



The effect of a Forest Biomass Supplement on Morphophysiological Parameters of Calves

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Abstract: Breeding, preserving, and successfully rearing young animals resistant to various environmental influences is one of the main parameters of effective livestock management. The study aimed to study the indicators of natural resistance and the growth rate of calves under the influence of dietary supplements. The studies were carried out on four groups of black-and-white calves, 10 heads each, at the age of 2-5 months. The dairy calves of the control group were fed according to the ration adopted on the farm (basic ration, including cereal-legume hay, whole milk, milk replacer, concentrates, chalk, salt). In addition to the main diet, the animals of the experimental groups were given a dietary supplement. At 2-3, 3-4, and 4-5, months of age, calves were given 12-23, 19-38, and 23-47 g.head⁻¹ of dietary supplement per day, respectively. The article deals with the effect of a forest biomass supplement on the morphophysiological parameters of calves. Biochemical blood analysis and the features of metabolic processes in the bodies of animals are studied. The results indicated that the use of a dietary supplement at a dosage of 30 g/head of cattle per day contributes to the highest increase (9.9%) in live weight gain of experimental animals and an improvement in natural resistance parameters (bactericidal activity of blood serum, phagocytic activity of neutrophils). Based on the data of biochemical and haematological blood tests, the authors conclude that the inclusion of a dietary supplement in the diet of calves has a positive effect on the indices of natural resistance and the course of nitrogen metabolism.

Keywords: dietary supplement, live weight gain, biochemistry, resistance

Introduction

The resistance of the animal organism to unfavourable environmental factors is a dynamic parameter and is determined by both the genetics and the effect of various environmental factors (Karamaeva & Zaitsev, 2010; Zaytsev, 2019). Because of this, it is possible to exert direct control over the development and manifestation of different body defence factors. Providing animals with good diet and favourable rearing environments, which conform as closely as possible to the biological features of the organism developed during evolutionary growth, aids in the production and manifestation of body defensive mechanisms (Gorelik & Barashkin, 2016; Kiselenko, 2020).

Newborns of all animal species have a physiological immune deficiency. A decrease in the parameters of nonspecific resistance of the organism (blood serum bactericidal activity (BSBA), blood serum lysozyme activity (BSLA), and phagocytic activity of blood neutrophils (PABN)) when rearing young animals in conditions of intensive technologies aggravates the manifestation of immunodeficiency. Compensation of physiological immune deficiency with an increase in natural resistance in new-born occurs due to cellular and humoral factors of colostrum (Loretts *et al.*, 2019).

In this regard, the formation and use of various dietary supplements aimed at increasing the resistance of the animal organism is an urgent issue for research and practice.

The goal of our experiment was to study the characteristics of metabolic processes in the calves' bodies based on biochemical blood tests and to analyse the growth rate of animals and parameters of natural resistance under the

influence of a combination of nutritional supplements.

Materials & Methods

General research scheme

The studies were carried out in normal work conditions on four groups of 10 calves in each group of the black-and-white breed aged 2 months with an average of live body weight of (72.65 kg). The study was in compliance with the International Recommendations of the European Convention for the Protection of Vertebrate Animals used in Experimental Research (Council of Europe, 1986). The live weight of calves when setting up for the experiment was 73.28 kg in the control group, 72.24 kg in experimental group 1, 72.33 kg in experimental group 2, and 72.75 kg in experimental group 3.

The milk-fed calves of the control group were fed according to the ratio adopted at the farm (basic diet — BD). Animals of the 1st, 2nd, and 3rd experimental groups, in addition to the BD, were given a dietary supplement. The supplement was administered orally by syringe daily in the morning feed.

The supplement was administered to calves: at the age of 2-3 months, in experimental group 1 12 g.head⁻¹ per day, experimental group 2 18 g/head, and experimental group 3 23 g.head⁻¹ per day; at the age of 3-4 months, in experimental group 1 19 g.head⁻¹ per day, experimental group 2 28 g/head, and experimental group 3 38 g/head per day; at the age of 4-5 months, in experimental group 1 23 g.head⁻¹ per day, experimental group 2 35 g.head⁻¹, and experimental group 3 47 g.head⁻¹ per day. The duration of the experiment equaled 90 days. Animals of the control and experimental groups were housed in the same facility.

Composition of the dietary supplement

The dietary supplement is a mixture of natural ingredients containing glycerin, pine extract, sugar, activated carbon, flaxseed, and table salt. Glycerin is the main component of all fats (triglycerides). It serves as a source of energy.

Pine or spruce needle extract is a natural vitamin source. Pine and spruce needles contain carotene, chlorophyll, xanthophyll, and other substances that are important for metabolism and in the synthesis of many vitamins in the body. Moreover, carotene, or provitamin A, is contained in pine and spruce needles in a dosage twice as high as in carrots. In winter and autumn, vitamin deficiency can be replenished by adding pine and spruce needles rich in vitamins A, C, B2, K, E, and P to the diet.

Sugar is not only a source of energy for ruminants but is also necessary for the normal functioning of the rumen microflora.

Activated carbon has a high ability to retain (adsorb) various substances, liquids, and gases on its surface. Activated carbon also adsorbs various toxic substances.

Flaxseed is rich in fat (36%) and protein (24%) and easily digestible. Therefore, it is especially valuable for young farm animals. Flaxseed swells in the gastrointestinal tract, forming slimy solutions that have excellent dietary properties. They were bred to improve the functioning of the gastrointestinal tract.

Table salt is necessary for animals to maintain the acid-base balance in the blood and water balance in the body. Without table salt, the correct functioning of the muscles, stomach, and nervous system of the animal is impossible. Salt

is not produced by the body and does not come in sufficient quantities from grass, hay, and animal feed. Salt is involved in metabolic processes, due to which each cell receives the right amount of nutrients. It enhances the taste of food, which affects the growth of the animal's appetite and has antibacterial properties, protecting the body from the development of harmful bacteria.

During the study period, we determined the chemical composition of the given feed. Live weight gains (gross, daily average) were calculated based on the individual weighing of experimental animals.

Methods

At the beginning and the end of the experiment, blood tests were carried out in the animals of the control and experimental groups. We measured the levels of their hemoglobin, red blood cells, white blood cells, haematocrit, total protein, albumin, globulins, creatinine, urea, total bilirubin, total cholesterol, calcium, phosphorus, alkaline phosphatase, glucose, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). We determined the level of nonspecific immunity in the blood of experimental animals ($n = 3$). BSBA was studied according to the method of O.V. Bukharin & Sozykin (1979) using a test culture of *E. coli* O₁₁₁.

BSLA was studied according to the method of Bukharin (1971) using a daily culture of *Micrococcus Lusoideicticus* (strain 2665 GKI named after L.A. Tarasevich).

PABN was determined according to the method of Ivanov & Chukhlovina (1967) using as a test culture of *E. coli* O₁₁₁, grown for a day on meat-and-peptone agar (MPA).

The data was analysed due to analysis of variance within a statistical program. Data were expressed as the arithmetic mean (M), the root-mean-square error ($\pm m$), and the significant difference (P). The study results were considered highly significant at $P < 0.001$ and significant at P

< 0.01 and $P < 0.05$. When $P > 0.05$, differences are not significant.

Results & Discussion

The data on the Live weight of calves of the control and experimental groups are presented in table (1).

Table (1): Productivity of milk-fed calves (n = 10/group, M \pm m).

| Parameter | Group | | | |
|------------------------------------|--------------------|-------------------|----------------------|-------------------|
| | control | 1st experimental | 2nd experimental | 3rd experimental |
| Live weight, kg: | | | | |
| at the beginning of the experiment | 73.28 \pm 2.0 | 72.24 \pm 1.6 | 72.33 \pm 1.7 | 72.75 \pm 1.4 |
| at the end of the experiment | 140.17 \pm 1.2 | 141.27 \pm 4.38 | 145.84 \pm 2.1* | 144.09 \pm 1.5 |
| Gross growth, kg | 66.89 \pm 1.41 | 69.03 \pm 1.66 | 73.51 \pm 1.05** | 71.34 \pm 1.23 |
| Average daily growth, g | 743.22 \pm 14.70 | 766.96 \pm 18.5 | 816.72 \pm 12.01** | 774.3 \pm 14.21 |
| In % to the control group | 100.0 | 100.8 | 104.0 | 102.8 |

Differences in comparison with control are statistically significant at * — $P < 0.05$, ** — $P < 0.01$, *** — $P < 0.001$

It was found that during the period of our experiment, calves of the 2nd experimental group developed the most intensively, receiving a dietary supplement in a dosage of 18-35 g.head⁻¹ of cattle per day. Thus, at the end of the experiment, the calves of the 2nd experimental group had exceeded the control group body weight by 6.6 kg, or 4.0%, and their average daily weight gain by 73.5 g or 9.9%, respectively, with a significant difference. The calves of the 1st and 3rd experimental groups had also exhibited higher (not significant) average daily weight gain by 23 g (3.2%) and 31.1 g (4, %), respectively, than those of control group.

The increase in the average daily gain in live weight of milk-fed calves in the experimental groups can be explained in the following way. The dietary supplement they received in various dosages supplied their bodies with nutrients and

biologically active and energetic substances. These substances had an antibacterial and immunomodulatory effect and promoted the development of beneficial microflora in the gastrointestinal tract.

The study of blood picture and biochemical parameters of the blood allows assessing the impact of physiological processes in the body (Ivanov & Chukhlovin, 1967; Koryakina & Borisov, 2016; Zemlyanukhina, 2016).

Blood picture and biochemical parameters of the blood of calves from the control and experimental groups at the beginning and end of the experiment are presented in tables (2) and (3). All figures were within the normal ranges of calves. Groups have not significantly influenced all traits except the concentration of urea at the end of the study ($P < 0.05$).

Table (2): Blood picture and biochemical parameters of the blood of experimental animals at the beginning of the experiment (M± m, n = 3/group).

| Parameter | Experimental Groups | | | |
|----------------------------------|---------------------|-------------|-------------|-------------|
| | control | 1st | 2nd | 3rd |
| White blood cells, $10^9/l$ | 10.20±0.65 | 9.34±1.06 | 9.56±0.48 | 9.64±1.16 |
| Red blood cells, $10^{12}/l$ | 11.88±0.96 | 10.46±0.34 | 9.74±1.57 | 10.46±0.34 |
| Hemoglobin, $g.L^{-1}$ | 99.3±3.16 | 93.9±4.16 | 88.9±8.20 | 89.9±4.10 |
| Total protein, $g.L^{-1}$ | 64.14±2.40 | 63.0±2.52 | 62.20±2.12 | 60.0±2.40 |
| Albumins, $g.L^{-1}$ | 26.9±0.62 | 26.75±0.39 | 27.15±0.90 | 26.65±0.42 |
| Globulins, $g.L^{-1}$ | 35.40±2.29 | 33.47±2.55 | 36.6±1.18 | 34.47±2.55 |
| A/G coefficient | 0.75 | 0.79 | 0.74 | 0.77 |
| Urea, $mmol.L^{-1}$ | 4.82±0.57 | 4.57±0.20 | 5.41±0.63 | 4.88±0.29 |
| Creatinine, $mmol.L^{-1}$ | 69.17±11.72 | 80.15±2.21 | 81.9±3.66 | 76.15±3.25 |
| ALT, $IU.L^{-1}$ | 8.33±1.09 | 7.37±0.28 | 7.01±0.35 | 7.37±0.28 |
| AST, $IU.L^{-1}$ | 72.8±16.12 | 60.93±5.98 | 49.46±2.71 | 60.93±5.98 |
| Alkaline phosphatase, IU/l | 942.0±167.8 | 948.1±128.0 | 844.7±177.5 | 908.1±152.0 |
| Total cholesterol, $mmol.L^{-1}$ | 3.83±0.38 | 4.79±0.26 | 4.39±0.42 | 4.79±0.26 |
| Glucose, $mmol.L^{-1}$ | 6.45±0.34 | 6.68±1.15 | 6.91±0.16 | 6.68±1.15 |
| Calcium, $mmol.L^{-1}$ | 2.49±0.13 | 2.57±0.04 | 2.38±0.10 | 2.57±0.04 |
| Phosphorus, $mmol.L^{-1}$ | 3.09±0.44 | 3.68±0.38 | 3.0±0.51 | 3.68±0.38 |
| Ca/P ratio | 0.80 | 0.70 | 0.79 | 0.70 |

Haemoglobin in the blood of animals acts as an oxygen carrier to the haematopoietic organs. The amount of haemoglobin in the calves of the control is $98.20 g.L^{-1}$. Hb of the 1st, 2nd and 3rd groups were higher than control by 9.2 (9.3%), 11.6 (11.8%), and 9, 0 g/l (9.1%).

RBC of 1st, 2nd and 3rd experimental group, were $11.89 \times 10^{12}.L^{-1}$, $11.82 \times 10^{12}.L^{-1}$, and $11.80 \times 10^{12}.L^{-1}$ respectively. Control recorded lower insignificant value ($10.94 \times 10^{12}.L^{-1}$).

Table (3): Blood picture and biochemical parameters of the blood of experimental animals at the end of the experiment (M± m, n = 3/group)

| Parameter | Experimental Groups | | | |
|-----------------------------------|---------------------|------------|------------|------------|
| | control | 1st | 2nd | 3rd |
| White blood cells, $10^9.L^{-1}$ | 9.98±0.14 | 10.60±0.71 | 10.69±0.22 | 10.67±0.71 |
| Red blood cells, $10^{12}.L^{-1}$ | 10.94±0.55 | 11.89±0.31 | 11.82±1.18 | 11.80±0.31 |
| Hemoglobin, $g.L^{-1}$ | 98.2±4.6 | 107.4±1.70 | 109.8±7.70 | 107.2±1.65 |
| Total protein, $g.L^{-1}$ | 67.06±2.21 | 70.87±0.38 | 73.72±0.51 | 71.87±0.38 |
| Albumins, $g.L^{-1}$ | 24.62±1.86 | 27.95±1.03 | 31.75±1.99 | 27.95±1.03 |
| Globulins, $g.L^{-1}$ | 42.44±2.14 | 41.92±1.30 | 41.97±1.81 | 41.92±1.30 |
| A/G coefficient | 0.58 | 0.67 | 0.76 | 0.67 |
| Urea, $mmol.L^{-1}$ | 6.29±0.06 | 4.62±0.28* | 4.72±0.19* | 4.80±0.24* |
| Creatinine, $mmol.L^{-1}$ | 93.5±7.34 | 84.23±1.83 | 82.44±2.23 | 84.0±1.83 |
| ALT, $IU.L^{-1}$ | 19.59±1.23 | 19.82±1.58 | 19.82±1.55 | 19.82±1.58 |

| Parameter | Experimental Groups | | | |
|--|---------------------|------------|------------|------------|
| | control | 1st | 2nd | 3rd |
| AST, IU.L ⁻¹ | 59.32±2.21 | 65.64±1.88 | 65.87±1.74 | 65.60±1.90 |
| Alkaline phosphatase, IU.L ⁻¹ | 354.9±19.3 | 323.3±20.4 | 272.1±27.8 | 323.3±20.4 |
| Total cholesterol, mmol.L ⁻¹ | 3.90±0.15 | 3.64±0.20 | 3.72±0.22 | 3.84±0.26 |
| Glucose, mmol.L ⁻¹ | 4.59±0.12 | 4.69±0.28 | 4.73±0.16 | 4.60±0.22 |
| Calcium, mmol.L ⁻¹ | 2.60±0.08 | 2.73±0.09 | 2.46±0.21 | 2.73±0.09 |
| Phosphorus, mmol.L ⁻¹ | 3.34±0.19 | 3.48±0.20 | 2.90±0.49 | 3.48±0.20 |
| Ca/P ratio | 0.78 | 0.78 | 0.85 | 0.78 |

*P<0.05

There was a slight increase in the number of WBC of calves of the experimental groups compared to the control group by 6.2-7.1%.

Proteins are the main constituent of the blood. They maintain osmotic pressure and blood pH, play an important role in the formation of hormones, carbohydrates, as well as lipids, and other essential substances. In the protective activity of the body, their role is vital. They participate in water metabolism, the transfer of nutrients and metabolic products, as well as blood clotting. The total protein content in the blood of calves in the control group was 67.06 g/l. In the 1st, 2nd, and 3rd experimental groups, this parameter exceeded the control group, respectively, by 3.81 g.L⁻¹ (5.6%), 6, 6 g.L⁻¹ (9.9%), and 4.81 g.L⁻¹ (7.2%).

At the end of the experiment, there was a tendency to an increase in the concentration of total protein in the blood of experimental animals in comparison with control animals (by 5.6-9.9%), and a decrease (P<0.05) in the level of urea by 23.3-26.5%.

The concentration of creatinine in the blood of calves from the experimental groups was lower than that in the control by 9.9-11.8%, which may indicate a more efficient nitrogen metabolism (Zaytsev, 2019).

In the blood of the animals of the experimental groups, there are higher parameters of the activity of ATSS, which carry out the transfer of amino groups from amino acids to keto acids. Thus, the ATS in the blood of the control group was 59.32 IU.L⁻¹. In experimental groups, this indicator was higher by 10.6% (group 1), 11.0% (group 2), and 10.5% (group 3).

An important role in the body also belongs to carbohydrate metabolism. In our studies, in the blood of calves receiving a dietary supplement, the glucose level was slightly higher (by 2.1% in group 1, by 3.0% in group 2, and by 0.2% in group 3), which may indicate a higher energy supply in their body.

It should be noted a decrease in the cholesterol content in animals of the 1st experimental group by 6.7%, in the 2nd experimental group by 4.6%, and in the 3rd experimental group by 1.5%, compared with the control group. This parameter may indirectly reflect the improvement in the functional activity of the liver.

The level of natural resistance of the animal organism and their adaptive abilities are of particular importance. In this regard, we have determined the factors of the natural resistance of animals in comparison with the control group (Tables 4 and 5).

Table (4): Parameters of broad-spectrum resistance of the blood of experimental animals at the beginning of the experiment (M± m, n = 3/group).

| Parameter | Experimental Groups | | | |
|--|---------------------|-----------|-----------|-----------|
| | control | 1st | 2nd | 3rd |
| Blood serum bactericidal activity (BSBA), % | 43.2±3.4 | 43.53±2.0 | 42.38±2.4 | 42.56±2.1 |
| Phagocytic activity (PA), % | 23.5±2.6 | 23.7±1.66 | 23.6±2.26 | 23.7±1.66 |
| Phagocytic number (PN), phagocytosed microbial cells | 0.36±0.12 | 0.33±0.07 | 0.31±0.04 | 0.31±0.07 |
| Lysozyme, mcg/ml of serum | 0.57±0.03 | 0.55±0.02 | 0.57±0.01 | 0.53±0.02 |

Many researchers have achieved an increase in the indices of natural resistance of newborn calves through the use of various biologically active substances (Koryakina & Borisov, 2016; Kochish *et al.*, 2019; Topuriya & Belyaeva, 2019).

In present study, it was noted that these parameters of different groups at the beginning of the experiment were similar (Table 4).

At the end of the experiment, some differences were observed in calves from the

control and experimental groups in terms of natural resistance. The BSBA of the calves of the experimental groups was higher than that of the control animals by 5.1% (the 1st experimental group), 13.5% (the 2nd experimental group), and 6.8% (the 3rd experimental group). The content of lysozyme in the blood serum in the experimental groups was at the level of 0.63-0.70 $\mu\text{g}\cdot\text{ml}^{-1}$.

Table (5): Parameters of nonspecific resistance of the blood of experimental animals at the end of the experiment (M± m, n = 3/group)

| Parameter | Experimental Groups | | | |
|--|---------------------|-----------|------------|------------|
| | control | 1st | 2nd | 3rd |
| Blood serum bactericidal activity (BSBA), % | 60.1±2.2 | 63.2±2.9 | 68.24±2.8* | 64.2±2.8 |
| Phagocytic activity (PA), % | 28.3±2.80 | 31.2±1.9 | 35.8±1.25* | 35.2±0.92* |
| Phagocytic number (PN), phagocytosed microbial cells | 1.1±0.01 | 1.2±0.01 | 1.10±0.01 | 1.1±0.01 |
| Lysozyme, mcg.ml ⁻¹ of serum | 0.63±0.02 | 0.66±0.07 | 0.70±0.07 | 0.68±0.07 |

Reliable for *P<0.05

One of the main cellular factors of the natural resistance of the calves' organism is the PABN, characterized by the number of bacteria captured by white blood cells. The highest PABN was noted in the experimental groups, especially in the 2nd and 3rd ones. The calves of the

experimental groups had exceeded the control group by 10.2-26.5%.

The PN, which is determined by the average number of phagocytosed bacteria by one neutrophil and determines the phagocytic activity

of neutrophils in the control and experimental groups, was at the level of 1.1-1.2.

Thus, based on the data of biochemical and haematological blood tests, it follows that the inclusion of a dietary supplement in the diets of calves has a positive effect on the course of nitrogen metabolism in the body of animals and parameters of natural resistance.

Conclusion

Based on our study, it can be concluded that the inclusion of a dietary supplement in a dosage of 18-35 g/head of cattle per day in the diet contributed to the greatest increase in live weight gain in experimental animals by 9.9% and an improvement in natural resistance parameters.

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Conflict of interests

The authors declare no conflict of interest.

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تأثير ملحق الكتلة الحيوية للغابات على المؤشرات الفسيولوجية المظهرية للعجول

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المستخلص: تعتبر تربية الحيوانات الصغيرة المقاومة للتأثيرات البيئية المختلفة والحفاظ عليها وتربيتها بنجاح أحد المعايير الرئيسية للإدارة الفعالة للثروة الحيوانية. يهدف البحث إلى دراسة مؤشرات المقاومة الطبيعية ومعدل نمو العجول تحت تأثير الإضافات الغذائية. أجريت الدراسة على أربع مجموعات من العجول البيضاء والسوداء، 10 رؤوس لكل منها، في سن 2-5 أشهر. تم تغذية عجول مجموعة السيطرة وفقاً للعليقة المعتمدة في المزرعة (العليقة الأساسية، بما في ذلك قشور الحبوب والبقوليات، والحليب كامل الدسم، وبدائل الحليب، والمركزات، والطباشير، والملح). تم إعطاء حيوانات المجموعات التجريبية مكمل غذائي. في عمر 2-3 أشهر، تم إعطاء العجول 12-23 غم. رأس⁻¹، في عمر 3-4 أشهر - 19-38 غم. رأس⁻¹، وفي عمر 4-5 أشهر - 23-47 غم. رأس⁻¹ مكمل غذائي يوميا. تناولت المقالة تأثير مكمل الكتلة الحيوية للغابات على المؤشرات الفسيولوجية المظهرية للعجول. تم دراسة تحليل الدم الكيموحياتي وخصائص عمليات التمثيل الغذائي في أجسام الحيوانات. أشارت النتائج إلى أن استخدام مكمل غذائي بجرعة 30 غم. رأس⁻¹ من الماشية في اليوم ساهم في تحقيق أعلى زيادة (9.9%) في زيادة الوزن الحي لحيوانات التجارب وتحسين معايير المقاومة الطبيعية (نشاط مبيد للجراثيم لـ مصل الدم، نشاط البلعمة من العدلات). استناداً إلى بيانات اختبارات الدم الكيموحياتي واختبارات الدم، يمكن الاستنتاج بأن إضافة مكمل غذائي في عليقة العجول الأساسية له تأثير إيجابي على مؤشرات المقاومة الطبيعية ومسار التمثيل الغذائي للنيتروجين.

الكلمات المفتاحية: الإضافات العلفية، الزيادة الوزنية، المؤشرات الكيموحيوية، المقاومة.