikolic et al.,* 2017; *Subotic et al.,* 2017). The aim of this study was to determine and compare the distribution of Cu, Zn, Fe and Mn in livers of chicken, pork, beef and lamb which were gathered from different meat processing facilities within the Serbian National Residue Monitoring Program in 2024.

***Corresponding author:** Jasna Đinović-Stojanović, *jasna.djinovic@inmes.rs*

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2. Materials and methods

2.1. Sample collection and preparation

A total of 69 liver samples of chicken (n=21), pork (n=23), beef (n=17) and lamb (n=6) were examined. Liver samples were acquired during regular slaughter and analyzed within the Serbian National Residue Monitoring in 2024. After collection, livers were labelled and stored in polyethylene bags and frozen at -18 °C. Frozen livers were thawed at 4 °C and homogenized, then approximately 0.5 g (wet weight) of sample was mineralized by adding 5 mL of nitric acid (67–70%, TraceMetal grade, Fisher Chemical, Belgium) and 5 mL deionized water, purity of 0.067 µS/cm, produced by a Purelab DV35 water purification system (ELGA, Buckinghamshire, UK). Microwave assisted digestion was performed in a MARS 6 iWawe Microwave Digestion System (CEM Technology, USA). After cooling at room temperature, the digests were quantitatively transferred into polypropylene volumetric flasks and diluted to 100 mL with deionized water.

2.2. Quality assurance

Inductively coupled plasma mass spectrometry (ICP-MS), (iCap Q mass spectrometer, Thermo Scientific, Bremen, Germany), was used for analysis of the ^{63}Cu, ^{66}Zn, ^{57}Fe and ^{55}Mn isotopes. The quality of the analytical process was verified by analysis of the certified reference material NIST 1577c (bovine liver, Gaithersburg, MD, USA). Reference material was prepared in the same way as test samples using microwave digestion. The obtained concentrations were in the range of certified values.

2.3. Statistical analysis

Statistical analysis of experimental data was performed using software Statistica 10.0 (StatSoft Inc., Tulsa, OK, USA). One-way analysis of variance (ANOVA) and Tukey's HSD test for comparison of means were used to analyze differences in the elements' levels in livers from different animal species. Principal component analysis (PCA) was used to group the observed samples and to discover the possible correlations among the element levels.

3. Results

3.1. Contents of micro elements

Contents of Cu, Zn, Fe and Mn measured in the analyzed chicken, pork, beef and lamb livers, expressed as mean±standard deviation, are presented in Table 1. Different superscripts within the same row indicate significant differences of mean element levels according to Tukey's HSD test ($p < 0.05$).

3.2. Principal component analysis (PCA)

PCA was applied to the correlation matrix, which included the four parameters of four sample groups of livers from the different animal species (*Hammer et al., 2001*). PCA was applied to group the observed the possible correlations among the measured Cu, Zn, Fe and Mn levels and the animal species (chicken, pork, beef and lamb liver) (Figure 1).

Table 1. Content of Cu, Zn, Fe and Mn (mg/kg; mean±standard deviation) in chicken, pork, beef and lamb livers.

Element	Chicken liver, n=21	Pork liver, n=23	Beef liver, n=17	Lamb liver, n=6
Cu	4.25^{a}±1.74	8.70^{a}±4.51	58.12^{b}±26.43	121.74^{c}±46.89
Zn	39.47^{a}±12.71	77.39^{b}±21.21	40.35^{a}±7.97	44.49^{a}±5.96
Fe	118.12^{a}±27.37	233.38^{b}±89.07	60.36^{a}±37.60	66.26^{a}±18.43
Mn	3.05^{a}±0.65	3.56^{a}±0.77	3.01^{a}±0.41	3.04^{a}±1.37

$^{a-c}$ *Values within the same row with different superscripts are significantly different (p<0.05).*

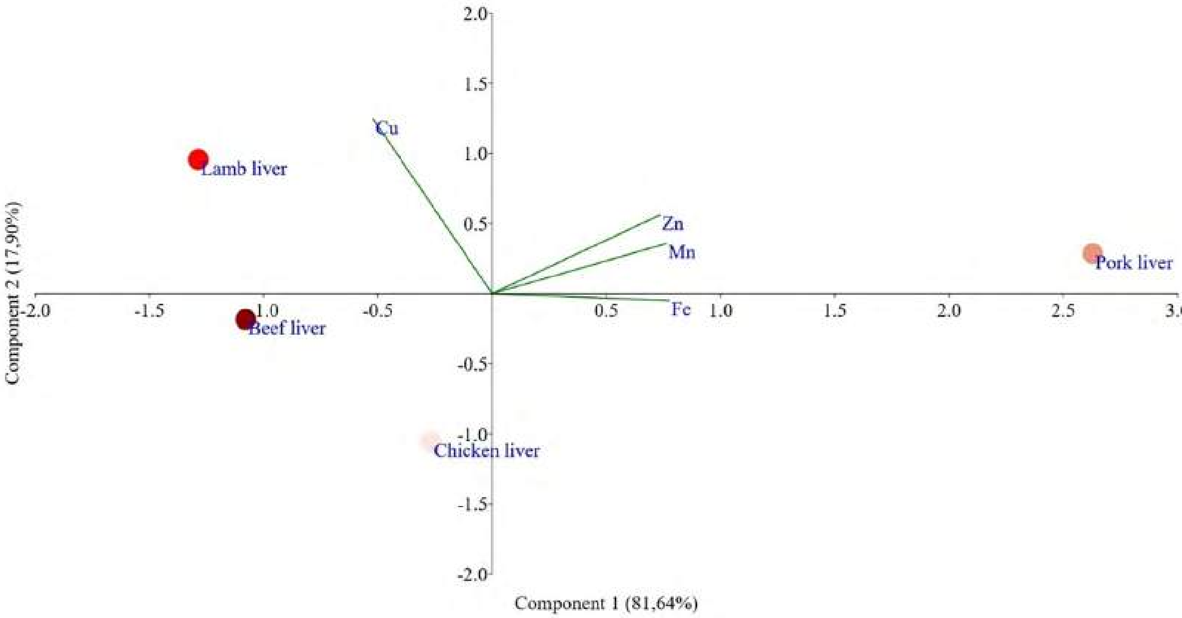

Figure 1. Bi-plot of principle component analysis (PCA) of Cu, Zn, Fe and Mn levels in livers from different animal species.

4. Discussion

4.1. Micro elements

The highest mean level of Cu was quantified in lamb liver (121.74 mg/kg) compared with significantly lower levels present in chicken, pork and beef liver. Levels of Cu were significantly higher in beef liver compared to chicken and pork liver. The mean Cu levels in beef (58.12 mg/kg) and pork (8.70 mg/kg) liver were similar to the average level reported by *Nriagu et al.* (2009) (63.9 mg/kg) and *Sola et al.* (1997) (6.1 mg/kg), respectively.

The highest mean Zn (77.39 mg/kg) and mean Fe (233.38 mg/kg) levels were established in pork liver, and they were significantly higher compared to other analyzed liver samples. The obtained levels were lower than those established in our previous investigation i.e., in livers of intensive and extensive pigs from Serbia, except for the Zn level in livers of extensive pigs (54.27 mg/kg) (*Nikolic et al.*, 2017). The mean levels of Zn and Fe in pork liver in the current study were similar to that reported by *López-Alonso et al.,* (2007) (they reported Zn – 81.3 mg/kg; Fe – 195 mg/kg).

The mean Mn levels (3.01-3.56 mg/kg) determined in liver samples were not statistically different between the four food animal species. Similar mean Mn levels were found by *Nikolic et al.*, (2017) and *López-Alonso et al.,* (2007) in pig liver, but our results for pork and chicken liver were higher than those reported by *Sola et al.* (1997) (they reported Mn – 1.2 mg/kg, pig liver; Mn – 0.8 mg/kg, duck liver).

4.2. PCA analyses

The first two components (PC1 and PC2) in the PCA resulting from the examination of the levels of Cu, Zn, Fe and Mn in liver from different animal species accounted for 99.54% of the total variance (PC1 81.64%, PC2 17.90%). The influence of different parameters that described the examined liver samples can be evaluated from Figure 1. The examined livers from the different animal species are located on different sides of Figure 1. Pork liver, in which the highest Zn, Mn and Fe levels were observed, is located on the upper right side of the figure. Lamb, beef and chicken liver are located on the opposite side of the figure. Lamb liver, in which the highest mean Cu level was observed in comparison with other livers, is located highest up on the upper left side (Figure 1).

5. Conclusion

This study aimed to provide information on levels of Cu, Zn, Fe and Mn in livers from different animal species (chicken, pork, beef and lamb) collected during the Serbian National Residue Monitoring Program in 2024. The concentration of Cu was significantly higher in lamb liver, while concentrations of Zn and Fe were significantly higher in pork liver. While most other authors researching this topic have published element levels in pork liver, this work highlights the importance of analyzing livers from different species of animals. Moreover, the differences seen in micro element levels imply differences in the benefits of the livers from domestic animal species for the human diet.

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Authors info

Jasna Đinović-Stojanović, https://orcid.org/0000-0003-4602-0835
Saša Janković, https://orcid.org/0000-0002-5223-6993
Ivana Branković Lazić, https://orcid.org/0009-0005-5844-9278
Danijela Vranić, https://orcid.org/0000-0002-7192-488X
Damjan Gavrilović, https://orcid.org/0009-0000-3786-496X
Munevera Begić, https://orcid.org/0000-0002-9425-8568