Experience of individual correction of elemental status of cows with reproductive disorder

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Abstract. The purpose of the study was to apply the method of individual correction of elementosis to increase the reproductive qualities of white-faced cows. The deficit of iodine and selenium in wool (I <0.28 mg/kg, Se <0.58 mg/kg) and low reproductive abilities (more than 2 months without estrus period) served the selection criterion of animals. The animals were divided by analogs into 2 groups – control (n=15) and experimental (n=15). On the 1st and 10th day the experimental animals were subjected to intramuscular injection of commercial formulations (10 ml) containing in 1 ml: iodine – 5.5–7.5 mg, selenium in organic form – 0.07–0.09 mg (corresponds to 0.16–0.20 mg of sodium selenite. It is found that the cows of the experimental group were characterized by the increase of I and Se content on the 28th day, which fell within permissible values (25–75 percentile), while the concentration of Ca, K, Mg, Na, Zn, Al, Sr, Pb, Hg decreased in relation to the beginning of the experiment. The assessment of reproductive qualities of cows showed that in the first month of the experiment 46.7% of cows came in season in the control group and 60.0% – in the experimental group. Within the next month the control group saw additional 20.0%, while the experimental group – 33.3%. During the first service of breeding 66.7% of cows were bred in the control group, 80.0% – in the experimental group. In the control group two cows were aborted, which reduced the number of living calves in the control group to 7 or 47%. The experimental group gave 14 calves or 93%.

1. Introduction

The reproductive function is the most important economic and biological feature of cattle, which depends on a number of factors – management and feeding conditions, calving, etc. [1].

The elemental status of animals is one of the key factors closely connected with the reproductive function of animals [2].

There is considerable amount of scientific data explaining close relation of reproduction of animals with exchange of some chemical elements, including iodine [3]; selenium [4, 5]; complex of elements [6].

Imbalances of microelements result in reproductive disorders of animals. Hence, the diagnostics and treatment of elementosis is a key tool in increasing the reproductive ability in livestock breeding. This can be achieved through individual assessment of the elemental status of animals according to multielement composition of bio-substrates. The method was widely adopted in medicine with the use of hair composition [7], which is confirmed by the number of appeals to medical centers that use new approaches to diagnostics and correction of elementosis [8].
The purpose of the study was to apply the method of individual correction of elementosis to increase the reproductive qualities of white-faced cows.

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

The first stage covered the study of the element composition of wool and the elemental status of beef cows raised in Orenburg Region (n=190), and 25 and 75 percentile values were defined and accepted according to recommendation [9] as “physiologically normal state”.

At the second stage out of 48 white-faced cows with reproductive disorders (more than 2 months without estrous period), with identified hypovarianism, on the basis of the elemental status analysis established according to element composition of wool 30 heads were selected, the wool of which contained iodine and selenium below earlier established norm (below 25 percentiles, I<0.28 mg/kg, Se<0.58 mg/kg). The animals were divided by analogs into 2 groups – control (n=15) and experimental (n=15). On the 1st and 10th day the experimental animals were subjected to intramuscular injection of commercial formulations (10 ml) containing in 1 ml: iodine – 5.5-7.5 mg, selenium in organic form – 0.07-0.09 mg (corresponds to 0.16-0.20 mg of sodium selenite).

During the experiment the animal feed included the following: hay of natural lands – 8 kg, alfalfa silage – 6 kg, concentrates: mixture of barley, wheat, oats, – 3.0 kg containing available energy – 106.2 MJ, dry matter – 12.1 kg, transferable protein – 1092 g, Ca – 123.2 g, P – 35.6 g, Mg – 15.6 g, K – 97.4 g, Fe – 3.91 g, Cu – 71.6 mg, Zn – 496.8 mg, Mn – 734.1 mg, I – 3.27 mg, Se – 1.25 mg.

Hair samples were collected by technique [10] on the 1st, 14th and 28th day of the experiment.

The elemental composition of hair was defined by 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). The elemental composition of biosubstrates was studied according to 25 indicators (Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, I, K, Li, Mg, Mn, Na, Ni, P, Pb, Se, Si, Sn, Hg, Sr, V, Zn).

2.1 Blood sampling and study
Blood sampling was made on the 1st, 14th and 28th day of the experiment, in the morning before feeding and drinking supply. Blood was taken in vacuum test tubes from a tail vein at the level of the average one third of the body of 2-5 tail vertebrae.

Blood for biochemical study, antioxidant status, and malondialdehyde was taken in vacuum test tubes APEXLAB with clot activator by Hebei Xinl Sky & Tech Co., Ltd, needle for blood sampling – Bodywin. The biochemical blood test was carried out by automatic biochemical analyzer CS-T240 (Dirui Industrial Co., Ltd., China). The biochemical analysis was performed using commercial biochemical sets for veterinary science DiaVetTest (Russia) and commercial biochemical sets Randox (USA) at the Federal Research Center of Biological Systems and Agro-Technologies of the Russian Academy of Sciences (accreditation certificate No. ROSS RU. 0001.21PF59 of 12.10.2015).

2.2 Definition of pregnancy
Ultrasonic diagnostics of cows to define pregnancy and futility was carried out via veterinary ultrasonography scanner IMAGO S (France) with rectal sector probe DB 355 M.

2.3 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. 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.

### Table 1. Concentration and percentile values of chemical elements in hair of cows of the experimental group, mg/kg

| Element | “Physiologically normal state” in percentile values | Concentration of elements in the experiment | 25 | 75 | 1 | 14 | 28 |
|---------|---------------------------------------------------|------------------------------------------|----|----|---|----|----|
|         |                                                   | Macrolelements                           |    |    |   |    |    |
| Ca      | 1593 2910                                        | 2029±86.1                                | 1715±125.9 | 1659±89.8** |
| K       | 806 3523                                         | 2815±233.3                                | 2109±234.7 | 2094±152.9* |
| Mg      | 425 980                                         | 535±26.6                                 | 440±48.1 | 427±33.0** |
| Na      | 405 1501                                        | 1406±119                                 | 1051±157.5 | 941±70.3** |
| P       | 168 298                                         | 241±9.2                                  | 272±3.5 | 258±7.4 |
|         |                                                   | Essential microelements                  |    |    |   |    |    |
| Co      | 0.05 0.12                                        | 0.078±0.01                               | 0.060±0.01 | 0.060±0.00 |
| Cr      | 0.13 0.28                                        | 0.44±0.06                                | 0.51±0.10 | 0.36±0.03 |
| Cu      | 4.87 6.61                                       | 11.39±0.54                               | 11.92±1.14 | 11.37±0.59 |
| Fe      | 38.25 95.63                                     | 396.72±81.10                             | 356.75±33.54 | 202.67±47.36 |
| I       | 0.28 0.69                                       | 0.27±0.01                                | 0.29±0.02 | 0.35±0.01*** |
| Mn      | 11.87 30.64                                      | 13.73±1.75                               | 16.19±0.44 | 16.45±0.30 |
| Se      | 0.58 1.07                                        | 0.56±0.03                                | 0.60±0.01 | 0.66±0.01** |
| Zn      | 107.0 153.0                                      | 198.9±19.25                              | 144.0±3.44 | 131.4±5.68*** |
|         |                                                   | Conditional essential microelements      |    |    |   |    |    |
| B       | 1.58 3.85                                        | 4.35±0.17                                | 2.97±0.22*** | 2.75±0.22*** |
| Si      | 10.75 27.38                                      | 18.31±1.18                               | 15.50±2.29 | 13.95±1.26* |
| Li      | 0.42 1.9                                         | 0.69±0.06                                | 0.47±0.04*** | 0.42±0.03*** |
| Ni      | 0.39 0.84                                        | 0.62±0.09                                | 0.45±0.05 | 0.35±0.04** |
| V       | 0.13 0.34                                        | 0.18±0.02                                | 0.15±0.04 | 0.07±0.01*** |
| As      | 0.08 0.17                                        | 0.07±0.005                               | 0.07±0.003 | 0.05±0.004 |
|         |                                                   | Toxic microelements                      |    |    |   |    |    |
| Al      | 26.74 58.42                                      | 81.88±12.97                              | 74.83±4.22 | 35.15±4.45*** |
| Sr      | 9.28 17.31                                       | 10.91±0.70                               | 9.04±1.60 | 6.90±0.89** |
| Pb      | 0.142 0.244                                      | 1.20±0.18                                | 0.86±0.20* | 0.41±0.03*** |
| Sn      | 0.009 0.018                                       | 0.013±0.001                              | 0.010±0.0042 | 0.02±0.006 |
| Cd      | 0.014 0.036                                      | 0.009±0.001                              | 0.006±0.001** | 0.006±0.001*** |
| Hg      | 0.002 0.009                                      | 0.01±0.001                               | 0.003±0.001** | 0.002±0.000*** |

* - P≤0.05; ** - P≤0.01; *** - P≤0.001 compared to the 1st day

### 3. Results

On the 14th day the content of toxic elements decreased in the experiment group: Pb – by 28.3% (P≤0.05), Cd – by 36.7% (P≤0.01), Hg – by 65.6% (P≤0.01), on the 28th day the concentration of I increased by 29.2% (P≤0.001), Se – by 17.9% (P≤0.01) with the decrease of macroelements: Ca – by 18.25% (P≤0.01), K – by 25.63% (P≤0.05), Mg – by 20.24% (P≤0.01), Na – by 33.09% (P≤0.01), essential macroelements: Zn – by 33.96% (P≤0.01), toxic: Al – by 57.07% (P≤0.001), Sr – by 36.76%
(P≤0.01), Pb – by 65.83% (P≤0.001), Hg – by 80.0% (P≤0.001) in relation to the beginning of the experiment (Table 1).

On the 28th day the elemental profile of cows within the compared groups revealed their significant changes on the basis of concentration of chemical elements in wool (Fig. 1).

![Image of chemical elements profile](image-url)

*a* - P≤0.05; *b* - P≤0.01; *c* - P≤0.001 in relation to the control group

**Figure 1.** Elemental profile of white-faced cows of the experimental group in relation to the control group on the 28th day, %

The use of iodine and selenium resulted in changes of interelement interactions in wool estimated according to the level of toxic and essential elements. At the beginning of the experiment there were 15 reliable relations, including two negative ones: Hg-Mn (r= -0.63) and Hg-Zn (r= -0.78). At the end of the experiment there were 9 relations, including negative ones: Se-Al (r= -0.65). For iodine none reliable correlation was recorded.

The assessment of antioxidant defense enzymes and catalase showed their increase in the experimental group on the 28th day of the experiment by 25.1 (P≤0.05) and 106.1 (P≤0.001) % respectively, in the control group these indicators did not change (Table 2).

| Indicator                        | Days          |
|----------------------------------|---------------|
|                                  | 1             | 14            | 28             |
| SOD, %                           | 318±21.3      | 377±17.2*     | 398±25.7*      |
| Catalase, µ H2O2/lxmin           | 3358±67.8     | 4370±83.3***  | 6922±76.5***   |
| Malondialdehyde, nm/ml           | 13.6±4.9      | 23.8±4.0      | 24.9±4.1      |

Control

SOD, %                           | 356±28.3      | 375±21.2      | 340±27.1      |
Catalase, µ H2O2/lxmin           | 4218±73.6     | 4494±91.1*    | 4352±84.5     |
Malondialdehyde, nm/ml           | 15.8±5.0      | 38.1±5.1***   | 38.8±4.9***   |

* - P≤0.05; ** - P≤0.01; *** - P≤0.001 compared to the 1st day
The study showed that during the first month after the beginning of the experiment in the control group 7 cows (46.7%) came in season, in the experimental group – 9 (60%). Within the next month the control group saw additional 3 (20%), the experimental group – 5 (33.3%). During the first service of breeding 6 (66.7%) cows were bred in the control group, 12 (80%) cows – in the experimental group. In the control group two cows were aborted, which reduced the number of living calves in the control group to 7 or 47%. The experimental group gave 14 calves or 93%.

4. Discussion
The study showed that the elemental status of animals concerning wool composition with subsequent comparison of data with the norm allows revealing cows with elementosis and treating them individually. In Orenburg Region of Russia wide circulation of elementosis by selenium and iodine is caused by environmental conditions [11]. In turn iodine and selenium are functionally connected among themselves and influence the production of thyroid hormones. Closely interacting with female sex hormones thyroid hormones ensure normal functioning of ovaries and maturation of ovum [12, 13].

It shall be noted that the elemental status of cows changes considerably with the introduction of selenium and iodine. On the 28th day of the experiment the element composition of hair of experimental cows was characterized by the increase of essential (Mn, Cu, I, Se) and decrease of toxic elements (As, Cd, Fe, Al, Pb, Sn), which may be explained by antagonism between Se and toxic elements [14, 15]. Perhaps this can also explain the increase of malondialdehyde content partially caused by the pathology of the reproductive system [16, 17].

The study showed the increase of malondialdehyde as lipid peroxidation indicator in comparison with the beginning of the experiment by 75.0 and 83.1% in the experimental and 141.1 and 145.5% in the control group respectively on the 14th and 28th day. In our opinion this is explained first of all by the change of the physiological state, since the samples were taken from cows in service and breeding. Earlier similar results were received by Mihu D. et al. 2012 [18].

In the experimental group the activity of free radical oxidation of lipids in comparison with the control group at the same time of the experiment on the 14th and 28th day was 33.0-34.9% lower (P≤0.05), which is explained by the introduction of iodine-selenium corrective additive, where the latter one has strongly pronounced antioxidant capacity, decreases the oxidative stress [19-21].

The study of enzymatic antioxidants showed reliable increase of superoxide dismutase of experimental cows. In comparison with the control group the increase of SOD concentration and CAT activity in blood serum indicates the efficiency of iodine-selenium drug, which improves antioxidant function and reduces the oxidizing stress. The confirming data of this effect were earlier obtained for dairy cows [22].

5. Conclusion
The deficit of iodine in wool lower than 0.28 mg/kg, selenium – 0.58 mg/kg requires correction by double intramuscular injection of 10 ml of commercial drug containing in 1 ml: iodine – 5.5-7.5 mg, selenium in organic form – 0.07-0.09 mg, which allows increasing the concentration in iodine in wool up to 0.35, selenium – up to 0.66 mg/kg, which corresponds to “physiologically normal state” (25-75 percentile) and improving reproductive qualities.

6. Acknowledgments
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