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The Reference Intervals of Hair Trace Element Content in Hereford Cows and Heifers (*Bos taurus*)

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Abstract The objective of the present study was to assess hair trace element content in Hereford heifers and cows (*Bos taurus*) living in South Ural region and calculate the site-specific reference intervals. Hair trace element content in 150 cows and heifers farmed in the Southern Urals of Russia was assessed using inductively coupled plasma mass-spectrometry. Dietary trace element content corresponded to the adequate values as estimated by recommendations of USSR State Agriculture Committee and U.S. National Research Council. Comparative analysis demonstrated that heifers are characterized by significantly higher hair Se (3-fold), Hg (4-fold), and Sn (46%) content, whereas cows had significantly higher levels of hair Co (56%), I (33%), Si (2-fold), V (27%), B (55%), Cd (19%), Pb (47%), and Sr (23%). At the same time, no significant group difference in hair Cr, Cu, Fe, Li, Mn, Zn, As, and Ni was detected between Hereford cows and heifers. The reference intervals and 90% confidence intervals for the lower and upper limits were calculated in agreement with the American Society for Veterinary Clinical Pathology Quality Assurance and Laboratory Standard Guidelines.

Keywords *Bos taurus* · Hair · Trace elements · Reference intervals · Heifers · Cows

Introduction

Trace elements play a significant role in the organism. In particular, essential trace elements are required for normal organism functioning through their regulatory, catalytic, and structural function. In particular, it has been demonstrated that essential metals play a significant role in immune [1] reproductive function [2] as well as embryonic and fetal development in livestock [3]. However, not only deficiency but also excess of essential trace elements may be harmful [4]. In particular, it has been demonstrated that high selenium (Se) intake due to consumption of Se-rich plants is associated with "alkali disease" in horses and cattle [5]. Moreover, excessive exposure to certain essential trace element may decrease bioavailability of other elements in ruminants [6].

In turn, toxic trace elements may have a negative impact on livestock health [7] through various mechanisms like inflammation and oxidative stress. In addition, it has been demonstrated that heavy metals may altered essential trace element status in cattle even in the low polluted areas [8]. The intake of toxic trace elements is increased in an industrial area. In particular, earlier studies have revealed a significant elevation of heavy metal levels in the organism of animals [9, 10].

Therefore, monitoring of trace element status of cattle is strongly required for prevention of diseases and high performance [11]. Chemical analysis of soil and feed does not always indicate the real intake of trace elements in animals due to the presence of insoluble complexes or antagonists that depress mineral bioavailability [12]. Hair was proposed as the potential substrate for assessment of trace element status in livestock [13]. Certain studies have demonstrated that hair

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content significantly correlated with blood concentration for certain essential and toxic trace elements [14–16]. However, various factors including sex and breed may have a significant influence on the results of hair trace element analysis [17]. Moreover, the absence of site-specific reference intervals for trace element content in hair of livestock also limits its use [11]. Therefore, the objective of the present study was to assess hair trace element content in Hereford heifers and cows (*Bos taurus*) living in South Ural region and calculate the site-specific reference intervals.

Material and Methods

The protocol of the present investigation was approved by the Local Ethics Committee of the Orenburg State University, Orenburg, Russia. All animal studies have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Animals

Hair samples were taken from clinically healthy animals within the years. The animals (*n* = 150) were cows (age = 3.2 ± 0.8 years old; *n* = 75) and heifers (age = 1.4 ± 0.4 years old; *n* = 75) of the Hereford breed (*Bos taurus*), cultivated by the nurse-cow technique in the Southern Ural region. In the stall period (October–May), the animals were fed mainly with wheat grass and sainfoin hay, haylage of Sudan grass and barley grains; in the pasture period (May–October), they grazed on cereal grasses. All animals were fed similar diet during all periods. Trace element content of the dietary items was generally adequate to the requirements but did not exceed maximal tolerable limits as estimated by recommendations of USSR State Agriculture Committee and U.S. National Research Council [18–20] (Table 1).

Sampling

Hair samples were collected from three to five sites in withers (5×5 cm^2) using ethanol-precleaned stainless steel scissors in a quantity of not less than 0.4 g. Only red hair samples were collected as hair color was shown to affect hair trace element content in cattle [13]. Proximal parts of the hair strands were used for trace element analysis in order to assess recent trace element status and reduce the rate of external contamination that may be observed in distal parts of the strands.

Trace Element Analysis

The collected hair samples were washed in acetone (ex.p., Khimmed, Russia) in 10–15 min and then rinsed thrice in deionized water (18 MΩ cm). The deionized water was

Table 1 Trace element content of the dietary items (μg/g dry weight)

Element	Wheat grass hay	Corn silage	Sudan grass	Grains	Cereal grasses	MAL[a]	MTL[a]	DR, μg/g
Co	0.225 ± 0.013	0.127 ± 0.009	0.152 ± 0.044	0.178 ± 0.062	0.186 ± 0.047	2.0	20	0.11
Cr	0.17 ± 0.03	0.49 ± 0.21	0.09 ± 0.05	0.11 ± 0.05	0.12 ± 0.06	0.5	100	–
Cu	24.1 ± 8.7	13.2 ± 4.8	8.5 ± 6.1	19.4 ± 1.6	13.4 ± 5.8	30.0	40	12 (15.7)
Fe	173 ± 93.2	159 ± 35	273.8 ± 30.2	143 ± 26	118.6 ± 43.4	100.0	500	118 (24)
I	0.54 ± 0.22	0.32 ± 0.16	0.36 ± 0.15	0.50 ± 0.19	0.37 ± 0.14	2.0	50	0.33
Li	0.13 ± 0.05	0.08 ± 0.03	0.11 ± 0.05	0.19 ± 0.06	0.09 ± 0.04		25	–
Mn	40.9 ± 5.7	13.0 ± 4.6	14.8 ± 5.4	39.8 ± 8.6	9.3 ± 1.5		2000	24.2 (16.7)
Se	0.45 ± 0. 9	0.26 ± 0.02	0.35 ± 0.14	0.40 ± 0.18	0.32 ± 0.14	1.0	5	0.3
Si	62 ± 19	67 ± 21	74 ± 22	55 ± 19	45 ± 14		0.2%	–
V	0.082 ± 0.044	0.013 ± 0.091	0.024 ± 0.010	0.032 ± 0.014	0.018 ± 0.009		500	–
Zn	23.1 ± 3.2	7.8 ± 1.5	15.1 ± 2.8	28.3 ± 8.7	10.1 ± 2.7	50.0	500	33 (63)
As	0.07 ± 0.03	0.05 ± 0.02	0.04 ± 0.01	0.05 ± 0.02	0.03 ± 0.01	0.5	30	–
B	19 ± 8	15 ± 7	13 ± 5	18 ± 6	12 ± 5		135	–
Cd	0.019 ± 0.008	0.015 ± 0.009	0.017 ± 0.011	0.021 ± 0.010	0.017 ± 0.007	0.3	10	–
Hg	0.018 ± 0.012	0.009 ± 0.006	0.018 ± 0.009	0.019 ± 0.013	0.012 ± 0.007	0.05	2	–
Ni	1.6 ± 6.2	1.0 ± 3.1	1.3 ± 4.3	1.2 ± 4.7	1.6 ± 4.8	3.0	100	–
Pb	0.12 ± 0.05	0.07 ± 0.03	0.11 ± 0.04	0.11 ± 0.03	0.09 ± 0.03	5.0	100	–
Sn	0.026 ± 0.016	0.020 ± 0.012	0.021 ± 0.011	0.024 ± 0.008	0.019 ± 0.010		100	–
Sr	18 ± 7	16 ± 5	16 ± 6	17 ± 5	12 ± 4		2000	–

MAL maximum allowable levels [20], *MTL* maximum tolerable levels [18], *DR* dietary requirements [19]

[a] Expressed as μg/g (ppm) for all elements except Si (%)

obtained by an electric distiller with combined membrane set DVS-M/1HA-1(2)-L (Mediana-Filter, Russia). After that, the samples were dried at 60 °C till air-dry condition. Appropriate weights of the samples (ca. 0.05 g) were digested with 5 ml of nitric acid (ex.p., Khimmed, Russia) in the microwave system Multiwave 3000 (PerkinElmer – A. Paar, Austria) using the following mode: 5 min temperature rise up to 200 °C, 5 min stable at 200 °C, then chilling down to 45 °C. The digested solutions were quantitatively transferred into 15 ml polypropylene test tubes; the liners and tops were rinsed thrice by deionized water with the rinses transferred into the correspondent test tubes. Then, the solutions were filled up to 15 ml with deionized water and thoroughly mixed up by shaking in the closed test tubes.

Determination of essential (Co, Cr, Cu, Fe, I, Li, Mn, Se, Si, V, Zn) and toxic (As, B, Cd, Hg, Ni, Pb, Sn, Sr) trace elements in the samples was performed using NexION 300D spectrometer (Perkin Elmer, USA). Graduation of the instrument was carried out using monoelement Universal Data Acquisition Standards Kits (PerkinElmer Inc., Shelton, CT 06484, USA). Internal online standardization was performed using 10 μg/l Yttrium (Y) Pure Single-Element Standard (PerkinElmer Inc., Shelton, CT 06484, USA). Analytical quality was verified by the certified reference material of hair GBW09101 (Shanghai Institute of Nuclear Research, Shanghai, China). The recovery rate for all studied trace elements was within the interval of 90–110%. All analytical procedures were performed in the laboratory of Center for Biotic Medicine (Moscow, Russia), the IUPAC company associate. The laboratory is also involved in the system of External Quality Assessment Schemes in the field of Occupational and Environmental Medicine (EQAS OELM).

Statistical Treatment

Statistical treatment of the obtained data was performed using Statistica 10.0 (StatSoft Inc., USA) software package. Normality of data distribution was assessed using Shapiro-Wilk test. As data on hair trace element content was not normally distributed, median and the respective 25 and 75 percentile boundaries were used as descriptive statistics. However, mean values and the respective standard deviations (SD) were also calculated. Group comparisons were performed using Mann-Whitney *U* test at the level of significance of $p < 0.05$.

The reference intervals were calculated using American Society for Veterinary Clinical Pathology Quality Assurance and Laboratory Standard Guidelines [21]. Briefly, after exclusion of outliers (percentile two-sided), the robust method was applied for assessment of reference intervals and 90% confidential intervals for the lower and upper limits. The calculations were performed using Reference Value Advisor for MS Excel [22].

Results

The obtained data demonstrate that cows and heifers are characterized by distinct hair essential trace element profile (Table 2). In particular, cows are characterized by significantly higher values of hair Co, I, and V, exceeding the respective values in heifers by 56, 33, and 27%. At the same time, the level of Si in hair of cows was more than 2-fold higher than the one observed in heifers. Oppositely, hair levels of Se were nearly 3-fold lower in cows than in heifers.

Similarly, hair toxic trace element content was also affected by the status of animals (Table 3). The hair content of B, Cd, Pb, and Sr in cows was higher than that in heifers by 55, 19,

Table 2 Hair essential trace element (μg/g) content in Herreford heifers and cows

Element	Heifers (n = 75)		Cows (n = 75)		*P* value
	Median (25–75)	Mean ± SD	Median (25–75)	Mean ± SD	
Co	0.118 (0.069–0.217)	0.154 ± 0.108	0.184 (0.125–0.261)	0.201 ± 0.121	0.008
Cr	0.216 (0.118–0.372)	0.305 ± 0.286	0.227 (0.159–0.372)	0.312 ± 0.230	0.390
Cu	4.700 (3.860–5.670)	4.846 ± 1.226	4.795 (3.900–5.440)	4.756 ± 1.090	0.754
Fe	67 (26–191)	129 ± 134	117 (39–207)	157 ± 142	0.111
I	0.681 (0.514–0.901)	0.775 ± 0.413	0.908 (0.606–1.260)	0.962 ± 0.439	0.009
Li	0.256 (0.180–0.349)	0.289 ± 0.178	0.278 (0.173–0.421)	0.326 ± 0.199	0.384
Mn	30 (19–43)	31 ± 16	30 (21–47)	37 ± 25	0.428
Se	0.665 (0.197–0.867)	0.572 ± 0.375	0.230 (0.201–0.365)	0.330 ± 0.219	0.013
Si	13 (7–27)	18 ± 14	29 (17–45)	34 ± 24	<0.001
V	0.293 (0.118–0.522)	0.367 ± 0.305	0.371 (0.251–0.597)	0.468 ± 0.318	0.026
Zn	101 (86–111)	102 ± 23	102 (88–110)	97 ± 18	0.586

Data expressed as median and 25 and 75 percentile boundaries, as well as mean values and the respective standard deviations (SD). The difference was considered significant at $p < 0.05$ according to Mann-Whitney *U* test

Table 3 Hair toxic trace element (μg/g) levels in Herreford heifers and cows

Element	Heifers (n = 75)		Cows (n = 75)		P value
	Median (25–75)	Mean ± SD	Median (25–75)	Mean ± SD	
As	0.109 (0.072–0.198)	0.137 ± 0.082	0.119 (0.080–0.175)	0.130 ± 0.058	0.926
B	3.3 (2.5–4.2)	3.7 ± 2.4	5.1 (3.0–8.3)	6.0 ± 4.1	0.001
Cd	0.031 (0.024–0.040)	0.034 ± 0.018	0.037 (0.027–0.052)	0.043 ± 0.024	0.020
Hg	0.008 (0.005–0.011)	0.011 ± 0.019	0.002 (0.002–0.007)	0.006 ± 0.009	<0.001
Ni	0.668 (0.460–0.923)	0.713 ± 0.324	0.616 (0.466–0.868)	0.719 ± 0.373	0.783
Pb	0.232 (0.161–0.397)	0.288 ± 0.161	0.340 (0.236–0.430)	0.338 ± 0.148	0.044
Sn	0.019 (0.013–0.048)	0.032 ± 0.028	0.013 (0.009–0.026)	0.023 ± 0.023	0.003
Sr	13 (10–16)	13 ± 6	16 (12–21)	17 ± 7	0.008

Data expressed as median and 25 and 75 percentile boundaries, as well as mean values and the respective standard deviations (SD). The difference was considered significant at $p < 0.05$ according to Mann-Whitney U test

47, and 23%, respectively. In turn, hair Sn levels in cows were decreased by 32% as compared to heifers. Moreover, the level of hair Hg was more than 4-fold lower in cows than that in heifers.

The reference intervals (Table 4) calculated in agreement with American Society for Veterinary Clinical Pathology recommendations (Quality Assurance and Laboratory Standard Guidelines) were distinct for heifers and cows, being in agreement with the results of group comparisons. For the majority of essential (Co, Fe, Li, Mn, Si, and V) and toxic trace elements (B, Cd, Ni, and Sr), the narrowest reference intervals were detected in heifers, whereas in cows the wider ranges were observed (except Cr, As, and Hg).

Discussion

The obtained hair levels of essential and toxic trace elements in cows are generally in agreement with the earlier published studies. In particular, the revealed values of hair Cu and Zn content are nearly similar to that published by Patra et al. (2006) for cattle from unpolluted areas, being 5.04 ± 0.13 and 106.3 ± 7.4 μg/g, respectively. At the same time, the hair levels of Co (1.39 ± 0.09 μg/g), Fe (206.2 ± 36.2 μg/g), Pb (3.00 ± 0.44 μg/g), and Cd (0.563 ± 0.170 μg/g), even in the unpolluted area of India, were higher than those in the present study. It is notable, that the strain of the animals was not specified in the study [15]. Examination of Polish Holstein-Fresian cows [23] demonstrated lower levels of hair As (~0.034 μg/g), B (~0.810 μg/g), Cd (~0.002 μg/g), Co (~0.058 μg/g), Cr (~0.075 μg/g), Cu (~2.2 μg/g), Fe (~15.9 μg/g), Li (~0.012 μg/g), Mn (~3.5 μg/g), Ni (~0.069), Pb (~0.033 μg/g), Si (~5.1 μg/g), Sr (~0.6 μg/g), and Zn (~33 μg/g) content as compared to the recently obtained values. In turn, the level of hair Hg (~0.083 μg/g), I (~9.7 μg/g), Se (~0.912 μg/g), and Sn (~0.113 μg/g) in the Polish study exceeded that obtained in the present investigation. The observed variance between the published and the obtained data may occur due to the difference in geographical location and strain of animals [11]. In particular, the obtained values of hair trace elements totally correspond to the earlier data on Cu (4.25–4.95 μg/g), Fe (59–89 μg/g), and Zn (119–131 μg/g) in Hereford cows [24]. Possible differences in dietary trace element content may also play a significant role in the observed variation as cows are maintained at diets primarily based on local products. Taking into account the fact that dietary trace element content was adequate to cover the nutritional requirements and did not exceed maximum tolerable levels, the examined animals are not characterized by trace element deficiency or toxicity. Therefore, the obtained data may be used as reference intervals for hair trace element content in heifers and cows living in South Urals.

The obtained data demonstrate that heifers and cows are characterized by distinct hair essential trace element content. Earlier studies have also revealed the influence of reproductive status on trace element content in cows. However, the data are insufficient and contradictory. In particular, certain studies demonstrated a significant group difference in hair [25] and serum [26] zinc, copper, iron levels between heifers and cows. In turn, the earlier investigation failed to reveal any effect of reproductive status on hair Zn content in Hereford cows [27].

The lower hair Se content in cows observed in the present study is contradictory to the previous studies. In particular, whole blood analysis demonstrated that cows have more than 2-fold higher levels of Se than heifers [28]. Similarly, Se-dependent glutathione peroxidase activity was also higher in cows [29]. Serum Se concentrations have been also shown to increase with age in livestock [30]. However, taking into account a potential role of hair as excretory mechanism for selenium [31], one can propose that elevated hair Se content in heifers may be indicative of increased excretion of the metalloid.

Table 4 The reference values of hair essential and toxic trace element content (μg/g) in Hereford heifers and cows in calculated in accordance with American Society for Veterinary Clinical Pathology Quality Assurance and Laboratory Standard Guidelines [21]

Element	Total				Heifers				Cows			
	n	Reference interval	Lower limit 90% CI	Upper limit 90% CI	*n*	Reference interval	Lower limit 90% CI	Upper limit 90% CI	*n*	Reference interval	Lower limit 90% CI	Upper limit 90% CI
Co	150	0.039–0.496	0.035–0.049	0.398–0.668	75	0.035–0.461	0.035–0.047	0.347–0.495	75	0.053–0.627	0.049–0.066	0.361–0.668
Cr	146	0.053–1.121	0.039–0.071	0.781–1.580	74	0.049–1.268	0.039–0.068	0.830–1.580	74	0.055–1.051	0.052–0.081	0.754–1.110
Cu	150	3.0–7.6	2.9–3.2	6.5–8.3	75	2.9–7.9	2.9–3.2	6.6–8.3	75	3.0–7.5	2.9–3.2	6.3–7.6
Fe	150	17.1–524.8	13.2–21.1	416.2–627.0	75	13.3–490.7	13.2–19.0	390.2–627.0	75	21.7–575.9	21.3–24.5	436.9–596.0
I	150	0.308–2.052	0.196–0.338	1.699–2.430	74	0.245–2.075	0.196–0.355	1.620–2.430	75	0.317–2.101	0.309–0.415	1.640–2.120
Li	149	0.105–0.906	0.092–0.119	0.726–1.180	73	0.103–0.975	0.097–0.118	0.522–1.180	75	0.099–0.924	0.092–0.122	0.725–0.931
Mn	149	7.5–100.1	6.5–9.3	66.2–122.0	75	7,3–65,7	6.5–9.2	58.3–66.2	75	7.0–117.3	6.5–11.5	89.7–122.0
Se	150	0.139–1.213	0.117–0.162	1.101–1.320	75	0.125–1.313	0.117–0.153	1.167–1.320	74	0.158–1.014	0.154–0.176	0.815–1.060
Si	149	0.9–73.9	0.4–3.5	59.8–121.0	75	0.5–58.1	0.4–2.3	47.3–58.7	75	5.5–111.3	5.4–6.7	71.3–121.0
V	150	0.072–1.282	0.048–0.086	1.070–1.480	75	0.055–1.297	0.048–0.081	0.973–1.340	75	0.086–1.422	0.077–0.114	1.036–1.480
Zn	149	60.2–130.3	52.9–69.4	127.0–231.0	74	71.0–165.0	69.5–76.8	128.6–231.0	75	54.2–124.4	52.9–61.9	117.0–126.0
As	150	0.040–0.304	0.029–0.043	0.258–0.381	75	0.029–0.344	0.029–0.040	0.261–0.381	75	0.043–0.258	0.043–0.054	0.230–0.266
B	147	1.2–16.9	1.0–1.7	12.0–18.2	73	1.2–14.0	1.2–1.6	6.5–16.8	75	1.2–18.2	1.0–1.8	12.0–18.2
Cd	148	0.013–0.098	0.009–0.015	0.080–0.149	74	0.011–0.098	0.009–0.015	0.067–0.127	74	0.014–0.135	0.013–0.019	0.085–0.149
Hg	145	0.002–0.042	0.002–0.002	0.026–0.153	72	0.002–0.073	0.002–0.002	0.027–0.153	72	0.002–0.044	0.002–0.002	0.029–0.047
Ni	150	0.280–1.575	0.209–0.321	1.380–2.100	75	0.247–1.519	0.206–0.294	1.250–1.570	75	0.317–2.007	0.311–0.335	1.360–2.100
Pb	150	0.096–0.681	0.075–0.107	0.597–0.787	75	0.082–0.684	0.075–0.113	0.590–0.787	75	0.098–0.688	0.096–0.118	0.553–0.691
Sn	149	0.005–0.091	0.004–0.007	0.079–0.170	74	0.008–0.108	0.007–0.009	0.076–0.170	72	0.004–0.094	0.004–0.005	0.082–0.095
Sr	150	3.4–29.1	2.6–4.9	26.4–35.6	75	2.9–26.6	2.6–4.5	23.1–26.7	75	5.3–34.8	4.8–8.3	26.4–35.6

n the number of samples included into analysis after exclusion of outliers. Distribution: non-Gaussian. Briefly, after exclusion of outliers (percentile two-sided), the robust method was applied for assessment of reference intervals and 90% confidential intervals for the lower and upper limits. The calculations were performed using Reference Value Advisor for MS Excel

The observed differences in essential trace element levels may be associated with different nutrient requirements in cows depending on reproductive status [32].

The majority of heavy metals tended to increase in hair of animals with age. An increase in hair toxic trace element content may occur due to external exposure and accumulation of metals in hair matrix. An earlier investigation performed in South Urals demonstrated a significant age-dependent increase in hair heavy metal content in urban occupationally non-exposed human population [33]. It is notable that hair mercury and tin content were decreased in cows as compared to heifers. Generally, this finding is in agreement with the earlier study by Lopez Alonso and the coauthors (2003) who demonstrated that cows did not accumulate mercury with age [34].

Generally, the obtained data demonstrate that heifers are characterized by significantly higher hair Se, Hg, and Sn content, whereas cows had significantly higher levels of hair Co, I, Si, V, B, Cd, Pb, and Sr. At the same time, no significant group difference in hair Cr, Cu, Fe, Li, Mn, Zn, As, and Ni was detected between Hereford cows and heifers.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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