The application of wool as a biosubstrate to assess the impact of various strontium levels on the biological characteristics and productive qualities of dairy cattle under conditions of increased technogenic load

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Abstract. Strontium is a natural component of aquatic and terrestrial ecosystems. However, due to anthropogenic activity, strontium concentrations locally increase to levels that create a potential environmental risk and can lead to disturbance of the elemental status and productive qualities of farm animals. In this regard, the aim of this study was to study the elemental status and productivity of Holstein cows with different levels of Sr in wool, bred in environmentally unfriendly areas of the Southern Urals. The studies were carried out among the Holstein cows whose wet weight was within 505–535 kg. The lactation stage was 30–50 days after calving. Dairy productivity was 18.8–31.4 liters per day. The research was carried out in Agrofirm Industrial LLC, Orenburg region. The animals were divided into three groups depending on Sr concentration in their coat. The range of Sr concentrations in the coat of cows Group I was from 0.716 to 1.69 \( \mu \)g/g, Group II – from 1.82 to 3.68 \( \mu \)g/g, and Group III – from 3.81 to 7.23 \( \mu \)g/g. When comparing the chemical elements concentrations in the coat among the groups under the experiment, it was found that an increase in Sr level was accompanied by the significant change in levels of Ca, K, Mg, P, Se, Mn, Cd, Ni, B. Moreover, as Sr content increased from the minimum to the maximum value, there was occurred a decrease in Cu concentration by 10.9–25.3 %; Zn – by 11.1–21.2 %, and As – by 10.0–30.0 %. The ratios calculated with the Spearman's rank correlation for the experimental animal groups revealed a reliable correlation between Sr concentrations and concentrations of Cu, Se, and Zn in the coat. As Sr content in the coat increased, there was a change in the indicators of the antioxidant status of the blood serum, which was expressed via an increase in the level of malondialdehyde by 2.2 and 2.6 times. The cows with Sr content in coat lower than 1.82 \( \mu \)g/g exceeded the analogues of Groups II and III in daily productivity calculated based on milking – 1 % milk by 32.8 and 32.3 %, respectively.

1. Introduction
The mineral elements are important components of an animal body and active components involved in organs and system functioning [1, 2]. However, the effects of certain pollutants on animals’ body, including some heavy metals, lead to disturbances in animal health and result in a decrease in their qualities and characteristics affected productivity [3, 4].

Getting into the body with inhaled air, with food and water, the heavy metals can negatively affect the health and productivity of farm animals [5], through the reduced enzyme activity [6], and...
metabolism disruption of some essential body elements [7], and etc. The danger caused by heavy metals lies in their ability to be accumulated in the bones and other tissues and organs of animals, even if they appear in the animals’ organism with a slight dose from the environment [8].

One of the most common heavy metals in the environment is strontium (Sr). Strontium is a natural component of aquatic and terrestrial ecosystems. Due to the human-induced activity, the level of this element in the environment can be raised locally to levels potentially hazardous to ecosystems. Pollution of Sr-saturated soils and waters is often caused by the prolonged use of phosphate fertilizers, strontium-containing reclamation and industrial waste in agriculture [9].

Sr is an alkaline-earth metal, an analogue of calcium, available for plants and which is carried up well along the food chain. For animals, this element is not essential, but can replace calcium when the latter is in lack. Against the background of high dosages and under certain conditions, this can lead to osteomalacia, characterized by a) impaired bone mineralization [10], b) a decrease of glycogen in a liver, c) an increase of kidneys and adrenal glands mass [11], d) some morphological changes in the organs of animals’ lymphatic system [12].

Thus, the aim of this research was to study the elemental status and productivity of the Holstein cows regarding the level of strontium (Sr) in their coat during milking.

2. Materials and methods
2.1. Object of research
The studies were carried out among the Holstein cows whose wet weight was 505-535 kg. The lactation stage was 30-50 days after calving. Dairy productivity was 18.8-31.4 liters per day. Animal services and experimental studies were carried out in accordance with the instructions and recommendations declared by the Order of the Ministry of Health of the USSR dated on July 27, 1978, No. 701 “On the additions to the Order of the Ministry of Health of the USSR dated on 12.08.77 No. 755” and “The Guide for Care and Use of Laboratory Animals” (National Academy Press, Washington, D.C. 1996). On the way of the experimental works, we undertook the efforts to minimize animals' suffering and to reduce the number of samples used.

2.2. Experiment scheme
The experimental works were carried out in Agrofirm Industrial LLC, Orenburg region. The animals (n=45) were divided into three groups depending on Sr concentration in their coat. Group I – up to the 25th percentile (n=12), Group II – within the limits of the 25-75th percentile (n=19), Group III – above the 75th percentile (n=14).

2.3. Selection and study on coat samples
The coat samples weighing at least 0.4 gr. were selected from the top of the animals’ withers in line with the method developed earlier [13]. Stainless steel scissors previously treated with ethanol were used to select the samples. The elemental composition of the coat was studied within 25 indicators (Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, I, K, Li, Mg, Mn, Na, Ni, P, Pb, Se, Si, Sn, Sr, V, Zn) by the mass spectrometry method and atomic-emission spectrometry method with inductively related plasma (AES-IRP) and AES Optima 2000 DV and Nexion 300 D (Perkin Elmer, USA). All the analytical procedures were performed in the laboratory of Center of Biotic Medicine (Moscow, Russia). The results of the chemical analysis carried out with the coat samples were compared with the intervals of “physiological norm (value)” established for the Holstein cow breed [14].

2.4. Selection of average milk samples
The cows were mechanically milked three times a day at 6 a.m., at 2 p.m., and at 6 p.m. The milk produced was weighed individually from each cow daily for two neighboring days. The raw milk samples were selected individually from each cow three times a day at each milking and then they were placed in the sterile containers, cooled (up to 5 degrees Celsius) and sent for the analysis to the Center for Collective Use of the Federal Scientific Center of Biological Systems and Agricultural
Technology of the Russian Academy of Sciences. The studies with the milk samples were carried out on the day of sampling from the animals. The amount of milk was adjusted for fat content – 1%.

The content of fat, protein and lactose in the milk was evaluated through using the FIL-IDF procedure via the milkoScan™ FT1 (Foss Electric, DK-3400, Hiller'd, Denmark).

2.5. Selection of average blood samples

The blood samples (9 ml) were taken from each cow (the day after milk sampling was undertaken) from the tail vein into a vacuum test tube. The concentration of malandialdehyde (MDA) was determined in heparinized blood through the reaction with the thiobarbituric acid by the spectrophotometric method [15].

The degree of the peroxide oxidation of the lipids was judged according to accumulation of the product reaction of small-mdehyde (Total-MDA) with the thiobarbituric acid. The activity of the enzyme superoxidedismutase was judged according to the attrition rate of the hydrogen peroxide in the incubation environment. The concentration of the hydrogen peroxide was determined by the reaction with ammonium molybdah.

2.6. Statistical analysis

The Shapiro-Wilk criterion was used to test the hypothesis about the normality in distribution of other quantitative characteristics. The distribution law regarding the numerical indicators differed from the standard (norm value), therefore, the reliability of the differences was tested with the Mann-Whitney U-criterion. To determine the strength of some functional connections existing between the parameters, we needed to calculate the Spearman correlation ratios (Ks). In all statistical analysis procedures, the level of value (R) was calculated, with a critical level of value being taken less or equal to 0.05. The tables show the averages of indicators (M) and their standard deviations (±STD). Statisticsa 10.0 application package (StatSoft, Inc., USA) was used to process the data.

3. Results

The actual data of differences with the concentration of the chemical elements in the coat between the groups of dairy cows, depending on the percentage interval of Sr concentration are presented in Table 1.

As seen from Table 1, the coat of the animal (Group I) contained Sr equal to 1.32 μg/g, that is by 2.1 (P≤0,001) and by 3.5 times (P≤0,001) less compared to Groups II and III. Wherein the range of Sr concentration in the cows’ coat belonging to Group I was within 0.716 and 1.69 μg/g, Group II was in the range of 1.82 and 3.68 μg/g, and Group III – 3.81 to 7.23 μg/g.

Compared to the chemical elements concentration contained in the coat of all groups, we concluded that the increase of Sr concentration was accompanied with the reliable change occurring in the levels of Ca, K, Mg, P, Se, Mn, Cd, Ni, B. In this case, with increasing Sr concentration from minimal to maximal value, there was observed the decrease in Cu concentration by 10,9–25,3 %; in Zn – by 11,1–21,2 % and in As – by 10,0–30,0 %.

The cows' elemental status evaluation in the context of the groups relative to the limits of the “physiological norm value” revealed that there were some significant changes in the concentrations of the number of the elements (Figure 1, a–c).

The data analysis showed that the general pattern for all the groups under study was a synchronous offset of the elemental profile with Sr level. Therefore, if the cows with the minimal Sr levels of the coat were characterized by lower concentrations on terms of 8 studied elements, then in the cows with maximum levels it was only on terms of 2 elements. Wherein, the cows with Sr levels within 25–75 percentile deviations from the norm were not observed.

The ratios calculation based on the Spearman's rank correlation for the experimental animal groups revealed a reliable correlation between Sr concentrations and concentrations of Cu, Se, and Zn in the coat (Table 2).
The cows with Sr content in the coat which is lower 25 percentile exceeded the analogues of Groups II и III per daily productivity that was calculated on milking of 1 % milk per 32.8 (P≤0.05) and 32.3 % (P≤0.01) respectively (Table 3).

**Table 1.** Content of chemical elements in coat of the Holstein cows, depending on the percentage interval of Sr concentration, (μg/g)

| Element   | Group I | Group II | Group III |
|-----------|---------|----------|-----------|
| Ca        | 643.9±174.0 | 1973.6±541.5 | 2629.3±589.4 |
| K         | 2913.2±656.9 | 3695.0±794.7 | 4360.3±895.0 |
| Mg        | 221.2±53.0 | 561.7±117.3 | 718.9±143.9 |
| Na        | 2520.7±945.8 | 2571.8±727.5 | 3596.2±2079.6 |
| P         | 212.0±25.4 | 266.9±39.47 | 285.0±42.83 |

**Table 2.** The correlation ratios of Sr concentration with the concentrations of essential elements in the coat from the withers of the Holstein cows

| Elements | Co | Cr | Cu | Fe | I | Mn | Se | Zn |
|----------|----|----|----|----|---|----|----|----|
| Sr       | 0.24 | 0.19 | -0.60* | 0.21 | 0.25 | 0.51 | 0.71* | -0.61* |

**Table 3.** The milk indicators of quantity and quality depending on the percentile interval of Sr concentration in the coat from the withers of the Holstein cows breed

| Indicator                                           | Group I                  | Group II                 | Group III                |
|-----------------------------------------------------|--------------------------|--------------------------|--------------------------|
| Fat yield, kg / day                                 | 0.855±0.065              | 0.644±0.2               | 0.646±0.113              |
| Protein yield, kg / day                             | 0.875±0.141              | 0.839±0.212             | 1.05±0.224              |
| Nonfat dry milk yield, kg / day                     | 2.32±0.318               | 2.18±0.536              | 2.63±0.532              |
| Average daily milk yield 1 % milk, liter / day      | 85.53±6.51               | 64.42±19.97*            | 64.64±11.29*            |
| Average daily milk yield 1 % milk, liter / day      | 25.76±3.16               | 24.100±5.87             | 28.3±5.62              |
а P≤0.05; б P≤0.01 compared to Group I.

Figure 1. Multiplicity of the deviations among the elemental composition of the coat from the withers of the holers among the Holstein breeds with the different levels of Sr concentration during milking time from the “physiological norm value” established within the limits of 25 and 75 percentiles [14]
(conducted via the method of Doctor Scalny): (a) – Sr concentration from 0.716 to 1.69 \( \mu \text{g/g} \); (b) – from 1.82 to 3.68 \( \mu \text{g/g} \); and (c) – from 3.81 to 7.23 \( \mu \text{g/g} \).

As Sr content in the coat increased, there was a change in the indicators of the antioxidant status of the blood serum (Figure 2, a–b), which was expressed in the increase along the level of malondialdehyde in the animals of Groups II and III by 2.2 and 2.6 times (\( P \leq 0.05 \)), respectively in relation to the individuals of Group I.

![Figure 2](image)

**Figure 2.** The indicators of the antioxidant status of the blood serum among the Holstein cows, depending on the percentile interval of Sr concentration in the coat from the withers: (a) superoxide dismutase activity, %; (b) – level of malondialdehyde, nmol / l, Average ± 0.95 confidence interval.

4. Discussion

When planning and carrying out the research, we proceeded from the fact that the animal coat can be used to characterize the elemental status of its organism [16]. According to the existing concept adopted in the official medicine, it is proposed to consider the interval between the 25th and 75th percentiles of the levels of chemical elements in the coat of a representative sample as “physiological normal value”. In line with this practice, the concentration of the chemical elements in human hair in the range from 10 to 25 percentiles and from 75 to 90 percentiles is considered as deviations that correspond to the state before the disease (pre-deficiency). The concentrations in the range up to the 10th percentile and above the 90th percentile are associated with a clear clinical demonstration of the syndromes and symptoms to be characteristic to dyslementosis [17].

In the studies carried out earlier, in accordance with the concept proposed above, we determined the reference intervals for the content of the chemical elements in the coat of the Holstein cows [14]. It is noteworthy that the obtained levels of the concentrations of the main and toxic microelements in the cows’ coat are consistent with the studies published earlier [18, 19].

A comparative data analysis allowed to state that the increase in the “exchange pool” regarding to Sr content (that is above 75 percentile), was accompanied by an excess of 75 percentile in the content of Sn, Ca, K, Mg, Na, Se, Cr, Fe, Ni, and B in the coat, wherein this level of Cu and Zn, decreased by less than 25 percentile (Figure 1, c).

The decrease of the essential elements (Cu and Zn) content in the animal coat against the background of a toxic growth can be explained by the result of antagonistic interactions between these elements. Earlier, the fact of the metabolism of the essential elements under the influence of the toxic metals was observed for Fe [20], Se [21], Zn and Mn [22, 23]. The proof of that there are the results showing up the negative correlation of toxic Sr with Cu and Zn that were obtained within our experimental studies (Table 2).

Sr and Ca are characterized by the similar chemical properties. Therefore, these elements are
antagonists and can replace each other in various biological systems. In our experiment, in the group with the maximum Sr concentration, the Ca level was in the range of 75 and 90 percentile, which, according to the existing concept, may indicate a state of pre-deficiency for this element. Another reason for the shift of Ca exchange pool towards the deficiency may be the increase in the metabolism of the latter against the background of milking yield – the period of maximum use of the body's reserves for milk production.

The increase in the exchange pool of Fe appeared the most significant, the concentration of which in the coat of the animals of Group III exceeded the values of 90 percentile. It is known that the concentration of Fe in the body is tightly regulated [24], including through activation of the synthesis of ferroportin [25-27]. It can be assumed that the ability of Ferroportin to transfer other metals could lead to a change in their total pool in the body of the cows when milking.

The highest milk productivity calculated by the average daily milk yield of 1 % milk was observed in the cows with Sr in the coat that was lower 25 percentile. As the content of Sr increased from minimum to maximum, the significant decrease in this indicator occurred.

One of the reasons for relatively low productivity among the cows with the increased Sr content in the coat is the negative effect of the toxic elements on the animals through oxidative stress development. Within our experiment, the fact confirming the development of oxidative stress is the increase in the level of malondialdehyde in the blood serum, among the cows with a high level of Sr in the coat (Figure 2, b).

5. Conclusion
The elemental composition of the coat is closely related to the productivity of the dairy cows. The excess of Sr concentrations in the coat from the withers that is above 1.82 $\mu$g / g leads to the significant decrease in the milk production. In this regard, it is necessary to further study the possibility of using reference intervals of the toxic elements concentrations in the coat of the dairy cows in order to improve productive qualities and preserve the animals' health.

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