Adaptive changes of the elemental status of Kalmyk cattle to conditions of biogeochemical province of the Republic of Sakha (Yakutia)

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Abstract. The purpose of the study was to compare adaptive changes of the elemental status and hematologic indicators of blood of the Kalmyk cattle brought from the Republic of Kalmykia to the Republic of Sakha (Yakutia). Three groups of animals were formed for this purpose: brought cows (n=75), first-generation calves (n=28) and second-generation calves (n=16) received in Yakutia. The body weight of cows during bio-substrate selection made 480.4±13.8 kg. Age – 2.3-8.0 years. Wool samples weighing at least 0.4 g were taken from the top withers of animals. The proximal part of hair 15 mm long from the root was taken for analysis. The element analysis of samples was carried out via ICP-AES and ICP-MS. Blood samples were taken from a tail vein. The study was conducted using automatic hematologic analyzer: URIT-2900 Vet Plus (URIT Medial Electronic Co., Ltd, China). It is found that the wool of cows brought to the Republic of Sakha (Yakutia) had smaller concentration of Ca, Na, K, I, Mn, Zn, Sr at higher Hg value in comparison with the first-generation cows and smaller Cu and Zn and higher n Cr, Si, Pb content in comparison with the second-generation animals. The study of blood revealed significant changes in their parameters. Thus, the increase of erythrocytes and lymphocytes is observed from one generation to another with the decrease of the average volume of erythrocytes and hemoglobin in a separate erythrocyte.

1. Introduction
In order to create the massif of meat cattle large quantities of animals are delivered from other regions of Russia and abroad to the farms of the Republic of Sakha (Yakutia). The cattle brought from other regions will acclimatize to severe climatic and economic conditions of the republic [1].

Successful adaptation to new conditions is accompanied by morphofunctional changes in the organism of animals [3], including at the level of mineral metabolism [4, 5].
These changes may be assessed according to wool composition [6] with subsequent interpretation of obtained data to evaluate the state of health of an animal [7], which is especially relevant in the study of acclimatization abilities of animals.

The specifics of pastures in beef farming defines the uniqueness of the elemental status in certain territories. Hence, the movement of cattle for long distances to other biogeochemical provinces may lead to pathologies and mortality. The range of measures to assess and correct the elemental status before and after the movement of cattle increases the overall livability of livestock.

The purpose of the study is to compare adaptive changes of the elemental status and hematologic indicators of blood of the Kalmyk cattle brought from the Republic of Kalmykia to the Republic of Sakha (Yakutia).

2. Materials and methods

2.1 Animals

The study covered the cows of the Kalmyk breed received in March-April, 2011 in the Republic of Kalmykia brought in 2014 to Solooun integrated agricultural production center of the Republic of Sakha (Yakutia) and their first-generation (February-March, 2014 of year of birth) and the second-generation (November-December, 2016 of year of birth) calves. The body weight of cows during bio-substrate selection made 480.4±13.8 kg. Age − 2.3-8.0 years.

The animals were kept and pilot studies were conducted according to instructions and guidelines of the 1987 Russian Regulations (Order No.755 on 12.08.1977 the USSR Ministry of Health) and the Guide for Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996). During the study the authors took all efforts to minimize the suffering of animals and to reduce the number of used samples.

2.2 Description of territories

The climate of the Republic of Kalmykia (lat/lon: 46.3078, 44.2558) is sharply continental. The average temperature of the cold month (January) is -9°C, hot (July) − +25°C, with the range of temperature from -35°C to +40°C. Droughts and hot dry winds are typical for this region. The annual precipitation does not exceed 250-300 mm. The grass of natural pastures consists of herd grass, smooth brome, cockfoot, fescue and feather grass.

The climate of the Republic of Sakha (Yakutia) (lat/lon: 61.710589, 129.466690) is sharply continental with long winters (negative temperature lasts about 8 months) and short summers. The average temperature of the cold month (January) -42°C, hot (July) − +18°C, with the range of temperature more than 100°C – from +40°C in summer to -65°C in winter. Winter in Yakutia is long, cold and dry, white summer is short, droughty with relatively high temperatures. The annual precipitation does not exceed 200-255 mm. Grass is mainly composed of mixed herbs agropyrum or sedge agropyrum mixed herbs with domination of couch grass and hardish sedge. The following plant species are typical for steppes: Kolyma sheep fescue, koeleria, feather grass, crested wheat grass, meadow grass, bent grass and others.

Industrial manufacturing directly affecting biogeochemical situation includes mining enterprises that extract iron and titanic ores, copper, zinc and gold. Gas-and-oil industry, including primary processing, is also developed in the region.

2.3 Design of experiment

The groups were formed from among clinically healthy cows of the Kalmyk breed. The elemental status, morphological indicators of blood and indicators of natural resistance of an organism of cows were compared: cows brought from the Republic of Kalmykia to the Republic of Sakha (Yakutia) (n=75), first-generation calves (n=28) and second-generation calves (n=16) received in Yakutia.

From the moment of their delivery the animals were fed by diets compiled according to recommendations [8].

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The diet of cattle during the stall-feeding period included hay of cereal mixed herbs (7 kg), concentrates (barley, oats, meal sunflower) – 3.0 kg, during the pasture season – grass of cereal mixed herbs.

2.4 Assessment of elemental status
Wool samples weighing at least 0.4 g were taken in March, 2019 from the top withers of animals according to technique [9].

The elemental composition of bio-substrates was studies according to 25 indicators via atomic emission and mass spectrometry (AES and MS) at the Test Laboratory of the ANO Center for Biotic Medicine, Moscow (Registration Certificate ISO 9001:2000, No. 4017 of 5.04.06). The biosubstrates were ashed using the MD-2000 microwave decomposition system (USA). The content of elements in the resulting ash was estimated using the Elan 9000 mass spectrometer (Perkin Elmer, USA) and the Optima 2000 V atomic emission spectrometer (Perkin Elmer, USA).

2.5 Blood sampling and testing
Blood samples (4 ml) were taken from a tail vein of each cow in a vacuum test tube with anticoagulant (EDTA), needle for blood sampling – Bodywin. Morphological indicators were defined by automatic hematologic analyzer: URIT-2900 Vet Plus (URIT Medial Electronic Co., Ltd, China). Laboratory tests were conducted in the Laboratory of Technogenic Nanomaterials Agroecology of the Federal Research Center of Biological Systems and Agro-Technologies of the Russian Academy of Sciences (accreditation certificate RA. RU.21PF59 of 02.12.15).

2.6 Statistical processing
The Shapiro-Wilk test was used to check the hypothesis of normality of distribution of quantitative criteria. A median (Me) was used to calculate the average values and as a measure of central tendency. The distribution law of studied numerical indicators was different from the normal one, therefore the significance of differences was checked by Mann-Whitney U-test. The Spearman correlation coefficients (Kc) were calculated to define functional relations between parameters. All procedures of statistical analysis calculated the reached significance value (p), at the same time the critical significance value in this study was accepted smaller or equal 0.05. Statistica 10.0 application software package (Stat Soft Inc., USA) was used for data processing.

Figure 1. Difference in the concentration of chemical elements in hair of the first-generation cows born in the Republic of Sakha (Yakutia) in comparison with imported from the Republic of Kalmykia, %

Note:*p ≤0.05; **p≤0.01
3. Results
The comparison of elemental composition of wool of the studied animal groups revealed its heterogeneity depending on cattle generation delivered to the Republic of Sakha (Yakutia) (Table 1).

Table 1. Concentration of chemical elements in hair of cows depending on generation, mkg/g

| Element | Imported Kalmyk cattle | Calves of imported Kalmyk cattle | I generation | II generation |
|---------|------------------------|----------------------------------|--------------|--------------|
|         |                        |                                  |              |              |
| Ca      | 1712±247               | 2603±516a                        |              | 2213±664.3   |
| Na      | 1770±634               | 3184±1110a                       |              | 2901±1304    |
| K       | 2751±736               | 4816±2136a                       |              | 3498±1538    |
| P       | 209.7±36.8             | 252.1±76.3                       |              | 268±64.6     |
| Mg      | 935.6±459.7            | 1417±636.9                       |              | 884±551.1    |
|         |                        |                                  |              |              |
|         | **Macroelements**      |                                  |              |              |
| Co      | 0.269±0.156            | 0.219±0.118                      |              | 0.173±0.077  |
| I       | 0.59±0.276             | 1.04±0.302a                      |              | 0.964±0.729  |
| Cr      | 2.17±0.376             | 1.46±0.877                       |              | 1.08±0.401b  |
| Cu      | 6.49±1.206             | 7.62±1.37                        |              | 7.96±0.689b  |
| Fe      | 694.4±225.3            | 473.3±190.3                      |              | 474.9±172.5  |
| Mn      | 16.27±6.43             | 44.04±16.67a                     |              | 20.86±15.46c |
| Se      | 0.283±0.082            | 0.333±0.062                      |              | 0.241±0.082c |
| Zn      | 86.5±16.7              | 114.8±23.1a                      |              | 109.6±13.3b  |
|         | **Essential microelements** |                                  |              |              |
| Si      | 297±211                | 130±116                          |              | 82.5±81.5b   |
| Ni      | 2.74±2.77              | 1.11±0.46                        |              | 0.94±0.41    |
| B       | 1.97±0.569             | 3.56±1.27a                       |              | 2.18±1.63    |
| Li      | 0.52±0.22              | 0.45±0.201                       |              | 0.40±0.174   |
| V       | 0.934±0.446            | 0.787±0.397                      |              | 0.669±0.315  |
| As      | 0.154±0.079            | 0.389±0.617                      |              | 0.083±0.037  |
|         | **Conditional essential microelements** |                                  |              |              |
| Al      | 436±194                | 317±183                          |              | 332±141      |
| Sn      | 0.835±1.49             | 0.151±0.263                      |              | 0.119±0.226  |
| Pb      | 0.439±0.129            | 0.424±0.112                      |              | 0.289±0.081bc|
| Cd      | 0.010±0.003            | 0.009±0.003                      |              | 0.008±0.003  |
| Hg      | 0.020±0.009            | 0.008±0.006a                     |              | 0.004±0.001c |
| Sr      | 8.65±2.09             | 12.38±3.84a                      |              | 8.82±4.88    |

a - P≤0.05 – I generation compared with imported;
b - P≤0.05 – II generation compared with imported;
c - P≤0.05 – II generation compared with I generation.
The table shows average values (M) and their standard deviations (± STD)

The comparative assessment of chemical composition of hair of the Kalmyk breed cows revealed a considerable difference in the concentration of elements depending on the generation of cattle. Thus, the hair of the first-generation cows contained more Ca, Na, K, I, Mn, Zn, B, Sr in comparison with imported animals. The difference regarding some elements exceeded 100% (Fig. 1).

The study of the concentration of elements in wool showed significant changes in mineralization of the second-generation cows in comparison with imported (Fig. 2).
Figure 2. Difference in the concentration of chemical elements in hair of the second-generation cows born in the Republic of Sakha (Yakutia) in comparison with imported from the Republic of Kalmykia, %

It is found that the wool of cows delivered to the Republic of Sakha (Yakutia) is characterized by smaller concentration of zinc – by 26.6% (P≤0.05) and copper – by 22.7% (P≤0.05) against the background of increased silicon content – by 72.3% (P≤0.05), chrome – by 50.5% (P≤0.001), and lead – by 34.2% (P≤0.05) in comparison with the second-generation received in this biogeochemical province.

The difference in hair mineralization was revealed by the sum of the amount of substances (Table 2).

Table 2. Amount of chemical elements in the hair of cows, mmol/kg

| Element                        | Imported Kalmyk cattle | Calves of imported Kalmyk cattle |
|--------------------------------|------------------------|---------------------------------|
|                                | I generation           | II generation                   |
| Macroelements                  | 235.3±27.28            | 393.0±117.78**                  | 315.8±108.11                  |
| Essential elements             | 14.2±4.18              | 11.2±3.45                      | 10.7±3.39                     |
| Conditional essential microelements | 11.0±7.50              | 5.6±5.14                       | 3.3±2.80*                     |
| Toxic elements                 | 16.83±7.44             | 12.30±7.02                     | 12.81±5.45                    |

Note:* p ≤0.05; **p ≤0.01 (compared with imported)

The table shows average values (M) and their standard deviations (± STD)

The volume of macrocells in the wool of the first-generation calves increased by 67.0 (P≤0.01) and the quantity of conditional essential microelements of the second-generation calves decreased by 70.0% (P≤0.05) in relation to the delivered cows.

Morphological indicators of blood had significant differences within the studied groups (Table 3). It shall be noted that the concentration of lymphocytes in blood of the cows delivered to the Republic of Sakha (Yakutia) was lower by 43.0% (P≤0.05) compared to cows of the II generation. The concentration of erythrocytes (RBC) had a stable (P≤0.01) tendency towards the increase (by 34.0-47.6%) from the generation of imported cattle to the following generations.
Table 3. Morphological indicators of blood of the Kalmyk breed cows depending on the generation of cattle delivered to the Republic of Sakha (Yakutia)

| Indicator                              | Imported Kalmyk cattle | Calves of imported Kalmyk cattle |
|----------------------------------------|------------------------|----------------------------------|
|                                        | I generation           | II generation                    |
|                                        |                        |                                  |
| Leucocytes, 10^9 kl/l                  | 8.42±2.79              | 9.73±2.71                        | 10.76±3.5 |
| Lymphocytes, 10^9 kl/l                 | 3.27±1.31              | 3.73±1.41                        | 5.74±2.11*|
| Granular leucocytes (GRA), 10^6 kl/l   | 4.17±1.53              | 5.32±2.57                        | 3.52±1.57 |
| Red blood cells (RBC), 10^{12} kl/l    | 6.03±0.803             | 8.08±1.08**                      | 11.5±3.78**|
| Haemoglobin (Hb), g/l                  | 103.3±15.25            | 109.9±9.48                       | 115±12.4 |
| Hematocrit (HCT), %                    | 32.65±5.32             | 34.39±2.98                       | 45.28±22.04|

Note:* p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001 (compared with imported)
The table shows average values (M) and their standard deviations (± STD).

The mean cell volume and the mean cell hemoglobin (MCH) had inverse dependence on their concentration and was reduced for cows of the I and II generations in relation to the imported cattle (Table 4).

Table 4. RBC indices of blood of the Kalmyk breed cows depending on generation delivered to the Republic of Sakha (Yakutia)

| Indicator                              | Imported Kalmyk cattle | Calves of imported Kalmyk cattle |
|----------------------------------------|------------------------|----------------------------------|
|                                        | I generation           | II generation                    |
|                                        |                        |                                  |
| Mean cell volume (MCV), fl             | 54.14±4.56             | 42.14±5.27***                    | 38.71±6.68***|
| Mean cell hemoglobin (MCH), pg         | 17.21±1.75             | 15.09±3.02                       | 10.91±3.39***|
| Mean cell haemoglobin concentration (MCHC), g/l | 317.9±10.67         | 319±5.07                         | 290.43±90.06|
| Red cell distribution width, coefficient of variation (RDW-CV), fl | 20.8±1.76             | 23.27±1.35*                      | 22.07±5.73 |

Note:* p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001 (compared with imported)
The table shows average values (M) and their standard deviations (± STD)

Table 5. Thrombocytic indices of blood of the Kalmyk breed cows depending on the generation delivered to the Republic of Sakha (Yakutia)

| Indicator                              | Imported Kalmyk cattle | Calves of imported Kalmyk cattle |
|----------------------------------------|------------------------|----------------------------------|
|                                        | I generation           | II generation                    |
|                                        |                        |                                  |
| Тромбоциты (PLT), 10^9 kl/l           | 427.9±81.83            | 448.9±83.76                      | 461.5±191 |
| Trombokrit (PCT), %                   | 0.610±0.538            | 0.313±0.05                       | 0.342±0.182|
| Average volume of platelets (MPV), fl  | 7.66±0.538             | 7.1±0.473                        | 6.72±0.977 |
| Relative width of distribution of platelets on volume (PDWc), fl | 34.39±1.78             | 35.73±1.85                       | 30.82±6.32 |

Note:* p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001 (compared with imported)
The table shows average values (M) and their standard deviations (± STD)
The distribution width of erythrocytes had the maximum value for cows of the first generation (11.9%; P≤0.05) in relation to the imported cattle.
The calculation of thromboytic indices for the studied groups of animals did not reveal statistically significant differences (Table 5).

4. Discussion
Data obtained during the experiment indicate significant changes in mineral metabolism of cows against the background of their adaptation. Thus, the wool of the first-generation animals was characterized by the increase of Ca, Na, K, I, Mn, Zn, B, Sr, and the second-generation cows − Cu, Zn in comparison with imported cattle. These changes may be explained by adaptation reorganization of an organism of cows to conditions of the Republic of Sakha (Yakutia) [10, 11] taking into account the fact that earlier conducted studies in this area indicate lack of age influence on the accumulation of chemical elements [12].

In particular, the zinc pool in the organism of cattle is directly connected with the content of zinc in the environment [13]. The study showed that the concentration of zinc in hair of imported cows was lower in comparison with the first-generation calves by 24.6% (P ≤ 0.05) and the second-generation calves – by 21.0%, which confirms both the stressful condition of animals [14, 15] and the speed of exchange processes in the organism of young and adult animals.

Earlier it was revealed that the concentration of zinc affects the quantity of erythrocytes and the Hb level [16]. In our study the statistically significant difference and positive correlation is only received for RBC (r = 0.53, P ≤ 0.01) and negative – for mean cell hemoglobin (MCH) (r = -0.47, P ≤ 0.001).

The conducted study indicates the competition between Cu and Zn [17], which was not revealed in our research and only positive correlation between the accumulation of these metals is revealed (r = 0.73).

The increase of Zn exchange pool in the organism of cows received in Yakutia in comparison with cows imported from Kalmykia may also be explained by the decrease of toxic elements: Hg and Pb, which act as antagonists to some essential elements including Zn [18, 19].

The recent study demonstrated the role of white blood cells in the adaptation of an organism to influences of adverse environmental conditions [20-22]. In our study we recorded the increase in the number of lymphocytes throughout the adaptation of animals to the conditions of the Republic of Sakha (Yakutia).

5. Conclusion
The elemental status of cattle against the background of adaptation to severe conditions of the Republic of Sakha (Yakutia) undergoes considerable changes caused by the accumulation of Ca, Na, K, I, Cu, Zn and reduction of Cr, Si, Pb, Hg.

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