

## PRELIMINARY RESEARCH OF SELENIUM AND COPPER CONCENTRATIONS IN BEEF CATTLE FROM DIFFERENT FARM TYPES

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**Abstract:** To maintain proper beef cattle health and performance, it is essential to provide adequate amounts of trace elements in their diet. A growing body of research has recently assessed the interaction between different microelements and macroelements, especially those needed by the body; such knowledge is important for assessing differences in their bioavailability. The aim of the study was to compare the selenium and copper content in the serum of cows raised on various types of farms, and to examine the interaction between these elements. Selenium content was determined using spectrofluorometry (Shimadzu RF-5001 PC spectrofluorimeter), while copper levels were studied using colorimetry (Marcel Media spectrophotometer). Statistical analysis was performed using Statistica 13.3 PL software. The accuracy of the analytical method was determined using Seronorm Trace Elements Whole Blood L-2 as reference material. The recovery ranged from 95 to 97% of the reference value. Our results found beef cattle from conventional farms to be characterized by higher serum levels of selenium ( $0.09 \pm 0.034$  vs.  $0.015 \pm 0.057 \mu\text{g}\cdot\text{ml}^{-1}$ ) and copper ( $0.834 \pm 0.14$  vs.  $0.658 \pm 0.176 \mu\text{g}\cdot\text{ml}^{-1}$ ) than those from organic farms. In addition, no statistically significant correlation was found between Se and Cu concentrations for organic or conventional farms. In order to ensure optimal coverage of the demand for essential elements, animal supplementation is recommended.

**Key words:** beef cattle, conventional farm, copper, organic farm, selenium.

## INTRODUCTION

Some trace elements, such as selenium, copper, magnesium, iron, zinc, and chromium, are needed for the proper functioning of the body. In addition to performing various regulatory, structural, and catalytic functions, they also influence the activity of the immune and reproductive systems, and play key roles in the fetal development of farm animals (Miroshnikov et al. 2017). Antioxidant vitamins and minerals protect the body by directly capturing free radicals or inhibiting the activity of oxidizing enzymes (Yang and Li 2015).

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Vitamin and mineral deficiency, usually due to a low supply of minerals in the diet or the presence of antagonistic factors, impairs the absorption or metabolism of nutrients and reduces the reproductive and productive capacity of animals (Karkoodi et al. 2012; Paolicchi et al. 2013); however, reproductive performance, healthy fetal development, and immunity can be promoted by enriching the diet with supplements. Micronutrient supplementation is known to alleviate bacterial infections associated with mastitis, reduce the number of somatic cells in milk, and minimize the risk of metabolic diseases caused by a negative energy balance (Żarczyńska et al. 2017). In contrast, excessive exposure to some essential trace elements in ruminants may reduce the bioavailability of other elements. Therefore, it is important to monitor the status of trace elements in cattle in order to prevent disease and maintain high performance (Miroshnikov et al. 2017).

One of the most important tasks in beef cattle management is to achieve the correct balance between trace elements in the diet. Proper nutrition is important for cattle production, and mineral status affects the entire body (Davy et al. 2019). In addition, animal supplementation can also increase the supply of selected nutrients in animal products, which can serve to provide a better source of micro and macro elements for consumers (Haug et al. 2018).

One of the most important elements in beef production is selenium (Se), which is needed by a number of physiological processes. Se excess and deficiency both have adverse effects on the body. Se deficiency is often associated with reproduction problems, including the death of fetuses, retention of the placenta, and impairment of reproductive capacity. It is also known to result in muscle degeneration and spasms, as well as diarrhea in young cattle (Kurek et al. 2011). Ruminants are particularly prone to low levels of Se, especially beef cattle. Such deficiencies have been attributed to grazing without the provision of additional supplementation, as well as the poor bioavailability of Se (Sgoifo Rossi et al. 2017; Surai et al. 2019).

Like Se, copper (Cu) is an important element in the rearing of beef cattle. Cu deficiency and excess both lead to physiological disorders of the body. It takes part in the synthesis of hemoglobin by triggering iron (Fe) reserves and the production of red blood cells. It also participates in cholesterol metabolism and bone mineralization, and regulates the synthesis of elastin and collagen (Klebaniuk and Grela 2008). Low Cu levels are associated with malnutrition, nephrotic syndrome, and anemia, as well as low Fe levels in the body. The symptoms of deficiency may include stiff gait, and swelling of the knees and ankles. In turn, excessive Cu concentrations have been associated with hyperthyroidism and hypothyroidism, chronic infections, and hemochromatosis (Onmaz et al. 2018; Mattioli et al. 2019). Furthermore, low Se and zinc (Zn) levels, and elevated serum Cu levels have been associated with rheumatoid arthritis (Ma et al. 2019), while Cu and Se deficiency are correlated with lameness in heifers (Edmondson et al. 2013).

A growing number of recent studies have examined the interaction between various microelements and macroelements, especially those needed by the body. This knowledge is crucial for assessing the differences in their bioavailability. Interestingly, the relationship between Se and Cu in living organisms demonstrates both antagonism and synergism. Se can ameliorate the toxic effects of excess Cu by locking it into metabolically-inactive conjugates. In a similar way, Cu can limit the toxic effects of Se by limiting its absorption and causing the accumulation of its non-toxic compounds in tissues. However, both elements are essential components of the antioxidant enzymes glutathione peroxidase (Se) and superoxide dismutase (Cu), both of which participate in protecting the body against oxidative stress caused by the presence of free radicals. Therefore, it is necessary to maintain a balance between the two elements to ensure homeostasis (Apsite et al. 2012).

The purpose of this study was to compare the Se and Cu content in the serum of cows raised on organic and conventional farms, and to identify any interactions between these elements.

## MATERIAL AND METHODS

### Samples

The research material consisted of random tail-vein blood samples taken from Limousine beef cattle that were kept on an organic farm and on a conventional farm in the Zachodniopomorskie Voivodeship. The study included material from cows. The cows were dry. A total of 30 blood samples were taken, 15 from the organic farm animals and 15 from the conventional farm animals. The material was collected in sterile tubes, which were then centrifuged after clotting to obtain serum. The samples were stored for 10 days at a temperature of  $-20^{\circ}\text{C}$  until analysis. Five samples from the conventional farm's animals traces of hemolysis were excluded.

The material was obtained in spring 2020 during the spring grazing of animals. Quarter grazing was used on farms. In accordance with good agricultural practice, animals were grazed at the time of the so-called pasture maturity, i.e. after the plants reach a height of 15–18 cm. Pastures were fertilized with manure every 4 years. It is assumed that by using this practice, animals are able to meet their nutritional needs. Animals from the organic farm were kept in a pasture and stall system. From early spring to late autumn, they stayed on the pasture during the day and were kept in livestock buildings at night. At the time of material collection, the cattle from the organic farm were fed green forage, and the buildings contained straw. The material came from animals aged 24–30 months. The animals from the conventional farm were also kept in a pasture and stall system; however, during the grazing period, they were maintained on pasture throughout the day. The cattle were fed green forage and straw. All material was obtained from animals aged 18–22 months. During the research period, no mineral supplements were used for cattle on both farms.

The blood was obtained during routine veterinary examinations. As the study was performed in accordance with the Polish law on livestock breeding (Resolution No. 22/2006 of the National Commission for the Ethics of Experiments on Animals, November 7, 2006), no consent was required from the local Ethics Committee to perform the study.

### Reagents

All reagents used in the tests were of analytical grade. Most were obtained from Chempur® (Piekary Śląskie, Poland); however, the 2,3-diaminonaphthalene (DAN) was purchased from Merck KGaA (Darmstadt, Germany).

### Chemical analysis

Total Se concentration was determined by spectrofluorometry using a Shimadzu RF-5001 PC spectrofluorimeter (Shimadzu Corporation, Tokyo, Japan) (Pilarczyk et al. 2009). Serum samples were digested in nitric acid V at  $230^{\circ}\text{C}$  for 180 minutes and then in perchloric acid at  $310^{\circ}\text{C}$  for 20 minutes. Selenium ( $\text{Se}^{6+}$ ) was reduced to selenium ( $\text{Se}^{4+}$ ) using 9% hydrochloric acid. Ethylenediaminetetraacetic acid (EDTA) and hydroxylamine were used as masking agents. The selenium concentration was then determined using 2,3-diaminonaphthalene at pH 1–2 by the formation of selenodiazole complex. This complex was extracted into cy-

clohexane, and the Se content was determined spectrofluorometrically at wavelengths of 376 nm for excitation and 518 nm for fluorescence.

The serum copper content was determined by direct colorimetry using 3,5-DiBr-PAESA and a Marcel Media spectrophotometer. A BioMaxima reagent kit was used for analysis.

The accuracy of the analytical method was determined using Seronorm™ Trace Elements Whole Blood L-2 as reference material. The recovery ranged from 95 to 97% of the reference value. All determinations of Se and Cu concentrations were performed in duplicate.

### Statistical data analyses

The results were statistically analyzed using Statistica 13.3 PL software. The results included arithmetic means, standard deviation, and minimum and maximum values (range). The normality of the data distribution was determined using the Shapiro-Wilk test. All measured sets of data were found to have a normal distribution ( $p > 0.05$ ). One-way ANOVA and Duncan's test were used to assess the differences in Se and Cu concentrations between farms. The correlation between Se and Cu serum concentrations for the different farms was determined using Pearson's ( $r$ ,  $r^2$ ,  $p$ ,  $y$ ) correlation coefficient.

## RESULTS AND DISCUSSION

Our findings indicate that the beef cattle from the organic farm had significantly lower serum Se concentrations ( $0.015 \pm 0.057 \mu\text{g}\cdot\text{ml}^{-1}$ ) than those from the conventional farm ( $0.09 \pm 0.034 \mu\text{g}\cdot\text{ml}^{-1}$ ) ( $p < 0.05$ ), i.e., six times lower for the organic farm than for the conventional farm (Table 1).

Table 1. Serum concentrations of the elements in beef cattle

Elements	Element concentration ( $\mu\text{g}\cdot\text{ml}^{-1}$ )		p value
	conventional farm	organic farm	
Selenium (Se)			
mean	0.09	0.015	0.00015
median	0.0336	0.0057	
range	0.033–0.128	0.007–0.028	
Copper (Cu)			
mean	0.834	0.658	0.1833
median	0.1398	0.1763	
range	0.64–1.081	0.487–1.083	

However, various recommendations exist in the literature regarding the suitable concentration of Se in the blood. For example, Dargatz et al. (1996) propose an appropriate range of 0.08 to 0.16  $\mu\text{g}\cdot\text{ml}^{-1}$ , while Villarda et al. (2002) suggest a range from 0.51 to 0.85  $\mu\text{g}\cdot\text{ml}^{-1}$ . The present study uses the standards published by Puls (1994), according to which, the beef cattle from the organic farm could be regarded as being selenium deficient, while those from the conventional farm would have selenium levels that are optimal or marginal (Table 2).

Table 2. Percentage of animals (%) with deficient, marginal, or optimal Cu and Fe concentrations ( $\mu\text{g}\cdot\text{ml}^{-1}$ ) in serum, based on Puls (1994)

Elements	Deficient	Marginal	Normal
Selenium	0.002–0.03	0.03–0.08	0.08–0.3
conventional farm	0	40	60
organic farm	100	0	0
Copper	0.1–0.55	0.55–0.8	0.8–1.5
conventional farm	0	30	70
organic farm	6.67	66.67	26.67

In the case of Cu, the mean concentration was  $0.658 \pm 0.176 \mu\text{g}\cdot\text{ml}^{-1}$  on the organic farm and  $0.834 \pm 0.14 \mu\text{g}\cdot\text{ml}^{-1}$  on the conventional farm (Table 1). Again, based on Puls (1994), the cattle from the organic farm could be regarded as deficient, marginal, or optimal for Cu, while those from the conventional farm demonstrate marginal or optimal levels (Table 2).

Similar levels of Se and Cu were reported by Miranda et al. (2019), i.e.,  $0.078\text{--}0.112 \mu\text{g}\cdot\text{ml}^{-1}$  for Se and  $0.658\text{--}0.896 \mu\text{g}\cdot\text{ml}^{-1}$  for Cu. Other studies have noted concentrations of  $0.076 \mu\text{g}\cdot\text{ml}^{-1}$  for Se and  $0.601$  for Cu (Denholm et al. 2019), or  $0.048 \mu\text{g}\cdot\text{ml}^{-1}$  (Se) and  $0.91 \mu\text{g}\cdot\text{ml}^{-1}$  (Cu) (Abuelo et al. 2016). These findings correspond to those of the present study. In contrast, Pereira et al. (2018) reported a significantly higher Se concentration than in the present study ( $0.268 \pm 0.070 \mu\text{g}\cdot\text{ml}^{-1}$ ) but a similar Cu concentration ( $0.812 \pm 0.063 \mu\text{g}\cdot\text{ml}^{-1}$ ).

In addition, Onmaz et al. (2018) recorded significantly lower mean concentrations of Se ( $0.03 \mu\text{g}\cdot\text{ml}^{-1}$ ) and Cu ( $0.017 \mu\text{g}\cdot\text{ml}^{-1}$ ) for a conventional farm compared to the present study. This may be due to the presence of other components in the feed that limit the absorption of both Se and Cu (Blanco-Penedo et al. 2009; Niwińska and Andrzejewski 2014). In addition, micronutrient deficiencies are common in ruminants, especially grazing animals that have access only to pasture and other naturally-occurring plants that represent their staple diet (Han et al. 2016). Further studies by Denholm et al. (2019) suggest that diet has a significant effect on the concentrations of microelements in animal serum.

Significant differences in serum Se concentrations were observed in the present study between beef cattle kept on organic farms and those kept on conventional farms ( $p < 0.05$ ). However, no statistically significant differences were found in the Cu concentrations between the two groups ( $p > 0.05$ ). Orjales et al. (2018) also reported differences in both Se and Cu levels between organic and conventional farms. This may be related to the farming management system and the greater potential for controlling agricultural production on conventional farms, which is regulated by law; however, no difference was reported between organically- and conventionally-farmed cattle with regard to Se or Cu levels, despite a high level of variability being observed among organic farms (Orjales et al. 2018). This suggests that organic production largely depends on specific local conditions.

In the present study, no statistically significant correlation was observed between Se or Cu concentrations for either the organic or conventional farms ( $p > 0.05$ ) (Table 3). Similarly, no such correlation was noted by Davy et al. (2019) ( $r = -0.12$ ,  $p = 0.01$ ) or Denholm et al. (2019) ( $r = 0.11$ ,  $p > 0.05$ ). However, in contrast, García-Vaquero et al. (2011) observed a strong positive correlation ( $r = 0.857$ ) between Se and Cu levels in meat from beef cattle.

Table 3. Correlation between Se and Cu concentrations in serum from farmed cattle

Correlation	r	r <sup>2</sup>	p	y
Conventional farm				
Se:Cu	-0.038	0.01	0.9232	0.8113 + 0.168*x
Organic farm				
Se:Cu	-0.261	0.0685	0.366	0.829 - 7.2009*x

These findings indicate that cattle raised on organic farms may be more exposed to mineral deficiencies than those on conventional farms. This may be due to the existence of legal restrictions that clearly define permitted methods of feeding and the possibility of regulating levels using mineral feed additives; however, it would also be determined by the vegetation available to the animals during grazing. This pasture is the main component in the diet; however, its microelement and macroelement composition may vary considerably depending on the geographical location of the farm. In addition, our findings indicate that there is no statistically significant correlation between Se and Cu. It seems appropriate, however, to undertake further studies in this regard, particularly considering the fundamental importance of both Se and Cu in the correct development and maintenance of homeostasis in cattle.

## CONCLUSIONS

The results show that the Se content was six times higher in the serum of animals from a conventional farm than from an organic farm (0.09 vs. 0.015 µg·ml<sup>-1</sup>). For copper, the difference was smaller. The average Cu concentrations for animals from the conventional farm was 0.834 µg·ml<sup>-1</sup> and from the organic farm 0.658 µg·ml<sup>-1</sup>. The higher element concentrations found on conventional farms may be due to the elements' bioavailability to plants being higher, which is related to the physico-chemical properties of the soil and different plant cover in the pasture. In addition, animal supplementation with Se mineral salts is recommended on organic farms. For copper, the use of copper oxide, copper sulfate pentahydrate, or basic copper carbonate monohydrate is allowed. However, the above studies indicate that these practices were not applied on organic farms, which negatively affected the levels of the elements being tested. Although knowledge about the demand for Se and Hg in cattle has already been well established, the problem of deficiency of these elements still exists. On organic farms, there is a greater problem with the deficiency of micro and macro elements in the feed. This may be related to legal restrictions, therefore special attention should be paid to supplementation with minerals approved for organic production. It is recommended to conduct experiments on broader research material.

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## **BADANIA WSTĘPNE NAD STĘŻENIEM SELENU I MIEDZI U BYDŁA MIĘSNEGO POCHODZĄCEGO Z RÓŻNYCH TYPÓW GOSPODARSTW**

**Streszczenie.** Odpowiednia zawartość pierwiastków śladowych u bydła jest bardzo ważna dla utrzymania prawidłowego stanu zdrowia oraz wydajności. W ostatnich latach coraz częściej podejmowane są badania mające na celu ocenę interakcji między różnymi mikro- i makroelementami, zwłaszcza tymi niezbędnymi dla organizmu. Ma to znaczenie dla oceny różnic w ich przyswajalności. Celem pracy było porównanie zawartości selenu i miedzi w surowicy krów hodowanych w różnych typach gospodarstw oraz interakcji między tymi pierwiastkami. Do analizy selenu wykorzystano metodę spektrofotometryczną i posłużono się spektrofotometrem Shimadzu RF-5001 PC, natomiast do analizy miedzi wykorzystano metodę kalorymetryczną i spektrofotometr Marcel Media. Wyniki analizowano statystycznie za



pomocą oprogramowania Statistica 13.3 PL. Dokładność metody analitycznej określono na podstawie materiału referencyjnego Seronorm Trace Elements Whole Blood L-2. Odzysk wynosił od 95 do 97% wartości odniesienia. Wykazano, że bydło mięsne z gospodarstw konwencjonalnych charakteryzowało się wyższym stężeniem selenu ( $0,09 \pm 0,034$  vs.  $0,015 \pm 0,057 \mu\text{g}\cdot\text{ml}^{-1}$ ) i miedzi ( $0,834 \pm 0,14$  vs.  $0,658 \pm 0,176 \mu\text{g}\cdot\text{ml}^{-1}$ ) w surowicy niż bydło pochodzące z gospodarstw ekologicznych. Dodatkowo nie zaobserwowano istotnych statystycznie korelacji pomiędzy stężeniem Se i Cu w gospodarstwach. W celu zapewnienia optymalnego pokrycia zapotrzebowania na pierwiastki niezbędne zaleca się suplementację zwierząt.

**Słowa kluczowe:** bydło mięsne, gospodarstwo konwencjonalne, miedź, gospodarstwo ekologiczne, selen.