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## Determining Trace Elements in the Hair of Beef Cattle as a Non-Invasive Method for Assessing Mineral Metabolism

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**Abstract** | The aim of the study is to assess the trace element status of cows by using hair mineral content as a non-invasive method and to identify correlations between the trace element levels in hair and blood serum in animals. The experiment was conducted on 3-year-old cows of the Ukrainian beef breed raised in the same biogeochemical region. The methodology included determining trace elements in hair samples taken from various body areas and in blood serum using electrothermal atomic absorption spectroscopy (ET-AAS). The study revealed significant differences in the concentration of trace elements in hair depending on the season (grazing and stall-feeding) and the sampling site. In conclusion, the correlation analysis showed strong associations between various trace elements under both feeding conditions. The results can contribute to improved monitoring and correction of the trace element status in cattle.

**Keywords** | Beef cattle, Blood, Trace elements, Hair samples, Cattle housing systems

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## INTRODUCTION

The exposure of livestock to trace elements has significant implications for human health. Meat and meat products, particularly by products, are major sources of trace elements, including toxic ones. The concentration of these elements in meat is directly related to their levels in animal feed. The addition of certain trace elements, especially those like cobalt (Co), iodine (I), and selenium (Se), for which there are no intestinal homeostatic absorption mechanisms, can lead to over-enrichment of animal products.

It is well established that trace elements are essential for

many biochemical processes in the body. They are active components of various enzymes, participate in biological processes, and influence both the health and productivity of livestock. Maintaining an optimal micronutrient balance in the diet is crucial for the normal functioning of the body, particularly in structural, physiological, catalytic, and regulatory processes.

Imbalances in both essential and toxic micronutrients pose a serious threat to animal health and productivity (Roug *et al.*, 2015). Cygan-Szczegielniak (2021) found significant correlations between element levels and their variability in different tissues and organs, noting that the accumulation of a particular metal in one tissue may reduce

its concentration in another.

Evaluating the exchange of chemical elements in the body is typically done by determining their concentrations in various tissues and organs, or indirectly, through studies of the biochemical processes in which these elements are involved. Unlike liquid bio substrates like blood, serum, and urine, hair reflects the elemental status of the organism over a period of 3-6 months and is recommended for biomonitoring large populations as well as for clinical diagnostics.

Wool, as a product of the mammalian epidermis, serves several functions depending on its structure and arrangement (Cygan-Szczegielniak and Stasiak, 2022). In animals, it primarily protects the skin from environmental factors and serves a thermoregulatory function, with its composition varying by season. The quality of wool is influenced by species and nutrition, with protein, minerals, and vitamins playing crucial roles (Patra *et al.*, 2006). Nutritional factors that impact hair growth, anatomical structure, and mineral composition are complex and interrelated, with micronutrient imbalances being one of the key factors affecting normal growth.

Although the trace element composition of blood is often the first to reflect increased concentrations of heavy metals, it does not provide a true picture of the body's overall heavy metal burden. Hair is considered a more metabolically active bio substrate than blood or urine and provides a long-term record of metabolic activity, including mineral metabolism. This property makes hair an effective medium for assessing micronutrient status (Hurst *et al.*, 2013).

In recent years, using hair to assess the mineral status of animals non-invasively has gained attention due to the ease of sample collection and storage. In contrast, blood samples may be misleading due to the body's homeostatic mechanisms, which can maintain stable blood levels by drawing on internal reserves. The mineral composition of hair reflects a longer-term intake of trace elements than blood, where concentrations can fluctuate daily. Hair is also easier to collect and does not require refrigeration, making it practical for field sampling (Król *et al.*, 2018).

The aim of this study was to determine the correlation between trace element content in serum and wool, with a view to using hair as a non-invasive method to assess the mineral metabolism of beef cattle.

## MATERIALS AND METHODS

### EXPERIMENTAL DESIGN

The research involved the development and validation of

methods for detecting, correcting, and preventing trace element deficiencies in beef cattle by analysing the trace element composition of wool.

In developing the selection methodology, we used wool samples obtained from adult Ukrainian beef cattle (3-years old cows) totalling 20 animals. These animals were homogeneous in breed, live weight, and physiological condition, and were born and reared in the same biogeochemical province (Dnipropetrovsk region, Mahdalynivka district).

The diet provided all the necessary nutrients, trace elements, and vitamins according to the feeding standards for beef cattle (Ibatullin *et al.*, 2007). While the cattle were housed indoors, their main ration consisted of concentrates, legume and cereal hay, straw, maize silage and haylage, and fodder beets. In winter, the animals had free access to automatic drinkers with electric water heating. During grazing periods, the animals consumed green forage.

The study was conducted in accordance with the recommendations of the Declaration of Helsinki and was approved by the Bioethics Commission of Dnipro State Agrarian and Economic University (Protocol No. 1/11, 14 November 2023).

### METHOD FOR DETERMINATION OF TRACE ELEMENTS IN WOOL AND BLOOD OF ANIMALS

Sampling was carried out from the same animals during the grazing (July) and stall periods (December), using a 5×5 cm section of wool. Samples were taken from six locations on the animal's body surface: (1) the occipital part of the head, (2) the upper part of the withers, (3) the sub-chest area, (4) the median projection of the twelfth rib, (5) the projection of the first caudal vertebra, and (6) the tail hand. Wool was cut 0.3 cm from the root using stainless steel scissors treated with ethyl alcohol to eliminate the risk of chemical contamination. For the study, the proximal part of the wool (up to 3 cm long) was selected.

The collected samples were stored in tightly sealed plastic bags in a dry, shaded place until further analysis. Wool contamination was assessed by weighing native wool samples before and after a cleaning process, which involved soaking for three hours in distilled water (50–60 °C), ultrasonic treatment (35 kHz), and subsequent washing with a cleaning solution and bidistilled water.

All wool samples were thoroughly washed before analysis to avoid contamination. Each sample was placed in a separate beaker and cleaned in four steps: (1) immersing the wool in 50 ml of acetone for 15 minutes, (2) rinsing it in 50 ml of distilled water for 15 minutes, (3) immersing

it again in 50 ml of acetone for 15 minutes, and (4) a final rinse with distilled water for 15 minutes. The wool samples were then dried in an oven at 80°C for 4 hours to prepare them for further research.

The concentration of trace elements in both wool and serum was determined using atomic absorption spectroscopy (ET-AAS). The analyses were carried out in a certified laboratory according to the proposed procedures (Chatt *et al.*, 1989).

**STATISTICAL ANALYSIS**

Statistical software package STATISTICA 10 (StatSoft, Inc., Tulsa, OK, USA) was used for statistical data processing. The distribution of almost all variation series did not meet the normality criteria. Therefore, nonparametric statistical methods were used in further analysis. Data are presented as mean and standard deviation. Differences between samples were determined using the Mann-Whitney U test and were considered significant at P<0.05.

**RESULTS AND DISCUSSION**

Determination of trace element concentrations in bio substrates is an important indicator for assessing the trace element status of animals. Especially this issue is relevant in relation to beef cattle, which are kept on pasture for a

long time without appropriate mineral supplementation. Tables 1, 2 show the indices of some trace elements in samples of cow hair both at pasture and stall keeping.

Table 1 shows that the highest concentrations of Mn, I, and Co were found in wool samples from the middle of the last rib (25.98±0.248, 0.70±0.026, and 0.084±0.0090 mg/kg, respectively); Se was highest in wool from the tail root (0.71±0.035 mg/kg); Cr in the withers (0.35±0.045 mg/kg); Cu in wool samples from the back of the head (12.33±0.643 mg/kg); and Zn in wool from the root (113.3±1.269 mg/kg) and the tail brush (113.06±0.712 mg/kg).

When cows were kept in stalls, the content of Mn, I, and Co was again highest in wool samples taken from the middle of the last rib (23.09±0.307 mg/kg; 0.62±0.028 and 0.048±0.0053 mg/kg, respectively); Se was highest in wool from the tail root (0.58±0.002 mg/kg); Cr in wool from the chest area (0.24±0.015 mg/kg); Cu in the tail brush (7.85±0.090 mg/kg); and Zn in wool from the back of the head (103.5±0.675 mg/kg). During grazing, there were positive correlations between Mn and Co (r=0.500; P ≤ 0.01), Cu and Cr (r=0.222; P ≤ 0.05), and Cu and I (r=0.311; P ≤ 0.05). In stalls, positive correlations were observed between Mn and Se (r=0.254; P ≤ 0.05), Mn and Co (r=0.491; P ≤ 0.05), and Se and Zn (r=0.517; P ≤ 0.05).

**Table 1:** Content of trace elements in samples of wool of cows on pasture, mg/kg.

Trace elements	Wool sample location						Average value
	Occiput	Tail root	Middle of last rib	Dewlap	Withers	Tail brush	
Mn	17.01±0.414	20.29±1.455	25.98±0.248	16.21±0.060*	19.10±0.422*	10.42±0.705*	17.71±0.854
Se	0.56±0.033	0.71±0.035	0.61±0.005*	0.32±0.024	0.58±0.029	0.62±0.022*	0.47±0.062
Cr	0.24±0.024	0.23±0.038	0.22±0.004*	0.28±0.015	0.35±0.045	0.19±0.042	0.31±0.016
Cu	12.33±0.143*	3.98±0.365	6.74±0.225*	8.43±0.546	7.97±0.234	8.56±0.115	8.31±2.016
I	0.59±0.085	0.66±0.002*	0.70±0.026*	0.56±0.073	0.62±0.033	0.54±0.009	0.54±0.007
Zn	109.8±0.475*	113.3±1.269	111.4±1.388**	110.7±1.734*	107.5±0.566	113.06±0.712	116.6±3.286
Co	0.062±0.0018	0.049±0.0039	0.084±0.0090*	0.044±0.0020	0.056±0.0015	0.059±0.0016	0.060±0.0027

\*P ≤ 0,05 relative to the average sample.

**Table 2:** Content of microelements in wool samples of cows kept in stalls, mg/kg.

Trace elements	Wool sample location						Average value
	Occiput	Tail root	Middle of last rib	Dewlap	Withers	Tail brush	
Mn	14.94±0.062*	16.23±0.115	23.09±0.307	14.64±0.214	17.92±0.345*	15.71±0.183	16.19±0.548
Se	0.45±0.018*	0.58±0.002	0.53±0.007	0.25±0.020*	0.56±0.004*	0.57±0.005	0.59±0.024
Cr	0.16±0.013	0.16±0.005	0,19±0.005	0.24±0.015	0.15±0.017*	0.12±0.011*	0.23±0.020
Cu	7.30±0.215	5.65±0.223	5.99±0.087*	7.20±0.279	7.65±0.220	7.85±0.090	7.07±0.211
I	0.47±0,023	0.54±0.025	0.62±0.028*	0.43±0.024	0.49±0.013	0.47±0.013	0.50±0.016
Zn	103.51±0.675	100.71±1.269	102.09±0.520	96.83±1.320*	102.51±0.613	102.75±0.547	103.4±0.904
Co	0.063±0.0019	0.050±0.0038	0.072±0.0085*	0.047±0.0031	0.048±0.0053	0.060±0.0029	0.062±0.0027

Note: \*P ≤ 0,05 relative to the average sample.

**Table 3:** Correlations (*r*) of the content of microelements in wool samples and blood serum of cows under different methods of management.

Trace elements	Wool sample location						Average value
	Occiput	Tail root	Middle of last rib	Dewlap	Withers	Tail brush	
<b>Stock grazing</b>							
Mn	0.454*	0.279*	0.323*	0.048	0.113	0.603*	0.529*
Se	0.366*	0.419*	0.634**	-0.516*	0.365*	0.337*	0.273*
Cr	0.448*	0.355*	0.205*	0.598*	0.095	0.737*	-0.303
Cu	0.409*	0.132	0.261*	0.144	0.491*	0.540*	0.453*
I	0.545*	-0.005	-0.190	0.026	0.119	-0.059	0.301*
Zn	0.398*	-0.299*	0.184	0.269*	-0.193	-0.042	0.058
Co	-0.242*	0.147	0.199	0.343*	-0.103	0.156	0.353*
<b>Stall housing</b>							
Mn	-0.336*	-0.201	0.363*	-0.439*	-0.273	-0.185	0.027
Se	0.022	-0.149	0.337*	0.100	0.254*	-0.323*	0.152
Cr	0.538*	0.020	0.097	-0.104	-0.094	-0.395*	-0.364*
Cu	0.323	0.270	0.060	-0.057	0.017	0.128	0.083
I	-0.152	-0.162	-0.054	-0.284	0.016	0.039	0.096
Zn	0.079	0.216*	-0.254*	0.386*	-0.025	0.284*	-0.075
Co	-0.453*	-0.242*	0.147	0.199	0.343*	-0.103	0.156

Note: \* $P \leq 0.05$

The strongest correlations for Mn and I were also found in wool samples from the tail root and the withers ( $P \leq 0.05$ ). In the case of Co, correlations were observed in the chest, root, and tail brush ( $P \leq 0.05$ ). For Se, correlations were noted at the tail root and the occipital part of the head ( $P \leq 0.05$ ). Cu and Zn content showed correlations at the middle of the last rib and the tail brush ( $P \leq 0.05$ ). Cr content had correlations at the withers and the tail brush ( $P \leq 0.05$ ).

In the coat, as is known, there is no homeostatic regulation of the composition of chemical elements. A direct relationship was revealed between the elemental composition of wool and the blood serum of animals in terms of the indicators of individual microelements, as evidenced by the detailed data presented in Table 3.

Table 3 presents the correlations between trace element concentrations in wool samples and blood serum of cows under two different management methods: stock grazing and stall housing. Under stock grazing conditions, notable correlations were observed for several trace elements: manganese (Mn) showed a significant positive correlation with wool from the tail brush (0.603,  $P \leq 0.05$ ) and the occiput (0.454,  $P \leq 0.05$ ); selenium (Se) exhibited strong positive correlations with the middle of the last rib (0.634,  $P \leq 0.01$ ) and the tail root (0.419,  $P \leq 0.05$ ); chromium (Cr) correlated positively with the occiput (0.448,  $P \leq 0.05$ ) and tail brush (0.737,  $P \leq 0.05$ ); copper (Cu) correlated with the tail brush (0.540,  $P \leq 0.05$ ) and withers (0.491,  $P \leq$

0.05); iodine (I) demonstrated a strong correlation with the occiput (0.545,  $P \leq 0.05$ ); and zinc (Zn) showed a positive correlation in the tail root (0.398,  $P \leq 0.05$ ), while cobalt (Co) had a negative correlation at the occiput (-0.242,  $P \leq 0.05$ ). In contrast, stall housing revealed different correlations: manganese (Mn) had negative correlations with wool from the occiput (-0.336,  $P \leq 0.05$ ) and dewlap (-0.439,  $P \leq 0.05$ ); selenium (Se) presented a positive correlation only at the middle of the last rib (0.337,  $P \leq 0.05$ ); chromium (Cr) had a significant positive correlation at the occiput (0.538,  $P \leq 0.05$ ); copper (Cu) showed weaker associations across different wool sample locations; iodine (I) displayed weak or negative correlations across all locations; zinc (Zn) showed a positive correlation in the tail brush (0.284,  $P \leq 0.05$ ) and dewlap (0.386,  $P \leq 0.05$ ), while cobalt (Co) had negative correlations at the occiput (-0.453,  $P \leq 0.05$ ) and tail root (-0.242,  $P \leq 0.05$ ). These findings suggest that management methods significantly influence the correlations between trace elements in wool and blood serum, with stock grazing providing a more reliable reflection of trace element status, particularly for manganese, selenium, and chromium, while stall housing may disrupt the expected relationships, highlighting the importance of management practices in livestock nutrition and health monitoring.

Although the mass fraction of micronutrients in animals is less than 0.01%, they play an indispensable role in maintaining normal physiological functions, including haemopoiesis, immune response, energy metabolism,

enzyme activity, and reproduction (Guyot *et al.*, 2009; Cao *et al.*, 2021; Oconitrillo *et al.*, 2024). Required in minimal amounts, these micronutrients are crucial for numerous biochemical pathways and essential for the life of all organisms, from bacteria to humans. Even minor imbalances can lead to significant metabolic consequences, with deficiencies and excesses impairing cell function or causing cellular damage (Weiss and Carver, 2018).

The concentrations of trace elements in blood serum and wool samples can vary based on factors such as ecotype, type of feed, soil mineral levels, and season. Differences in mineral levels during stall housing may be partially explained by a more balanced dietary intake of trace elements compared to pasture feeding. Generally, plants reflect the mineral profile of the soil; thus, those growing in mineral-deficient soils may lead to trace element deficiencies in animals, potentially resulting in trace elementosis (Hurst *et al.*, 2013; Joy *et al.*, 2015). While quantifying trace element levels in organs, primarily the liver and kidneys, is considered standard, this method typically involves invasive biopsy or lethal sampling, which is particularly challenging with beef cattle due to their fearful nature. Additionally, blood collection procedures can induce stress in the animals (Massányi *et al.*, 2020; Hoffmann *et al.*, 2021; Skliarov *et al.*, 2023). Standard methods usually measure trace element concentrations in blood or serum, but correlations with liver and/or kidney values for most minerals are often negative (Vermunt and West, 1994).

Cygan-Szczegielniak (2021) revealed significant correlations among trace element levels, highlighting high variability in individual tissues and organs. The accumulation of certain trace elements in specific tissues can reduce their concentration in others or contribute to increased levels elsewhere. Compared to plasma, faeces, urine, and other tissues, hair analysis is non-invasive and provides long-term information on trace element accumulation (Dunnett and Lees, 2004). However, the relationship between hair mineral levels and other biosubstrates (such as organs and blood) is not fully understood, and established reference levels are lacking, complicating interpretation (Roug *et al.*, 2015). Moreover, factors such as season, gender, and age can influence trace element deposition in hair, further complicating analysis (Davies *et al.*, 2017; Król *et al.*, 2018). Correlations between trace mineral content in blood and hair have confirmed differences based on the breed and age of cattle (Patra *et al.*, 2006; Cygan-Szczegielniak and Stasiak, 2022).

The evaluation of chemical element concentrations in wool (hair) is regarded as a suitable method for assessing elemental status and health conditions in various farm and wild animal species (Patra *et al.*, 2006; Rębacz-Marón

*et al.*, 2013). Our studies indicate differences in trace element concentrations in serum and wool samples under varying housing methods. Notably, wool growth in cows begins in late spring or early summer and continues until late autumn. Consequently, our wool samples reflect the trace element status of cows between seasonal moults, highlighting differences in mineral status between stall housing and pasture.

The discrepancies between previously published results and our findings may be attributed to differences in the biogeochemical provinces where the evaluated animals are bred, as well as variations in feeding levels related to their physiological states. Elemental status is characterised by high lability and is influenced by many factors.

## CONCLUSIONS AND RECOMMENDATIONS

Based on the presented results, it has been established that the concentrations of trace elements in wool samples from cows kept on pasture and in the stall vary depending on the housing conditions. In pasture-based systems, high concentrations of manganese, iodine, and cobalt are observed, particularly in wool samples from the area of the last ribs, which may indicate insufficient intake of these elements in the animals' diets. Conversely, in stall housing, the concentrations of trace elements such as selenium and chromium show higher values in the tail and nape areas, indicating a more balanced supply of trace elements through feed. Correlation studies reveal that pasture-based housing better reflects trace element status, especially for manganese, selenium, and chromium, compared to stall housing, emphasising the importance of feed management in ensuring normal metabolism and animal health. Furthermore, the study results confirm that wool analysis is a less invasive and effective method for monitoring mineralisation in cattle, allowing for the avoidance of stress associated with traditional blood sampling methods.

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## NOVELTY STATEMENT

This study provides novel insights into the use of hair mineral content as a non-invasive method to evaluate the trace element status in cows. The strong correlations between trace elements in hair and serum offer a valuable approach for improved monitoring and management of mineral imbalances in cattle, enhancing animal health and productivity.

## AUTHOR'S CONTRIBUTION

All authors contributed equally and all authors are in agreement with the content of the manuscript.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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