Prenatal and postnatal correction of the immunobiological status of calves with a drug based on cyclic nucleotides

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Abstract. The resistance of young cattle to diseases is formed during intrauterine development. Oral administration of a drug that is made from the spleen of cattle and contains nucleosides and nucleopeptides at a dose of 0.1 g/kg of live weight to mother cows once within 5 days before calving increased the triglyceride level in the blood serum of newborn calves by 17.6-35.3%, calcium by 8.9-10.3%, magnesium by 2.3-1.5%, globulins by 8.54-9.45%, sum of immunoglobulins, percentage and absolute concentration of immunoglobulins A and G. The drug neutralised the negative effects of vaccination of cows and calves on the calves’ liver. The introduction of this drug to calves within 5 days after birth in the same dose as to cows stimulated the protein-synthetic function of the liver, an increase in total protein due to the globulin fraction, the total concentration of serum immunoglobulins by more than 2% and the absolute and percentage content of immunoglobulins A and M. The drug contributed to an increase in glucose by 12.8%, triglycerides by 8.7%, magnesium by 12.6%, calcium by 11.2%, stimulated structure-forming functions, transmembrane transport due to phosphorylation of enzymes and structural proteins. Thus, the supplement based on nucleosides and nucleopeptides controlled the course of various physiological processes, metabolism, membrane transport mainly associated with the exchange of Ca and Mg. The results obtained provide grounds to recommend the drug for the correction of the immunobiological status of calves in the prenatal and postnatal development periods.

1. Introduction

Ensuring economic growth and increasing the competitiveness of Russian good producers in the market of meat and dairy products is possible only under the condition of accelerated development of the basic agriculture industries, among which livestock breeding is the main supplier of raw material for these industries [1; 2]. The livestock industry is of critical importance for the Russian economy and for the livelihoods of its population. The production of competitive products in dairy and beef breeding provides for the breeding of cattle adapted to specific climatic conditions characterised by high productivity [3].

Modern technology of the industry makes high demands for the adaptive capabilities of young cattle. The attention of scientists and practitioners is focused on increasing the resistance of newborn calves and first-day calves to endogenous and exogenous factors. Various biologically active compounds are used for this. Among them, drugs made from the spleen are promising since this organ is capable of producing immunoglobulins, opsonins and other biologically active substances that are important for providing immune homeostasis and stimulate the phagocytic and metabolic activity of leukocytes and macrophages. The spleen is part of a powerful natural filtering body system, which plays an important role in the capture and elimination of xenobiotics, including immune complexes [4]. It is known that
Spleen-Active increases nonspecific resistance and acts as an immunomodulatory drug [5]. The ECXSF drug (extracts of cryopreserved xeno-spleen fragments) leads to the restored parameters of nonspecific and specific immunity, primarily local mechanisms of bronchopulmonary defence by activating alveolar macrophages, segmented neutrophils and free neutrophils of bronchoalveolar secretions, which ensures the restoration of the functional activity of phagocytic cells, an increase in the secretion of lysozyme and immunoglobulin A and alpha interferons [6]. The drug-gel made from the spleen of cattle embryos has not only immunomodulatory but also antihypoxic and reparative activity [7]. A very valuable growth activator is a drug made from the spleen of cattle based on organic peptides and nucleotides. Nucleotides are universal regulators of biochemical processes in living cells, the main role of which is to stimulate the phosphorylation of ribosome proteins, which is catalysed by protein kinases, which, in its turn, affects the nature and amount of synthesised proteins in the cell [8]. Due to the phosphorylation of a number of enzymes and structural proteins, nucleotides control the course of various physiological processes, the metabolism of glycogen, fatty acids, membrane processes associated with the exchange of electrolytes and, first of all, calcium, the functioning of the cell genetic apparatus, muscular fibre metabolic processes, etc. [9]. Some authors consider nucleotides to be immunomediators [10].

A number of studies have demonstrated a positive effect of food nucleotides on the immune response, the level of high density lipoproteins in blood plasma, in parallel with a decrease in the level of very low density lipoproteins, as well as an increase in the degree of iron absorption and stimulation of the small intestine mucosa maturation [11]. Therefore, nucleotides can play a polyvalent role [12].

Under certain conditions, the body needs more significant amounts of nucleotides than those that can be formed as a result of its own synthesis or metabolism. For instance, such special conditions include rapid body growth [13]. In the process of absorption in the intestine, 90% of the nucleotides are transformed into nucleosides and subsequently are subjected to degradation. Therefore, their effect is mainly manifested at the level of the mucous membrane of the small intestine. Experiments have shown that the diet supplemented with nucleotides leads to longer villi and deeper crypts [14]. Further, it has a trophic effect, and increases the activity of enzymes in the brush border [15; 16], promotes the mucous membrane reparation [17], has a positive effect on humoral and cellular immunity [18].

2. Objective of research
Based on the analysis of literature, the introduction of a drug that contains nucleotides should entail a change in the synthesis rate and content of several biological compounds. Therefore, the objective of this research was to assess the effect of the nucleosides- and nucleopeptides-containing drug on the immunological and biochemical parameters of calves' blood serum.

3. Materials and research methods
The research and production experience included calves from cows of Kostroma cattle breed. The cows were 3-4 years old, with the same live weight, health status and productivity parameters. In accordance with the scheme of veterinary interventions, all cows were vaccinated with OKZ associated inactivated vaccine against colibacillosis, salmonellosis, Klebsieliollosis and Proteus infection of young farm livestock and fur-bearing animals 30 days before calving, and revaccinated 14 days later per the instructions for use. All newborn calves were vaccinated as well.

The control group of cows received the basic diet adopted on the farm. The experimental group received a supplement containing low molecular weight biologically active compounds, nucleosides and nucleopeptides at a dose of 0.1 g/kg of live weight.

Before the first portion of colostrum was fed, blood samples were taken from all newborn calves, and their biochemical parameters were analysed.

Then three groups of 13 calves in each were formed.

According to the feeding scheme, the control group of calves obtained from the control group of cows was being received only colostrum for 5 days.

The calves obtained from experimental cows were divided into 2 groups: 1st experimental group received a feed supplement containing nucleosides and nucleopeptides at a dose of 0.1 g/kg of live
weight orally once a day, 2nd experimental group received only colostrum like the control group. After the end of the colostrum period, the biochemical parameters of the 5-day-old calves' blood were re-analysed. The blood was taken in the morning, before feeding the animals.

The experiment was carried out in August-September 2019. All cows and calves that participated in the experiment were kept under the same conditions. All animals were clinically examined twice a day.

Laboratory studies were performed at the Department of Obstetrics, Surgery and Non-Contagious Animal Diseases of the Ivanovo State Agricultural Academy. The blood serum was analysed with BA-88A Semi-Auto Chemistry Analyser biochemical analyser, AIFR-01 UNIPLAN enzyme immunoassay analyser and an i-SMART 30 Vet electrolyte analyser.

The biometric results were processed in an Excel spreadsheet.

4. Results and interpretation

In the initial period of their life, young cattle are the most sensitive to stress and adverse environmental factors [19], which is associated with a poorly developed system of nonspecific body defence.

The main parameter of metabolism in newborn calves and its vitality is the content of total protein in the body.

The content of total protein in the blood serum of newborn calves did not have significant differences and was in the range of 67.5-67.8 g/l. In the control calves, albumin (34.7 g/l) prevailed in the proteinogram, while in the experimental calves – globulins (35.6-35.9 g/l) prevailed. The concentration of globulins in the 1st and 2nd experimental groups of calves is higher in comparison with the control ones by 9.45 and 8.54%, respectively (p≤0.05). The protein coefficient in the control group was 1.06, in the 1st experimental group – 0.90, in the 2nd experimental group – 0.89 (figure 1).

![Figure 1](image)

**Figure 1.** The percentage of albumin and globulins in the calves' serum.

In 5-day-old calves of the control group, there was a decrease in total protein compared to the initial parameter by 8.89%, mainly due to a decrease in the globulin fraction, the content of which decreased by 13.4%. The calves of the 1st experimental group showed a tendency to increase the total protein and globulin fraction; the calves of the 2nd experimental group have the opposite trend, the level of globulins decreased by 15.2% against the background of an insignificant decrease in the total protein. The protein coefficient in the control group of 5-day-old calves was 1.16; in the 1st experience group – 0.88; in the 2nd experience group – 1.17.
A significant portion of globulins is represented with immunoglobulins. The clinical status of newborn calves directly depends on the concentration of serum immune globulins and their catabolic efficiency [20]. In the blood serum of newborn calves, immunoglobulin G prevailed in percentage terms, but its share in the control group was less than in the animals of the experimental groups (figure 2). The absolute and percentage content of serum immunoglobulin A in calves of the experimental groups is higher than in the control group, while the content of immunoglobulin M is higher in the control group of animals (table 1).

**Table 1.** The content of immunoglobulins in the calves’ blood serum, M±m, g/l.

| Parameter          | Control group | Newborn calves | 5-day-old calves |
|--------------------|---------------|----------------|------------------|
|                    | 1st experimental group | 2nd experimental group | Control group | 1st experimental group | 2nd experimental group |
| Ig A               | 0.3099±0.0004 | 0.3205±0.0009 | 0.3275±0.0007 | 0.3107±0.0001 | 0.3683±0.0003 | 0.3515±0.0004 |
| Ig G               | 12.55±0.50   | 12.83±0.36     | 12.78±0.29      | 12.49±0.42      | 13.06±0.10      | 12.44±0.37     |
| Ig M               | 2.65±0.05    | 2.57±0.04      | 2.59±0.06       | 2.57±0.05       | 2.66±0.03       | 2.59±0.06      |
| Total Ig content   | 15.5099      | 15.7205        | 15.6975         | 15.3707         | 16.0883         | 15.3815        |

**Figure 2.** Percentage of immunoglobulins A, M and G in the calves’ blood serum.

In 5-day-old calves of the control group, an increase in the percentage of immunoglobulins G and A was noted, while the total content of immunoglobulins tended to decrease. In the 1st experimental group, an increase in the percentage concentration of immunoglobulins A and M and the total content of immunoglobulins was established. In the 2nd experimental group, as well as in the 1st group, there was an increase in the percentage concentration of immunoglobulins A and M, while the total content of immunoglobulins tended to decrease. An increase in the level of immunoglobulins M and A in the blood serum of clinically healthy animals indicates their protective function [20].

Energy metabolism is usually accompanied by the release of energy, which is subsequently used for the synthesis of complex substances. As a result of catabolic reactions, there is a change in glucose,
cholesterol and triglyceride level in calves. The calves of the control group are distinguished by higher glucose content and lower triglyceride level (table 2).

**Table 2. Parameters of energy metabolism in calves, M±m, mmol/l.**

| Parameter       | Newborn calves | 5-day-old calves |
|-----------------|----------------|------------------|
|                 | Control group  | 1st experimental | 2nd experimental | Control group  | 1st experimental | 2nd experimental |
| Glucose         | 4.90±0.50      | 2.50±0.20        | 3.20±0.30        | 3.80±0.10      | 3.20±0.05        | 3.30±0.09        |
| Cholesterol     | 1.00±0.20      | 1.02±0.06        | 0.73±0.05        | 1.03±0.09      | 0.68±0.03        | 0.90±0.05        |
| Triglycerides   | 0.17±0.02      | 0.23±0.05        | 0.20±0.03        | 0.23±0.02      | 0.25±0.02        | 0.28±0.03        |

The glucose level in newborn calves was within the physiological standard. Despite the fact that the lowest glucose parameter in the blood serum in the calves of the 1st experimental group, the level of cholesterol and triglycerides is higher than in the calves of the control and 2nd experimental groups. The lowest cholesterol level was in the calves of the 2nd experimental group, and the triglyceride level – in the calves of the control group, which is possibly associated with hypoxia of newborns or dystocia.

Nevertheless, animals that were in the same conditions of feeding and keeping have different adaptive capacity for metabolic changes in the transition period against the background of the biologically active compound introduction.

In five-day-old calves of the control group, a decrease in glucose by 22.4%, an increase in triglycerides by 35.3% and a tendency to an increase in cholesterol concentration were noted. Thus, in calves, the energy deficit manifested by a decrease in glucose levels is compensated by an increase in triglycerides and cholesterol [21]. The calves of the 1st experimental group showed an increase in glucose by 28.0% and triglycerides by 8.7%, which indicates the effect of the drug on bioenergetic processes and its ability to reduce catabolic processes in the body. The decrease in cholesterol concentration by 33.3% is based on its direct participation in trans-membrane function [22]. The calves of the 2nd experimental group showed a tendency to increased glucose, a higher concentration of cholesterol and triglycerides, by 23.3% and 40.0% (p≤0.01), respectively.

Optimal balance of biological elements is a topical issue for the normal life of newborns and young cattle [23]. Magnesium is an essential element of the Ca, P, Mg triad [24]. Phosphorus, together with magnesium, promotes the absorption of calcium. The ratio of these mineral substances is of great importance for the body (table 3). Thus, in newborn calves of the control group, the Mg : P : Ca ratio was 1 : 2.8 : 2.5, which indicates the predominance of inorganic phosphorus in the blood serum and is associated with the functional rearrangement of the endocrine system in mother cows during pregnancy. The relatively low calcium content in the body of newborns is probably due to the fact that the morphological and functional development of the kidneys has not yet been completed by the time of birth [25]. As for the calves of the experimental groups, in animals of the 1st group the ratio of Mg : P : Ca reached 1 : 1.8 : 2.4, in the calves of the 2nd group - 1 : 1.9 : 2.7, respectively. The data obtained indicate a positive effect of Nucleostim drug, introduced to cows, on the active incorporation of P into transphosphorylation and the cycle of metabolic processes. This is provided by a complex neurohormonal polyorganic mechanism [26].

In 5-day-old calves of the control group, a quantitative change in the content of mineral substances was observed, a tendency to an increase in magnesium and a decrease in phosphorus and calcium was specified, where the ratio of Mg : P : Ca was 1 : 2.4 : 2.2. The calves of the 1st experimental group showed an increase in the level of magnesium and calcium by 12.6% and 11.2% and a decrease in phosphorus by 8.4%. In the calves of the 2nd experimental group, an insignificant change in the level of magnesium, a decrease in total calcium by 15.7% and an increase in phosphorus by 19.8% (p≤0.05) were revealed. The ratio of mineral substances in the 1st group was 1 : 1.4 : 2.4, in the second – 1 : 2.4:
2.4, respectively, Mg : P : Ca [27]. Objectively, the concentration of phosphate is controlled less rigidly than the concentration of calcium [28], and the amount of calcium depends on its consumption and the amount of dissolved calcium available for absorption [29].

Table 3. The level of Mg, P and Ca in calves, M±m, mmol/l.

| Parameter | Control group | 1st experimental group | 2nd experimental group | Control group | 1st experimental group | 2nd experimental group |
|-----------|---------------|------------------------|------------------------|---------------|------------------------|------------------------|
| Mg        | 0.86±0.05     | 0.95±0.04              | 0.88±0.05              | 0.90±0.06     | 1.07±0.02              | 0.83±0.05              |
| P         | 2.45±0.22     | 1.67±0.05              | 1.67±0.10              | 2.12±0.06     | 1.53±0.02              | 2.00±0.07              |
| Ca        | 2.13±0.06     | 2.32±0.08              | 2.35±0.06              | 2.00±0.04     | 2.58±0.02              | 1.98±0.02              |

One of the leading enzymes that provide calcium-phosphorus metabolism is alkaline phosphatase [30]. The activity of alkaline phosphatase in newborn calves of the control group exceeded such in calves of the experimental groups by 12.7-15.8% and amounted to 732.6±46.8 U/l. In 5-day-old calves of the control group, the enzyme activity decreased by 17.2% (p≤0.05). In the 1st and 2nd experimental groups, there was a tendency towards a decrease in alkaline phosphatase, while its activity was 621.8±9.9 and 616.85±7.83 U/l, respectively.

5. Conclusion
According to the research, the problem of correcting the immune status in calves should be addressed starting from the prenatal period [31]. After examining the blood serum parameters of the calves that received nucleosides- and nucleopeptides-containing biologically active supplement during their intrauterine development, a higher content of globulins and immunoglobulins G and A, triglycerides, total calcium and magnesium was revealed.

Oral administration of the drug for five days after the birth stimulated protein, mineral and energy metabolism in calves, the total content of immunoglobulins, the percentage concentration and absolute content of immunoglobulins A and M, which certainly gives evidence of the stimulation of defence mechanisms.

Thus, the drug that contains nucleosides and nucleopeptides from the spleen of cattle controlled the course of various physiological processes, metabolism, membrane processes primarily associated with the exchange of calcium and magnesium, which gives reason to recommend it for correcting the immunological and biological status of calves in the prenatal and postnatal development periods.

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