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Serum Mineral Levels in Dairy Cows Transiting from Feedlot to Pasture

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Abstract

The objective of the present study was to evaluate trace element and minerals levels in the serum of cows transiting from diets consumed in feedlot or under grazing. A total of 30 healthy 5–6 years old cows of the Red Steppe breed were involved in the study. Blood samples were collected at the end of the feedlot period (end of April) and during the pasture period (end of June). Serum essential trace element and mineral levels were evaluated using inductively coupled plasma mass spectrometry. The obtained data demonstrate that serum K levels in cows during the feedlot period exceeded those in the pasture period by 50%, whereas serum P values in the pasture period were signifcantly higher than in the feedlot period by 20%. Serum Li levels in cows during the feedlot feeding period were nearly 3-fold higher than the respective values in a pasture period. In addition, serum B, Sr, and Zn concentrations in cows during a pasture period exceeded those observed upon feedlot feeding by 38%, 40%, and 13%, respectively. In contrast, serum I and V levels in a feedlot period were 32% and 77% higher when compared to the respective values in a pasture period. Multiple regression analysis demonstrated that Cr, Cu, I, Na, and V are positively associated with feedlot feeding. At the same time, serum Zn and to a lesser extent Sr values were directly associated with the pasture period. Therefore, the results of the present study demonstrated that feedlot and pasture rations have a signifcant impact on trace element and mineral metabolism in dairy cows.

Keywords Cow housing · Production system · Transition · Trace elements · Minerals

Introduction

Trace elements and minerals are essential for ruminant health, and their defciency is associated with impaired growth, infertility, and immune defciency [[1\]](#page-6-0). In parallel with the overall health effects of trace elements and minerals on cattle health, adequate essential trace element and mineral supply in dairy cows is associated with milk productivity [\[2\]](#page-6-1) and quality [\[3](#page-6-2)]. Lactation is associated with increased requirements for essential trace elements and minerals [\[4](#page-6-3)]. In addition, milk is considered a source of essential micronutrients including minerals, playing a signifcant role in human micronutrient intake [\[5\]](#page-6-4). Therefore, assessment of trace element status and subsequent supplementation may be considered a valuable tool for the improvement of milk productivity and quality [[6\]](#page-6-5).

A number of factors were shown to infuence cattle trace element and mineral status with housing type being one of the most important [\[7](#page-6-6)]. Generally, cows are housed indoors or outdoors while grazing on pastures [[8\]](#page-6-7). Mixed types with access to pastures while housed indoors are also used, especially in the summer period [\[9](#page-7-0)]. Pasture feeding is more characteristic of organic dairy farms, whereas conventional farms more frequently use feedlot feeding [\[9](#page-7-0)].

Pasture-based housing systems are beneficial for animal health and behavior [[8\]](#page-6-7), although outdoor pasture feeding may be associated with a higher risk of insufficient mineral and trace element intake [[10](#page-7-1)]. Therefore, in conventional dairy farms trace elements are supplemented in concentrate feeds [[11\]](#page-7-2), which results in signifcant diferences in dietary trace element and mineral intake [[12\]](#page-7-3). Previous studies demonstrated that indoor feeding results in higher milk I, Cu, and Se levels [[13,](#page-7-4) [14](#page-7-5)], whereas pasture feeding is associated with higher milk Ca and P concentration [\[12](#page-7-3)]. At the same

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time, the existing data on the impact of feeding systems on serum trace element and mineral levels are insufficient and contradictory. In a study of serum Cu, Zn, and Fe levels, Noaman et al. [\[15](#page-7-6)] demonstrated that winter serum Cu and Zn concentrations signifcantly exceeded those in the summer period, whereas Dar et al. [[16](#page-7-7)] demonstrated nearly opposite results by indicating higher serum P, Mg, Cu, and Zn levels in summer. Moreover, the infuence of season and feeding systems on serum levels of B, Cr, Li, Sr, and V also has a significant impact on cattle health $[17–20]$ $[17–20]$ $[17–20]$ is nearly unstudied. Therefore, the objective of the present study was to evaluate trace element (B, Cr, Cu, Fe, I, Li, Mn, Se, Sr, V, Zn) and mineral (Ca, K, Mg, Na, P) levels in serum of cows transiting from indoor to outdoor housing periods. Assessment of cattle nutritional status in diferent periods and types of housing is essential for adequate supplementation and prevention of adverse health efects altogether resulting in high animal productivity [[21\]](#page-7-10).

Materials and Methods

The protocol of the current study was approved by the Local Ethics Committee (No. 03.04.2020-2) of Orenburg State University, Orenburg, Russia. A total of 30 healthy 5–6 years old (3–4 pregnancies) cows of the Red Steppe breed, cultivated by the nurse-cow technique in the Southern Ural region were examined. Only animals with a weight of 450–450 kg (427 \pm 10 kg) out of the dry period were involved in the study. Average milk production in the indoor and pasture periods were 18.2 ± 1.08 and 20.13 ± 0.83 L/ day, respectively. Blood samples were collected at the end of the feedlot period (end of April) and during the pasture period (end of June). During the feedlot period, the cows were mainly fed with hay from natural pastures (8 kg), corn silage (6 kg), and 3 kg of a mixture of barley, wheat, and oat in equal quantities, while grazing outside on a pasture that was not previously fertilized (Table [1](#page-1-0)). The pasture of the steppe zone was predominantly formed by *Poa pratensis* (Kentucky bluegrass), *Poa bulbosa* (bulbous bluegrass), *Stipa lessingiana* (Lessing feather grass), *Stipa pennata* (European feather grass), *Stipa capillata*, *Elymus* species (wild rye grass), *Elytrigia repens* (common couch), *Festuca valesiaca* (Volga fescue), and *Festuca pratensis* (meadow fescue) [\[18](#page-7-11)]. During pasture feeding, the animals consumed natural grass mixture ad libitum; therefore, the intake cannot be evaluated. In addition, salt lick block (99% NaCl) was provided to animals irrespectively of the production system ad libitum. Water was provided from a similar source ad libitum during both production systems.

Blood was sampled from the coccygeal vein prior to milking and collected in VACUETTE® CAT serum separator

Table 1 Trace element (B, Cr, Cu, Fe, I, Li, Mn, Se, Sr, V, Zn) and mineral (Ca, K, Mg, Na, P) content of the main dietary items consumed during indoor and outdoor periods (g/kg)

Element	Hay	Silage	Chow	Grass
Ca	3051	1253	557	5514
K	11039	3241	4259	22598
Mg	3319	478	1382	1845
Na	664	202	208	29
P	1281	687	3836	2114
B	9.99	3.8	2.6	7.79
Co	0.056	0.021	0.0486	0.14
Cr	0.52	0.28	0.125	0.63
Cu	3.24	2.5	5	5.06
Fe	54.78	32	54.41	195
I	0.435	0.11	1.22	0.31
Li	0.396	0.292	0.0794	0.49
Mn	27.39	11.1	35.7	46.74
Se	0.119	0.07	0.334	0.33
Sr	28.44	10.17	4.89	27.16
V	0.0786	0.032	0.0487	0.045
Zn	13.02	4.3	25.89	25.58

clot activator tubes. The studies were performed in the center for collective usage.

The obtained serum samples were diluted with an acidified diluent ($pH = 2.0$, 1:15; v/v) consisting of 1% 1-butanol (Merck KGaA, Germany), 0.1% Triton X-100 (Sigma-Aldrich, Co., USA), and 0.07% HNO3 (Sigma-Aldrich, Co., USA) dissolved in distilled deionized water (Merck Millipore, USA). Microwave digestion was performed in 65% nitric acid (Sigma-Aldrich Co., St. Louis, MO, USA) for 20 min with a maximal temperature of 170–180 °C using Berghof SpeedWave-4 DAP-40 (microwave frequency, 2.46 GHz; power, 1450 W) microwave system (Berghof Products + Instruments GmbH, 72800 Eningen, Germany). Trace element and mineral levels were evaluated using inductively coupled plasma mass-spectrometry (ICP-MS) at NexION 300D spectrometer (PerkinElmer Inc., Shelton, CT, USA) equipped with ESI SC-2 DX4 autosampler (Elemental Scientific Inc., Omaha, NE, USA). The ICP-MS system was calibrated using different concentrations of trace elements prepared from stock solutions of Universal Data Acquisition Standards Kit (PerkinElmer Inc., Shelton, CT, USA). Internal online standardization was performed using standard solutions $(10 \mu g/L)$ of yttrium-89 and rhodium-103 prepared from yttrium (Y) and rhodium (Rh) pure single-element standard (PerkinElmer Inc.). Laboratory quality control of trace element and mineral analysis was performed using the certified reference materials of plasma (ClinChek® Plasma Control, lyophil., for trace elements, Levels 1 and 2, RECIPE Chemicals + Instruments GmbH, Munich, Germany). The recovery rates for all studied elements varied from 92% to 109%.

The obtained data were processed using Statistica 10.0 software (StatSoft, Tulsa, OK, USA). Assessment of data distribution normality was performed using the Shapiro-Wilk test. Due to the Gaussian distribution of serum trace element and mineral levels, the mean values and the respective standard deviations were used as descriptive statistics. Group comparisons were performed using the Sign test for dependent variables. Spearman's correlation analysis was used for the estimation of the association between single trace elements in cow's serum. Multiple regression analysis and principal component analysis (PCA) were used for the evaluation of the association between production systems (dependent variable) and serum trace element and mineral levels as independent variables. The level of signifcance was set as $p < 0.05$ for all analyses.

Results

The obtained data demonstrate that serum mineral levels are significantly affected by the production system (Table [2](#page-2-0)). Specifically, serum K levels in cows during the feedlot period exceeded those in the pasture period by 50%. In contrast, serum P values in the pasture period were signifcantly higher than in the feedlot period by 20%. No significant difference in serum Ca, Mg, or Na levels in diferent production systems was revealed.

Essential trace element levels in blood serum difered signifcantly between the feeding periods (Table [3](#page-2-1)). Serum Li levels in cows during a feedlot feeding period were nearly 3-fold higher than the respective values in a pasture period. In addition, serum B, Sr, and Zn concentrations in cows during a pasture period exceeded those observed upon feedlot feeding by 38%, 40%, and 13%, respectively. In contrast, serum I and V levels in a feedlot period were 32% and 77% higher when compared to the respective values in a pasture period.

Table 2 Serum Ca, K, Mg, Na, and P levels in dairy cows in indoor and outdoor periods (μg/L)

	Indoor	Pasture	Difference	p value
Ca	100.378 ± 5.545	$103.001 + 6.653$	$+3%$	0.251
K	$306.533 + 61.118$	$200.667 + 18.699$	$-53%$	$< 0.001*$
Mg	$24.051 + 3.394$	$25.401 + 1.87$	$+6%$	0.188
Na	$3097.333 + 281.352$	$3220 + 435.984$	$+4%$	0.102
P	$164.067 + 23.933$	$196.333 + 33.181$	$+20%$	$0.005*$

Data expressed as mean \pm SD; *significance of group difference according to the Sign test

Table 3 Serum B, Cr, Cu, Fe, I, Li, Mn, Se, Sr, V, and Zn levels in dairy cows in indoor and outdoor periods (μg/L)

	Indoor	Pasture	Difference	<i>p</i> value
B	0.194 ± 0.039	$0.268 + 0.049$	$+38%$	$< 0.001*$
Co	0.0011 ± 0.0005	$0.0010 + 0.0008$	-10%	0.896
Сr	0.0115 ± 0.0031	$0.0132 + 0.0012$	$+15%$	0.057
Сu	$0.67 + 0.155$	$0.764 + 0.182$	$+14%$	0.138
Fe	$2.598 + 1.277$	$2.762 + 0.487$	$+6%$	0.646
Ι	$0.065 + 0.014$	$0.049 + 0.008$	$-25%$	$< 0.001*$
Li	0.013 ± 0.006	$0.041 + 0.021$	$+215%$	$< 0.001*$
Mn	0.0045 ± 0.0045	0.0043 ± 0.0019	$-4%$	0.878
Se.	$0.088 + 0.016$	0.091 ± 0.015	$+3\%$	0.578
Sr	0.092 ± 0.018	0.129 ± 0.018	$+40%$	$< 0.001*$
V	0.0092 ± 0.0022	$0.0052 + 0.0004$	$-43%$	$< 0.001*$
Zn	0.991 ± 0.168	$1.123 + 0.149$	$+13%$	$0.030*$

Data expressed as mean \pm SD; *significance of group difference according to the Sign test

Correlation analysis demonstrated a signifcant relationship between certain trace elements and minerals in dairy cows (Table [4](#page-3-0)). Negative correlations were most characteristic for serum I levels, being inversely associated with circulating B, Cu, Li, and Sr levels. Serum K concentration was also found to be inversely correlated with B, Cr, Li, Mg, and Sr serum levels. It is also notable that inverse associations with B, Ca, Cr, Li, Mg, Na, and Sr were characteristic of serum V concentrations. A negative correlation was also observed between Mg and K levels, as well as Mn and Ca serum concentrations. The most signifcant positive associations were observed between Li and B, Sr and B, as well as V and K. No signifcant correlation with serum Co was revealed (data not provided).

Multiple regression analysis (Table [5](#page-4-0)) with feeding period as a dependent variable and all studied elements as independent predictors (Model 1), demonstrated that Cr, Cu, I, Na, and V are directly associated with indoor feeding. At the same time, serum Zn and to a lesser extent Sr values were positively associated with the pasture period. After adjustment for variations in daily milk yield (Model 2) only serum Cr, I, and V remained signifcantly inversely associated with pasture period.

Trace elements and minerals characterized by a signifcant group diference or signifcant relationship with the production system in regression models were included in the principal component analysis model (Fig. [1\)](#page-4-1). After the exclusion of P, characterized by a weak contribution into the model, the fnal PCA model was responsible for 70% of production system variability. Principal component 1 (PC1) was determined by the values of serum B, Cr, I, K, Li, Sr, and V, whereas Zn and to a much lesser extent Cr and I contributed to PC2.

Table 4 Correlation analysis of the association between trace elements and minerals in dairy cows

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Table 5 Multiple regression analysis of the association between period of housing (dependent variable) and serum trace element and mineral levels (independent variables) in a crude model (Model 1) and after adjustment for daily milk yield (Model 2)

508 E. A. Sizova et al.

Data expressed as regression coefficient (*β*), partial correlation coefficient (PC), and *p* value; *association is significant at $p < 0.05$

Discussion

The obtained data generally correspond to the fndings from animals maintained upon diferent production systems. These findings are generally in agreement with the observed lower level of milk I in cows grazing on grass pastures as compared to feedlot-fed cows consuming a total mixed ration [[13\]](#page-7-4). The I concentration in milk samples from Ireland was found to be higher in the feedlot period as compared to the pasture grazing period, as also observed for Cu levels [[14](#page-7-5)]. Correspondingly, cattle raised at pastures were characterized by lower plasma I content [[22](#page-7-12)]. Certain studies demonstrated seasonal variability in iodine contents in milk samples. Analysis of milk samples from UK herds demonstrated that winter milk I levels signifcantly exceeded the summer values

Fig. 1 Principal component analysis (PCA) of the association between production system and serum trace element and mineral levels. **A** contribution of the studied elements into the model (loadings) plotted on the PC1 vs. PC2 plane. **B** Loadings of the principal components PC1 and PC2 accounting for 53.77% and 16.49% parameter variability)

[[23\]](#page-7-13). Similar findings were obtained in Bosnia and Herzegovina [\[24\]](#page-7-14) and Poland [[25\]](#page-7-15). Milk I levels in the summer months were also lower than in other periods of the year in Northern Ireland [[26](#page-7-16)]. Taken together, current fndings and the existing data demonstrate lower I intake during the pasture period due to lower I content in pastures, as well as consumption of I antagonists from plants [[27\]](#page-7-17). This suggestion is also confrmed by the higher I content in hay, silage, and chow consumed during the feedlot period as compared to grass in the pasture period. In view of signifcant diferences in serum I levels, maintenance of adequate I intake during the outdoor period is of particular importance due to the role of I defciency not only in hypothyroidism and goiter, but also in stillbirth, weakened ofspring, reproductive disorders, hoof diseases, as well as mastitis [[28](#page-7-18)].

A significant increase in serum P and Zn levels was directly associated with pasture grazing. Correspondingly, plasma P and Zn levels in cattle grazing in Kashmir were found to be signifcantly higher in the summer period as compared to the winter season, being in agreement with the current study results [[16\]](#page-7-7). The observed diferences may be associated with higher dietary P content in the pasture period. In particular, pasture feeding was shown to contain a higher content of P and K as compared to alfalfa silage and especially corn silage [[29](#page-7-19)]. Similarly, P levels of the studied hay and silage contained lower P levels as compared to grass. In another study, higher time spent on pastures (12 h) vs $(8 \text{ h or } 4 + 4 \text{ h})$ was positively associated with higher intake of P, Mg, K, Na, and Cu [[30](#page-7-20)]. Silage consumption was shown to result in increased P and lower Cu levels in blood plasma [[31](#page-7-21)]. In turn, mixed feedlot ration consumption also resulted in lower P, as well as Ca, Fe, and Mn levels in milk as compared to perennial ryegrass pasture-based rations [\[32](#page-7-22)]. At the same time, the efects on serum P may be also mediated by an increase in 25-hydroxycholecalciferol levels during the transition to pasture from feedlot conditions $[33]$ $[33]$. In turn, P insufficiency in reduced bone mineral density, reproductive disorders, developmental delays in the ofspring, and decreased milk productivity [\[34](#page-7-24)].

The obtained data also demonstrate that serum Zn levels are increased in a pasture period. These fndings are contradictory to the earlier indications of higher Zn content in feedlot rations [\[33](#page-7-23)]. At the same time, no signifcant efect of pasture grazing time on dietary intake of Zn or Fe was observed in Mexico [\[30](#page-7-20)]. A study in cattle grazed in Isfahan province, central Iran, revealed signifcantly increased serum Cu and Zn levels in winter as compared to summer period $[15]$. At the same time, no significant group difference in serum Cu, Zn, or Fe was observed in dairy cattle in summer and winter seasons [[15\]](#page-7-6). Therefore, the existing data are rather contradictory presumably due to diferent Zn content in pasture grass. At the same time, the observation of higher Zn levels during the pasture period is generally in agreement with the fndings of higher grass Zn content as compared to hay and especially silage. Given the role of Zn in cattle milk performance, immune response, rumen fermentation [[35](#page-7-25)], growth, and reproduction [[36](#page-7-26)], Zn supplementation may be required to address dietary insufficiency at certain production periods.

Although group comparison did not reveal a signifcant diference in cow serum Cu levels in diferent production systems, multiple regression analysis demonstrated that serum Cu concentration is directly associated with feedlot feeding. This observation corresponds to indications of a high rate of Cu supplementation to feedlot-fed animals [[11\]](#page-7-2), as well as observation of higher plasma Cu concentrations in healthy cattle raised indoors [\[12\]](#page-7-3). However, both hay and silage Cu contents were lower when compared to pasture grass. Although Cu supplementation is benefcial to maintain adequate Cu intake and prevent Cu defciencyassociated disorders, Cu overload also possesses signifcant adverse effects on cattle health, and excessive supplementation is to be avoided [[37](#page-7-27)].

A signifcant direct association of serum Li, B, and Sr levels with the outdoor period was observed. The increase in serum Li levels may be indicative of higher dietary intake of the metal $[38]$ $[38]$, that may be accumulated by plants $[39]$ $[39]$ including those grown on the pastures. Correspondingly, grass Li content was higher as compared to that of hay, silage, and chow. Although no physiological efects of Li in cattle were described, Li overexposure may induce toxic effects $[40]$ $[40]$.

Plasma B concentration is also indicative of increased dietary B intake [\[41](#page-8-2)]. Adequate B intake in cattle is considered protective against hypocalcemia and hypomagnesemia [[42](#page-8-3)]. Increased B intake may also increase P absorption through modulation of vitamin D-related pathways [[43](#page-8-4)], thus possessing a protective efect against osteoporosis, as well as immune deficiency [[18\]](#page-7-11).

Increased serum Sr levels may be also indicative of increased dietary Sr intake, as stable Sr was shown to be transferred from grass to cow milk, although at rather low coefficients $[44]$ $[44]$. Although both B and Sr levels in pasture grass were nearly similar to that in hay, the contents of these elements in silage and chow were rather low that may also at least partially underlie the observed diferences.

It is also notable that consistent fndings were obtained from a comparison of bovine trace element and mineral nutrition from organic and conventional farms, with the latter being characterized by lower grazing rate and higher use of concentrates. Specifcally, conventional farm milk contained signifcantly higher levels of Cu, Zn, I, and Se due to a high supplementation rate $[11]$ $[11]$. At the same time, Li levels were found to be higher in milk produced in organic farms [[45](#page-8-6)]. Due to these fndings, it has been proposed that chemometric analysis of milk may be used for the diferentiation of organic and conventional milk [\[46](#page-8-7)].

Together with lower I content in cow serum, a signifcant elevation of Li, B, and Sr is observed in a pasture period that may at least partially contribute to lower I status, especially in view of revealed negative correlations between these elements and I. Specifcally, excessive Li exposure was shown to result in altered thyroid hormone levels [\[47\]](#page-8-8) and ultimately hypothyroidism [\[48](#page-8-9)]. Boron was also considered a potential goiterogen due to its impact on thyroid structure and endocrine function, as well as certain similarities of chemical properties with iodine [[49](#page-8-10)]. At the same time, a recent study demonstrated that B supplementation in rams may promote thyroid functioning and increase T3 levels [[50\]](#page-8-11). Although data on the relationship between Sr and I metabolism are insufficient, it has been demonstrated that free T4 levels in rural adults are inversely associated with urinary Sr concentrations [\[51](#page-8-12)].

Given the role of the studied trace elements and minerals in cattle wellbeing [\[1](#page-6-0)], it is proposed that its alteration due to dietary transition may have a signifcant impact on bovine health. Specifcally, the results of a detailed study demonstrate that modulation of trace element status upon transition may be associated with signifcant alteration of immune reactivity [[33\]](#page-7-23).

At the same time, the study has certain limitations that should be addressed in future investigations. First, the sample size is relatively small and increasing the number of observations could be benefcial for the estimation of the additional associations. Second, the study was performed only using animals of the Red Steppe breed, although the effects of transition between housing systems may be signifcantly modulated by animal breeds. Using milk, hair, and other biosamples would provide additional information on trace element and mineral metabolism in cows during transition between the production systems. Finally, the use of functional markers of trace element and mineral metabolism including specifc enzymes would be useful for the estimation of the clinical value of the observed changes.

Conclusion

Therefore, the results of the present study demonstrated that indoor and outdoor rations have a signifcant impact on trace element and mineral metabolism in dairy cows. Serum P, B, Li, Sr, and Zn levels were signifcantly higher in cows during outdoor pasture grazing, whereas elevated serum concentrations of Cr, Cu, K, I, Na, and V were directly associated with indoor rations. It is assumed that the observed diferences occur due to diferent trace element and mineral content in main dietary items and supplements. Given the adverse effects of dietary trace element and mineral insufficiency in dairy cows including reduced productivity, reproductive disorders, bone diseases, and impaired immunity, mineral supplementation to meet the recommended dietary intake is required in both periods. However, in view of signifcant diferences in serum trace element and mineral levels during indoor and outdoor periods, the composition of the concentrates needs to be period-specifc. Such an approach may be also benefcial for the prevention of excessive intake of certain elements that may be also hazardous to animal health and productivity. Taken together, the obtained data also stress the importance of nutritional status monitoring during diferent feeding periods in order to maintain an dequate supply of essential trace elements and minerals for high milk productivity and health.

Author Contribution SAM, AVS—supervision; EAS, SVN, AAT data acquisition and analysis; EAS, SVN, AAT—manuscript draft preparation; SAM, AVS—manuscript review and editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability Data are available on reasonable request.

Declarations

Competing Interests The authors declare no competing interests.

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