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Retrospective diagnosis of intrauterine diselementosis in newborn calves

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Abstract. One of the methods in diselementosis diagnosis, i.e. in determination of the trace elements content excess, deficiency or imbalance in the organism, is screening the hair elemental composition. It is known that the tail brush hair growth in cattle begins from the 7th month of gestation. The authors suggested that the tail brush hair elemental composition in newborn calves could be used for integral evaluation of the trace elements supply to the fetus in the last months of its intrauterine development. In order to elaborate criteria for retrospective diagnosis of the intrauterine diselementosis, 125 clinically healthy 1-day-old calves (67 male and 58 female animals) were examined; they were obtained from Simmental cows with a physiological course of gestation under biogeochemical conditions of the Central Black Earth region of the Russian Federation. The concentration of 11 essential (Co, Cr, Cu, Fe, I, Li, Mn, Se, Si, V, Zn) and 8 toxic (As, B, Cd, Hg, Ni, Pb, Sn, Sr) trace elements were studied in samples of unpigmented guard hairs from the animal tail brush by inductively coupled plasma mass spectrometry (Nexion 300D, Perkin Elmer, USA). The results were expressed as mean ± standard error of mean, median, minimum, and maximum. Reference values of essential and toxic trace elements concentration in the hair of newborns were determined using the percentile scales separately for the groups of male and female animals. Group comparison was conducted using the independent samples Mann-Whitney U test. Research results demonstrated that physiologically determined the range of the trace elements concentration in the hair of newborn calves corresponded to the interval between the 25th and 75th percentiles. Values beyond the specified interval boundaries were proposed to be considered as deviations corresponding to the diselementosis.

1 Introduction

The term "diselementosis" in biological literature is accepted to designate violations in the chemical elements homeostasis manifested by their content excess, deficiency or imbalance in the organism [1, 2]. Diselementosis in animals could course both latently and manifest itself in various clinically pronounced pathological conditions [2, 3].

Recent studies showed that intrauterine diselementosis in cattle is a risk factor provoking a number of diseases [3, 4] and metabolic disorders in the postnatal ontogenesis [5]. In this connection, evaluation of the fetus bioelemental status in cows, especially in the last trimester of pregnancy, acquires important diagnostic and prognostic value.

To evaluate the bioelemental status in cattle, hair, blood, urine, liver and other biopsy tissues are examined, in lactating animals – milk [6, 7, 8]. In this case, both the direct content of chemical element itself in organs and tissues [7, 8] and its metabolites [9, 10] could be evaluated in animals. For example, to assess the selenium status in cows, activity of the selenium-dependent glutathione peroxidase in blood is determined [11].

When selecting a biosubstrate for research, it is important to understand that the chemical elements concentration in the organism liquid media (blood, urine, milk) mainly reflects short-term and deep alterations in the bioelemental status [7, 8]. At the same time, the elemental composition of the solid tissues (hair and bones) characterizes disorders formed over a long period from several months to several years [12, 13]. For some chemical elements (arsenic, cadmium, lead, calcium, copper, zinc), its content determination is informative both in the organism liquid and in solid media [14, 15]. For others (aluminum, barium, boron, iron, magnesium, phosphorus, strontium), it is preferable to study their content in solid media, for example, in the hair [16, 17].

In the last 10–15 years, hair as the research object to evaluate the cattle elemental status gained significant popularity [18, 19]. Reference chemical elements

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concentration ranges in the hair of heifers and cows of meat and dairy breeds were determined [20, 21]. Influence on the cattle hair elemental composition by sex and age [22, 23], productivity level [16, 24, 25], features of the region, season of the year and animal rearing technology [14, 19, 26] was studied.

Hair analysis in cattle diselementosis diagnosis has a number of advantages over traditional research objects, such as blood, urine and liver biopsy specimens [7, 8, 18]. The most important of them include the low level of stress load on animals during biomaterial sampling (compared to blood sampling or biopsy) and the elemental composition stability during long-term storage and transportation of the samples [12, 18, 27].

It was shown that chemical elements concentration in hair samples taken from the cattle body various parts were differing significantly [18]. The content of calcium, magnesium, copper, manganese, molybdenum, cobalt, nickel, strontium and cadmium in the tail brush guard hair was significantly higher, while zinc and potassium content, on the contrary, was lower in the samples taken from the tail brush compared to hair from other parts of the body (withers, dorsum) [18, 27]. High correlation was established between the chemical elements concentration in the tail brush integumentary hair of dairy cows and in the tissues of their parenchymal organs [27]. For lactating Black-and-White, Simmental and Holstein cows, indicative norms for the chemical elements content in the tail brush integumentary hair were elaborated [27]; sampling procedure, sample preparation, and analysis were described [21, 28].

Study of the chemical elements content in the tail brush integumentary hair in newborn calves is of great interest [5]. It is known that hair growth of the tail brush in cattle starts from the 7th month of gestation [29, 30]. In this connection, chemical elements concentration in the tail brush integumentary hair samples obtained from calves immediately after partition could be considered as integral indicators of the fetus supply with the studied elements in the last months of intrauterine development [5].

Objective of this study was to elaborate criteria for retrospective intrauterine diselementosis diagnosis in the Simmental newborn calves by chemical elements content in the tail brush integumentary hair.

2 Material and methods

2.1 Animals

Simmental cattle (*Bos taurus taurus*) was the research object. Research was conducted during the stall period (October-May) of keeping the animals in conditions of the Rechnoye Farm, Khlevensky District, Lipetsk Region of the Russian Federation. The sample included 125 clinically healthy 1-day-old calves (67 bulls and 58 heifers) weighing 43–55 kg and obtained from cows of 2–4 lactations with productivity of 5860–8338 kg. The trace elements content in the mother cows' ration generally met the animals' needs. It was not exceeding the maximum acceptable limits established by the USSR State Agriculture Committee [31] and the U.S. National Research Council [32, 33].

2.2 Sampling

Samples of integumentary unpigmented hair were obtained from the newborn calves tail brush using stainless steel scissors pre-treated with ethyl alcohol. From all the tail brush hairs cut as close as possible to the skin surface, an average sample weighing at least 0.5 g was taken. Hair samples were immersed in the clear for analysis acetone (Khimmed, Russia) for 15 minutes, then were washed three times with the deionized water (18 MΩ cm) and further were dried at the temperature of 60°C to the air-dry state followed by grinding into 2–3 mm long fragments.

2.3 Trace element analysis

Hair sample preparation and actual trace elements analysis were carried out, as described earlier [20], in the Micronutrients LLC accredited laboratory (Moscow, Russia) being the IUPAC associated company. 5.0 ml of high-purity concentrated nitric acid (Khimmed, Russia) was added to the 50.0 mg sample of hair. The mixture was decomposed in the Multiwave 3000 microwave device (PerkinElmer, Austria) in the following mode: 5 minutes – raising temperature to 200 °C, 5 minutes – stabilizing temperature at 200 °C, then cooling down to 45 °C. After that, the content was quantitatively transferred into the polypropylene tubes. The volume was adjusted to 15 ml by adding deionized water (18 MΩ cm) and thoroughly mixed by shaking in tubes closed with lids. The quantitative determination of chemical elements, including iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), cobalt (Co), chromium (Cr), selenium (Se), iodine (I), boron (B), silicon (Si), nickel (Ni), vanadium (V), arsenic (As), lithium (Li), tin (Sn), strontium (Sr), lead (Pb), cadmium (Cd) and mercury (Hg), in the samples was carried out by inductively coupled plasma mass spectrometry on the Nexion 300D spectrometer (Perkin Elmer, USA). The instrument was calibrated using the monoelement Universal Data Acquisition Standards Kit (CT 06484, PerkinElmer, USA), and for internal standardization - the single-element pure yttrium standard of 10 µg/L (Y, Perkin Elmer, USA). The GBW09101 hair sample from the Shanghai Institute for Nuclear Research (China) was used as the reference. Extraction degree in the studied chemical elements varied from 90 to 110 %.

2.4 Statistical analysis

Experimental data was statistically processed using the IBM SPSS Statistics 20.0 software (IBM Corp., USA). The entire data were expressed as mean ± standard error

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of mean (SEM), median, minimum (Min) and maximum (Max). Reference chemical elements concentration values in the tail brush hair of newborn calves were determined by the 25th (lower limit) and 75th percentiles (upper limit) [20, 21]. Group comparisons were made using the independent samples Mann-Whitney U test; differences were considered significant at $P < 0.05$.

3 Results

Results of the essential and toxic trace elements quantitative determination in the tail brush integumentary hair of newborn calves are presented in Table 1 and Table 2, respectively.

Table 1. Hair essential trace element content (μg/g) in Simmental newborn calves.

Element	Mean±SEM	Median	Min–Max
Co	0.072±0.006 0.063±0.005	0.067 0.056	0.020–0.239 0.016–0.167
Cr	0.076±0.014 0.050±0.012	0.021 0.017	0.010–0.330 0.011–0.260
Cu	7.19±0.26 6.94±0.29	7.34 7.41	2.20–11.44 2.10–10.60
Fe	46.1±3.6 50.7±3.6	40.0 42.6	13.5–151.0 11.9–123.5
I	0.236±0.035 0.354±0.072	0.220 0.340	0.130–0.560 0.190–0.530
Li	0.025±0.005 0.019±0.003	0.020 0.020	0.010–0.080 0.010–0.030
Mn	8.17±0.29 9.64±0.40	8.43 8.69*	4.30–11.60 5.60–17.40
Se	0.298±0.018 0.263±0.016	0.270 0.260	0.060–0.734 0.080–0.526
Si	22.4±3.1 20.1±3.5	21.6 18.5	4.2–45.0 8.3–35.4
V	0.166±0.011 0.144±0.016	0.160 0.120	0.120–0.250 0.110–0.230
Zn	114.5±4.1 115.5±3.6	115.5 112.7	68.7–191.0 59.0–173.0

Above the line – group of male animals (n = 67), under the line – group of female animals (n = 58). * Differences between the groups are statistically significant at $P = 0.026$.

Table 2. Hair toxic trace element content (μg/g) in Simmental newborn calves.

Element	Mean±SEM	Median	Min–Max
As	0.074±0.009 0.068±0.010	0.057 0.057	0.006–0.233 0.002–0.211
B	0.881±0.346 0.446±0.080	0.510 0.450	0.270–4.93 0.260–0.860
Cd	0.013±0.002 0.014±0.003	0.010 0.010	0.002–0.082 0.002–0.077
Hg	0.034±0.004 0.029±0.004	0.030 0.030	0.020–0.060 0.020–0.050
Ni	0.585±0.087 0.873±0.138	0.350 0.500	0.060–2.79 0.090–3.11
Pb	0.522±0.105 0.634±0.141	0.220 0.480	0.030–2.00 0.040–1.73
Sn	0.029±0.004 0.028±0.008	0.020 0.020	0.006–0.060 0.009–0.070
Sr	0.740±0.071 0.820±0.089	0.600 0.600	0.320–2.93 0.320–2.80

Above the line – group of male animals (n = 67), under the line – group of female animals (n = 58).

Tables 3 and 4 present reference values of the studied trace elements concentration in the hair of newborn bulls and heifers determined on the basis of percentile scales [20, 21].

Table 3. The reference values* of hair essential trace element content (μg/g) in Simmental newborn calves.

Element	Males (n = 67)	Females (n = 58)
Co	0.048–0.098	0.040–0.084
Cr	0.015–0.153	0.015–0.275
Cu	5.55–9.08	5.45–8.63
Fe	30.1–53.1	31.7–58.2
I	0.140–0.295	0.190–0.530
Li	0.010–0.030	0.010–0.020
Mn	7.38–9.27	8.44–11.25
Se	0.200–0.405	0.160–0.367
Si	16.0–28.6	11.7–28.0
V	0.130–0.205	0.110–0.160
Zn	90.1–133.8	96.8–128.0

* Lower boundary corresponds to the 25th percentile, upper boundary – to the 75th percentile.

Table 4. The reference values* of the hair toxic trace element content (μg/g) in Simmental newborn calves.

Element	Males (n = 67)	Females (n = 58)
As	0.020–0.110	0.020–0.106
B	0.370–0.590	0.270–0.530
Cd	0.003–0.016	0.008–0.011
Hg	0.025–0.040	0.020–0.030
Ni	0.218–0.690	0.200–1.40
Pb	0.068–0.893	0.060–1.28
Sn	0.020–0.040	0.010–0.040
Sr	0.428–0.830	0.500–1.00

* Lower boundary corresponds to the 25th percentile, upper boundary – to the 75th percentile.

According to content in the hair of newborn calves (μg/g), trace elements under study could be ranked in the following descending series: Zn (114.7), Fe (41.3), Si (20.4), Mn (8.58), Cu (7.37), Sr (0.600), B (0.500), Ni (0.390), Se (0.260), Pb (0.235), I (0.230), V (0.150), Co (0.060), As (0.057), Hg (0.030), Li (0.020), Sn (0.020), Cr (0.019), Cd (0.010).

Female animals demonstrate higher concentrations of manganese in the hair than males do (9.64±0.40 versus 8.17±0.29 µg/g, P = 0.026). Hair concentrations of other essential and toxic trace elements did not differ statistically significant between the samples of male and female animals.

4 Discussion

For the first time, we carried out comprehensive evaluation of the 19 trace elements content in tail brush integumentary hair of the 1-day-old Simmental calves. Comparison of the obtained results with literature data [20, 21, 22, 27] showed that elemental profile of hair in newborn calves differed from that in the adult cattle in the lower content of iron, iodine, lithium, selenium, boron and strontium, and higher content of manganese, silicon, vanadium and mercury. Concentrations of other essential (cobalt, copper, zinc) and toxic (arsenic, cadmium, nickel, lead and tin) trace elements under study in the hair of newborns were comparable with the reference values described for the lactating cows [21, 27, 28].

Newborn Hereford and Holstein calves demonstrated manganese (2–4 µg/g) and iron (15–31 µg/g) concentrations in hair lower than our data (Table 1), but comparable in regard to copper (10–12 µg/g) and zinc (128–142 µg/g) [34]. The observed difference between the data published previously [34] and our data could be associated both with breed characteristics of the animals and with differences in biogeochemical conditions in their cultivation. Within the same biogeochemical zone in the Central Black Earth Region of the Russian Federation, hair concentrations of cobalt (0.057–0.097 µg/g), copper (7.40–10.60 µg/ g), iron (20.3–65.0 µg/g), manganese (8.11–12.80 µg/g), selenium (0.398–0.595 µg/g) and zinc (91.3–138.1 µg/g) in 1-day-old calves obtained from the Red-and-White cows with physiological course of pregnancy [5] were comparable with our results.

Table 1 shows that newborn heifers were characterized by a higher (by 18.0%, P = 0.026) concentration of manganese in the tail hair brush compared to the bulls. These results are of particular interest, since it was previously demonstrated that manganese concentration in the hair correlates with the level of physiological maturity in the newborns [5] and depends on the productivity direction [22]. It was established [22] that the manganese content in the dairy animals hair is significantly higher (by 48.6%, P < 0.05) compared to that of beef cattle.

S.A. Miroshnikov et al. (2015) found that the 5-8-months-old Simmental heifers, compared to bulls of the same age, were characterized by a higher content in their hair of not only magnesium, but also of cobalt, chromium, iodine, vanadium, arsenic, nickel, lead, strontium, and by a lower level of silicon [22]. Our study showed that statistically significant differences between the groups of 1-day-old male and female animals were found only in the content of magnesium in the hair, but not in other trace elements. Probably, characteristic features of the hair elemental profile in bulls and heifers are formed at the older age.

We used percentile scales in the present study, when determining reference values of the chemical elements concentration in the hair of newborn calves. M.G. Skalnaya et al. were the first to pioneer this approach (2003) [35]. The authors demonstrated that physiologically determined range of the trace elements concentration in human hair corresponded to the interval between the 25th and 75th percentiles in the representative sample, and values beyond the 25th and 75th percentiles indicated presence of the diselementosis [35]. Diselementosis diagnosed on the basis of these criteria could be latent in a patient (as a rule, if values are in the range from the 10th to the 25th percentile or from the 75th to the 90th percentile) or with characteristic clinical signs of deficiency, excess or imbalance in the trace elements content in the organism. As a rule, if the values go beyond the 10th or 90th percentile boundaries [1, 35]. Eligibility of using this approach in diselementosis diagnosis in cattle is confirmed by research results provided by the scientific group of Professor S.A. Miroshnikov [20, 21, 28, 36, 37].

In the present study, we evaluated the chemical elements concentration in the average sample from the tail brush guard hairs obtained from calves on the 1st day after birth. Since the tail brush hair growth in cattle begins from the 7th month of gestation [29, 30], results of this analysis make it possible to assess fetus supply with elements under study in the last months of intrauterine development, diagnose diselementosis, predict metabolic disorders [5, 9] and correct them in a timely manner.

We physiologically determined the concentration range of essential (Table 3) and toxic (Table 4) trace elements in the hair of newborns separately for the groups of male and female animals. This approach makes it possible to more accurately diagnose diselementosis in newborn calves taking into account their gender.

5 Conclusion

As a result of research using percentile scales, reference intervals were determined for essential (cobalt, chromium, copper, iron, iodine, lithium, manganese, selenium, silicon, vanadium, zinc) and toxic (arsenic, boron, cadmium, mercury, nickel, lead, tin, strontium) trace elements in the hair of newborn Simmental bulls and heifers in biogeochemical conditions of the Central Black Earth Region of the Russian Federation. Values beyond the 25th and 75th percentiles are proposed to be considered as deviations indicating intrauterine diselementosis.

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