Influence of the prebiotic feed additive "Vetokislinka" the microflora of the feces and hematological parameters of calves of milk period

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Abstract. The results of studying the effect of the prebiotic feed additive "Vetokislinka" on the microflora of feces, hematological parameters and the growth rate of calves of the milk period are presented. Calves of the control group received the basic diet without the inclusion of the studied prebiotic. The calves of the experimental groups were fed with the prebiotic Vetokislinka in a dose of 0.8 in addition to the basic diet; 1.0 and 1.2 ml per 1 liter of drinking water from 6-10 to 90 days of age, daily frequency, 2 times a day (50% of the daily value). According to the research results, it was found that an effective dose of the prebiotic feed additive “Vetokislinka” was a dose of 1 ml per 1 liter of drinking dose, which had a positive effect on the microbial landscape of feces in calves, in which there was a significant increase in the number of normal flora: lactobacilli and bifidobacteria, as well as a decrease in Escherichia. Within the physiological norm, the use of the prebiotic "Vetokislinka" promoted an increase in erythrocytes by 9.1%, hemoglobin by 11.3%, platelets by 15.2% compared to the first control group (p < 0.05). The average daily gain of calves was significantly higher by 9.8%, while reducing feed costs per 1 kg of live weight by 8.8%. The economic efficiency from the introduction of the prebiotic supplement "Vetokislinka" into the diet of calves, at a dose of 1 ml / head per 1 liter of drinking water, per calf was 561.6 rubles.

1. Introduction
Fundamental research in the field of modern biological and medical Sciences and advances in the knowledge of multidimensional aspects of the relationship between macro-organisms and microorganisms have allowed the development and implementation of probiotics, a new class of drugs that is based on living microbial cultures and has complex beneficial properties for microorganisms, in healthcare and veterinary medicine. [1-10]. The use of intensive technologies in animal husbandry leads to an increase in the stress resistance of animals, a decrease in their immune status and the development
of pathological conditions. To increase the safety of young animals, including by reducing their morbidity and loss of cattle from diseases, feed additives have been developed that include probiotics, prebiotics and other components that stimulate the immunological resistance of the animal body, their growth and hormonal development [11-15]. Prebiotics and probiotics are an effective alternative to the use of synthetic antibiotics in the diet. The mechanisms by which prebiotics affect the immune system have not yet been studied in detail. Most of the effects are associated with an increase in innate and acquired immune responses [16-20]. Probiotics and prebiotics have the ability to modulate the balance and activity of the gastrointestinal (GI) microbiota and are thus considered beneficial to the host animal and are used as functional food. Numerous factors, such as dietary and management restrictions, have been shown to significantly affect the structure and activity of intestinal microbial communities in livestock animals. Previous studies have reported the potential of probiotics and prebiotics in animal nutrition; however, their effectiveness often varies and is controversial, perhaps in part because the dynamics of the GI community have not been taken into account. Under stressful conditions, directly fed microbes can be used to reduce the risk or severity of flushing caused by disruption of the normal intestinal environment. The observed benefits of prebiotics may also be minimal in healthy calves, where the microbial community is relatively stable. However, probiotic yeast strains were introduced to increase the efficiency of rumen fermentation by modulating microbial fermentation pathways. This review focused on the benefits of probiotics and prebiotics for the GI microbial ecosystem in ruminants that are deeply involved in animal nutrition and health [19-23]. Prebiotics are offered as an alternative to antibiotics when raising animals. Fermentable substances such as inulin or lactulose have been proposed to stimulate the immune system and health by modulating the intestinal flora and its fermentation products. This study examined the effects of inulin and lactulose on intestinal health and Hematology in calves. Both prebiotics significantly reduced the number of platelets in the peripheral blood. Only inulin was able to increase the concentration of hemoglobin and hematocrit. The total number of white blood cells was reduced using lactulose, while both prebiotics tended to reduce the proportions of monocytes. The expression of RNA markers of inflammation in the intestine was also influenced by both prebiotics, indicating a reduced inflammatory status. This may be due to a possible decrease in intestinal pathogenic load, which remains to be tested. Only the amount of interleukin-8 RNA was increased by lactulose in the mesenteric lymph nodes. In the ileum, expression of the proliferation marker was increased by inulin, while the apoptosis-related gene was increased by both prebiotics. The results of this study showed a clear effect of prebiotics on certain parameters related to animal health and performance, which have yet to be studied in detail in future studies [24-35]. LLC BashInkom Scientific and Innovative Enterprise (Russia, Ufa, 2019) offered livestock breeding a new prebiotic feed additive Vetokislinka, produced according to technical conditions TU 10.91.10-120-20672718-2019. The Vetokislinka feed additive is designed to reduce the level of pathogenic and opportunistic microflora in water for drinking and to optimize digestion processes in farm animals, including calves of the dairy period. The prebiotic "Vetokislinka" contains copper sulfate, organic acids: acetic, formic, propionic, lactic, citric, stabilizer glycline and solvent (water in certain proportions) as active ingredients. It is recommended to introduce 0.8-1.0 liters of acidifier per ton of tap water (pH = 7.0) to maintain the optimal pH of the water (4.0-4.5). Thus, the use of acidifiers (prebiotics) in the diets of dairy calves is a reserve for improving the quality of drinking water, dairy feed, the development of beneficial intestinal microflora, improving the digestibility of nutrients in the diets and, ultimately, increasing their productive qualities.

The aim of the work was to study the effect of the prebiotic "Vetokislinka" on the microflora of feces, hematological parameters and the growth rate of calves of the dairy period.

2. Materials and methods

The research was carried out in the conditions of LLC Agrofirma "Nikolaevskaya" of the Ufa region of the Republic of Bashkortostan on black-and-white calves of the dairy period for 83 days, where comfortable conditions for keeping and feeding calves were created. The prebiotic "Vetokislinka" for scientific and economic experiments on calves was provided by LLC BashInkom Scientific and
Innovative Enterprise. For scientific and economic experiments, calves in four groups were selected by the method of pairs-analogues (by sex, date of birth, live weight) at the age of 6-10 days, 10 heads in each (50% bulls + 50% heifers). In each experiment, the conditions of keeping and feeding were the same and corresponded to the rations adopted in the farms. Calves of the first control group received the basic diet without the inclusion of the studied prebiotic. Calves of the second experimental group, in addition to the basic diet, were fed the prebiotic "VetoKislinka" at a dose of 0.8 ml per 1 liter of drinking water, calves of the third experimental group - 1.0 ml, calves 4 of the fourth group - 1.2 ml of the prebiotic "VetoKislinka" also per 1 l of drinking water. Frequency - daily, twice a day (50% of the daily requirement). Prebiotic "VetoKislinka" was pre-diluted with drinking water at a ratio of 1: 3. Up to 3 months of age (inclusive) during the experiment, per 1 head, 18 liters of whole milk, 323 liters of whole milk replacer "Eurolac Turbo", 17.5 kg of vetch-oat hay, 30.8 kg of herb haylage, 85.4 kg of a mixture of concentrates were consumed. The consumption of digestible protein was 148 g per 1 energy feed unit.

All calves were weighed once a month at the same time, 2 hours before feeding. Blood samples were taken from the jugular veins on the 22nd, 53rd and 83rd days, 3 hours after the morning feeding, and delivered to the laboratory. To establish the level of metabolism and assess the immune status of the experimental calves, blood samples were taken from 3 heads from each group before weighing. Blood sampling was carried out from the jugular vein into vacuum tubes separately for whole blood and serum for biochemical and immune analysis. Serum was separated from blood by centrifugation at 2000 rpm for 10 minutes.

Biochemical analysis of blood serum was performed on a Stat Fax semiautomatic analyzer, and hematological parameters on an Abacus Junior B automatic hematology analyzer using Olvex test kits separately for each analyzed parameter according to approved procedures for analyzers.

Microbiological examination of calf feces on the 90th day of the experiment included the determination of the composition of the intestinal microflora and the typification of microorganisms (lacto- and bifidobacteria, Escherichia). Fecal samples were collected from the rectum with sterile rubber gloves and placed in sterile plastic tubes. Samples were stored in a freezer at -20 °C until analysis for E. coli, lactobacilli and bifidobacteria. In vitro survival and enumeration of E. coli were determined by the method of Gue, et al. (2006).

Statistical analysis was performed using Statistica 10 (Stat soft company). Quantitative data are presented as the arithmetic mean and its standard error (X ± Sx). The reliability of intergroup differences was judged using the value of the Student’s t test. Differences in the compared groups were considered statistically significant when the level of the first level error (p) was less than 0.05.

3. Results
When monitoring the safety indicators of raw smoked sausages, the indicator of water activity is of great importance. This indicator characterizes the binding energy of moisture for microorganisms, and also affects the biochemical and physicochemical processes that occur in the finished product. For raw smoked sausages, indicators have been determined that guarantee the microbiological safety of the product. Figure 1 shows the values of water activity in the studied samples of raw smoked sausages.

The microbiocenosis of feces of calves at three months of age is presented in table 1.

| Indicator     | 1st control group | 2nd experimental group | 3rd experimental group | 4th experimental group |
|---------------|-------------------|------------------------|------------------------|------------------------|
| Lactobacillus | 4.66±0.42         | 5.82±0.36              | 6.06±0.26*             | 6.82±0.56*             |
| Bifidobacteria| 5.22±0.36         | 6.02±0.48              | 6.92±0.38*             | 7.44±0.48*             |
| Escherichia   | 4.48±0.34         | 4.02±0.28              | 3.24±0.24*             | 3.18±0.28*             |

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Table 2. Morphological composition of the blood of calves (X ± Sx, n = 3).

| Indicator          | 1st control | 2nd experimental | 3rd experimental | 4th experimental |
|--------------------|-------------|------------------|------------------|------------------|
| Erythrocytes, 10^12 / l | 9.5±1.01    | 8.5±0.19         | 9.5±0.54         | 9.1±0.12         |
| Hemoglobin, g / l    | 102.3±7.05  | 97.7±2.72        | 99.3±5.04        | 102.0±1.45       |
| Hematocrit, %        | 32.1±1.87   | 28.2±1.02        | 28.0±2.84        | 31.0±0.77        |
| Leukocytes, 10^9 / l | 15.6±0.62   | 14.7±0.47        | 14.4±1.36        | 15.0±1.51        |
| Lymphocytes, 10^9 / l| 7.2±1.04    | 7.8±0.84         | 7.2±1.28         | 8.2±1.01         |
| Monocytes, 10^9 / l  | 1.3±0.03    | 1.3±0.06         | 1.2±0.17         | 0.9±0.23         |
| Neutrophils, 10^9 / l| 4.3±0.13    | 4.6±0.32         | 5.3±0.52         | 4.4±0.51         |
| Platelets, 10^9 / l  | 455.7±13.42 | 452.0±11.68      | 458.3±16.25      | 455.0±23.06      |

| Indicator          | Experiment start | Experiment end |
|--------------------|------------------|----------------|
| Erythrocytes, 10^12 / l | 8.8±0.16         | 9.3±0.52       |
| Hemoglobin, g / l    | 94.9±2.62        | 94.6±2.62      |
| Hematocrit, %        | 38.8±0.52        | 39.4±0.44      |
| Leukocytes, 10^9 / l | 9.9±0.62         | 9.9±0.42       |
| Lymphocytes, 10^9 / l| 5.4±0.32         | 5.7±0.28       |
| Monocytes, 10^9 / l  | 0.12±0.02        | 0.15±0.03      |
| Neutrophils, 10^9 / l| 3.0±0.22         | 3.1±0.24       |
| Platelets, 10^9 / l  | 399.2±15.04      | 412.8±22.32    |

As studies have shown, the introduction of the prebiotic Vetokislinka into the diets of calves contributed to an increase in the number of lacto- and bifidobacterial by 30.0 - 46.4 and 32.5 - 42.5%, respectively, with a decrease in Escherichia by 27.7-29.0%, compared with the first control group (P<0.05).

Table 2-3 provide information on the morphological composition and biochemical parameters of the blood of experimental calves.

According to the table 2 it can be seen that all the studied indicators of the morphological composition of the blood of calves were within the physiological norms. Within the physiological norm, the use of the prebiotic "Vetokislinka" in a dose of 1 ml per 1 liter of drinking water promoted an increase in erythrocytes by 9.1%, hemoglobin by 11.3%, platelets by 15.2% (experimental group 3) compared to the first control group (p<0.05). In the fourth experimental group, where the prebiotic "Vetokislinka" was used at a dose of 1.2 ml per 1 liter of drinking water, an increase in erythrocytes by 9.1% and hemoglobin by 12.5% was found, as compared with the first control group.

Data analysis from table 3 showed that all the studied biochemical parameters of the blood of calves were within the physiological norms for the age of animals indicated in the reference literature. Within the limits of physiological norms, there was an increase in the total protein content in calves of the third experimental group by 3.2%, glucose by 15.0%, calcium by 16.0%, phosphorus by 26.7% compared with the first control group.

Diseases and deaths during the scientific and economic experiment were not observed in the control and experimental groups. The safety of calves in all groups was 100%. The average daily gain of calves in the third experimental group was significantly higher by 9.8%, while reducing feed costs per 1 kg of live weight by 8.8% compared to the first control group.

A production check showed that the cost-effectiveness of the introduction of the prebiotic additive Vetokislinka into the diet of calves, at a dose of 1 ml / head per 1 liter of drinking water, per head was 561.6 rubles.
Table 3. Biochemical parameters of the blood of calves (X ± Sx, n = 3).

| Indicator          | 1<sup>st</sup> Group | 2<sup>nd</sup> Group | 3<sup>rd</sup> Group | 4<sup>th</sup> Group |
|--------------------|-----------------------|-----------------------|-----------------------|-----------------------|
|                    | control               | experimental         | experimental         | experimental         |
| Total protein, g/l | 53.6±0.63             | 54.7±1.66            | 55.4±1.82            | 55.4±0.47            |
| Albumin, g/l       | 36.3±3.14             | 36.3±0.87            | 36.1±0.89            | 36.4±0.81            |
| Glucose, mmol/l    | 4.5±0.07              | 4.8±0.32             | 5.2±0.44             | 5.3±0.22             |
| Urea, mmol/l       | 3.3±0.14              | 3.5±0.24             | 3.7±0.28             | 3.8±0.21             |
| AST, units/l       | 41.6±7.11             | 44.9±0.52            | 44.2±0.91            | 43.0±0.83            |
| ALT, units/l       | 27.7±0.76             | 27.5±2.91            | 27.0±1.01            | 28.2±1.32            |
| Calcium, mmol/l    | 2.8±0.12              | 2.7±0.25             | 2.8±0.21             | 3.0±0.23             |
| Phosphorus, mmol/l | 1.5±0.15              | 1.5±0.12             | 1.6±0.31             | 1.6±0.18             |
| Bilirubin, mmol/l  | 3.0±0.18              | 2.8±0.16             | 2.9±0.18             | 2.9±0.21             |
|                   |                       |                       |                       |                       |
| Total protein, g/l | 60.1±0.41             | 60.6±0.84            | 62.0±0.42*            | 61.2±0.82            |
| Albumin, g/l       | 28.3±0.96             | 30.8±0.87            | 28.1±0.15            | 29.3±0.32            |
| Glucose, mmol/l    | 4.0±0.12              | 4.3±0.18             | 4.6±0.13*            | 4.1±0.14             |
| Urea, mmol/l       | 3.4±0.08              | 3.7±0.15             | 3.6±0.12             | 3.6±0.15             |
| AST, units/l       | 42.7±0.24             | 43.8±0.92            | 42.8±1.53            | 43.7±1.04            |
| ALT, units/l       | 29.9±1.28             | 26.6±1.13            | 29.0±1.47            | 31.3±1.31            |
| Calcium, mmol/l    | 2.5±0.06              | 2.6±0.07             | 2.9±0.09*            | 2.9±0.09             |
| Phosphorus, mmol/l | 1.5±0.06              | 1.7±0.08             | 1.9±0.09*            | 1.5±0.05             |
| Bilirubin, mmol/l  | 2.9±0.24              | 3.3±0.34             | 2.8±0.38             | 3.0±0.24             |

4. Discussion
In studies by Terré M, Calvo M A, Adelantado C, Kocher A, Bach A [30], Riddell J B, Gallegos A J, Harmon D L, Mcleod K R [33], Timmerman H M, Mudler L, Everts H, Vanespan D C [34] there were no differences in the level of productivity, health, the number of bacteria in feces and the presence of microorganisms in it from the use of mannan oligosaccharides (MOS).

Other research results show that microbial diversity in feces is closely related to calf age, immune status and growth rate. As a result of the introduction of probiotics into the body in calves, a significant increase in the number of lactobacilli and bifidobacteria was observed, while the number of E. coli decreased. Our data are consistent with the data of Mohamadi P, Dabiri N [32], which conducted a study to assess the effect of probiotic, prebiotic and synbiotic as a feed additive on the amount of microflora of calves' feces. According to the results of our research, it was found that the prebiotic "Vetokislinka" had a certain effect on the microbiocenosis of the intestines of calves. The introduction of the probiotic Vetokislinka into the diets of calves promoted an increase in the number of lacto- and bifidobacteria by 30.0 - 46.4 and 32.5 - 42.5%, respectively, with a decrease in Escherichia by 27.7–29.0%, compared to the first control group (p<0.05).

The change in the biochemical parameters of blood serum was within the physiological norms, the differences were not significant. Similar data are presented in studies by Dar A H et al. [26] in which it was shown that no significant effect of probiotic, prebiotic, and synbiotic on glucose, AST and ALT levels has been established.

Within the physiological norm, the use of the prebiotic "Vetokislinka" in a dose of 1 ml per 1 liter of drinking water promoted an increase in erythrocytes by 9.1%, hemoglobin by 11.3%, platelets by 15.2% (experimental group 3) compared to the first control group (p<0.05). In the fourth experimental group, where the prebiotic "Vetokislinka" was used at a dose of 1.2 ml per 1 liter of drinking water, an increase in erythrocytes by 9.1% and hemoglobin by 12.5% was found, as compared with the 1st control group. There was an increase in the content of total protein in calves of the third experimental group by 3.2%, glucose - 15.0%, calcium - 16.0%, phosphorus - 26.7% also in comparison with the first control group. A similar pattern is confirmed in the works of Dar A H, Singh S K [28], Franklin S T, Newman M C, Newman K E, Meek K I [31].
Calves that received the prebiotic "Vetokislinka" in a dose of 1 ml per 1 liter of drinking water increased the growth rate. The average daily gain of calves in the third experimental group was significantly higher by 9.8%, while reducing feed costs per 1 kg of live weight by 8.8% compared to the first control group. Similar results have been obtained by several authors: Riddell J B, Gallegos A J, Harmon D L, Mcleod K R [32], Bayatkouhsar J, Tahmasebi A M, Naserian A A, Mokarram R, Valizadeh R [35].

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