Influence of the prebiotic feed additive "VetoKislinka" on the immune resistance of the blood and the intensity of growth of calves of milk-feeding period

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Abstract: The results of studying the effect of the prebiotic feed additive "VetoKislinka" on the immune resistance of the blood and the growth rate of calves of the milk-feeding period are presented. Calves of the control group received a basic diet without the inclusion of the studied prebiotic. The calves of the experimental groups, in addition to the basic diet, were fed the prebiotic VetoKislinka at a dose of 0.8, 1.0 and 1.2 ml per 1 liter of drinking water from 6-10 to 90 days of age, daily, 2 times per day (50% of the daily value). According to the research results, it was found that the effective dose of the prebiotic feed additive "VetoKislinka" was a dose of 1 ml per 1 liter of the drinking dose, where the phagocytic activity of the blood was 5.4 abs % higher than in the first control group. 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 additive "VetoKislinka" into the diet of calves, at a dose of 1 ml/head per 1 liter of drinking water, per 1 calf was 561.6 rubles.

1. Introduction
Fundamental research in the field of modern biological and medical sciences and advances in understanding the multidimensional aspects of the relationship between macroorganisms and microorganisms have made it possible to develop and introduce 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 livestock leads to an increase in stress resistance of animals, a decrease in their immune status and the development of pathological conditions. To improve the safety of young animals, including by reducing their morbidity and loss of cattle from diseases, feed additives have been developed, including probiotics, prebiotics and other components that stimulate the immunological resistance of the animal body, their growth and hormonal development [11-15]. Prebiotics and probiotics represent an effective alternative to the use of
synthetic antibiotics in the diet. The mechanisms by which prebiotics affect the immune system are not yet fully understood. 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 used as functional foods. Numerous factors, such as dietary and management restrictions, have been shown to significantly affect the structure and activity of the gut microbial communities of livestock animals. Previous studies have reported the nutritional potential of probiotics and prebiotics; however, their effectiveness is often variable and controversial, perhaps in part because the GI community dynamics has not been taken into account. Under stressful conditions, directly fed microbials can be used to reduce the risk or severity of flushing caused by disturbed intestinal conditions. The observed benefits of prebiotics may also be minimal in healthy calves, in which the microbial community is relatively stable. However, probiotic yeast strains have been introduced with the aim of increasing the fermentation efficiency in the rumen by modulating microbial fermentation pathways. This review focused on the benefits of probiotics and prebiotics for the microbial ecosystem of the GI tract in ruminants, which are deeply involved in animal nutrition and health [19-23]. Prebiotics are offered as an alternative to antibiotics in animal raising. 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 gut health and hematology in calves. Both prebiotics significantly reduced the number of platelets in a peripheral blood. Only inulin was able to increase the hemoglobin concentration and hematocrit. The total leucocyte count was reduced by lactulose, while both prebiotics tended to decrease the proportion 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 RNA of interleukin-8 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 NVP Bashlnkom (Russia, Ufa, 2019) offered livestock breeding a new prebiotic feed additive VetoKislinka produced according to 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 glycine 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 way for improving the quality of drinking water, dairy feed, the development of beneficial intestinal microflora, improving the digestibility of nutrients in rations and, ultimately, increasing their productive qualities.

The purpose of the work was to study the effect of the prebiotic "VetoKislinka" on the immune resistance of the blood and the growth rate of calves of milk-feeding period.

2. Materials and method
The studies were carried out for 83 days on black-and-white calves of the dairy period in the conditions of LLC Agrofirma "Nikolaevskaya" of the Ufa region of the Republic of Bashkortostan, where comfortable conditions for keeping and feeding calves were created. The prebiotic "VetoKislinka" for scientific and economic experiments on calves was provided by LLC NVP "Bashlnkom". For scientific and economic experiments, calves were selected into four groups by the method of analogs (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 a basic diet (BD) 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 of the fourth group - 1.2 ml of the prebiotic VetoKislinka also calculated for 1 liter of drinking water. Frequency - daily, twice a day (50% of the daily value). Prebiotic "VetoKislinka" was pre-diluted with drinking water at a ratio of 1: 3. Up to 3 months of age (inclusive) for the period of the experiment, per 1 head, 18 liters of whole milk, 323 liters of whole milk replacer "Eurolac Turbo", 17.5 kg of vetch-oats hay, 30.8 kg of herb haylage, 85, 4 kg of a mixture of concentrates. The consumption of digestible protein was 148 g per 1 energetic feed unit (EFU).

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 was taken 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.

Phagocytic activity of neutrophils was determined using inert particles of polystyrene latex (Sigma, USA) with a diameter of 0.80 microns with a 1:10 dilution with Hanks medium.

The amount of IgA, IgM and IgG was determined by the method of radial diffusion in a gel according to Mancini. Immune serum against antibodies of a certain class (IgG, IgM, IgA) was introduced into a melted agar gel. After the agar solidifies, the ABs are evenly distributed in it. The test material (TM) introduced into the well diffuses radially into the gel. Since the concentration of AB is the same everywhere, as a result of the TM-AB reaction, not precipitation bands are formed in the equivalence zone, but precipitation rings around the well with TM. The diameter of the precipitation ring is directly proportional to the concentration of TM in the test fluid. For the quantitative determination of circulating immune complexes, the blood taken from the calves from the jugular vein was defended for 30 min at room temperature. The resulting blood serum was centrifuged at 400 rpm for 10-15 minutes. The method is based on selective precipitation of antigen-antibody complexes with a 3.75% solution of polyethylene glycol M-6000 (PEG), followed by photometric determination of the precipitate density at a wavelength (λ) of 450 nm.

Statistical analysis was performed using Statistica 10 software (Stat soft). 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 if the level of the first level error (p) was less than 0.05.

3. Results
In studies of blood serum on the resistance of the body of calves, results were obtained proving the immunostimulating effect of the probiotics under study on the indices of nonspecific and humoral immunity factors (table 1).

| Indicator                      | 1st control | 2nd experimental | 3rd experimental | 4th experimental |
|-------------------------------|-------------|-----------------|------------------|------------------|
| Phagocytic activity, %        | 70.8±1.24   | 72.2±0.94       | 76.2±1.12*       | 75.4±0.68        |
| IgA, mg/ml                    | 4.2±0.32    | 4.6±0.42        | 4.4±0.38         | 4.6±0.52         |
| IgM, mg/ml                    | 3.5±0.52    | 3.9±0.44        | 3.9±0.48         | 3.8±0.38         |
| IgG, mg/ml                    | 21.6±1.22   | 20.6±1.22       | 21.6±1.22        | 19.8±1.32        |
| IgE total, MU/ml              | 33.6±4.12   | 34.8±4.22       | 46.8±4.14        | 48.0±4.14        |
| Circulating immune complexes, units | 52.6±0.94 | 54.6±1.32       | 56.8±0.84*       | 56.4±1.22        |
As a result of the research, it was found that the phagocytic reaction in the blood serum of the calves of the third experimental group was enhanced, which meant a high response of the body to the probiotic feed additive VetoKislinka. Thus, the excess of phagocytic activity in the group with the prebiotic "VetoKislinka" was 5.4 abs. % than in the first control group. In addition, in the 3rd experimental group of calves, an increase in circulating immune complexes by 8.0% was found, also in comparison with the first control group.

Table 2. Calf raising results for the experiment period (X ± Sx, n = 10).

| Group                          | 1st control | 2nd experimental | 3rd experimental | 4th experimental |
|-------------------------------|-------------|------------------|------------------|------------------|
| Live weight at the beginning of the experiment, kg | 35.3±1.05  | 35.7±0.84        | 36.8±1.17        | 36.5±2.28        |
| Live weight at the end of the experiment, kg | 81.1±1.46  | 84.3±1.23        | 87.1±1.65*       | 85.4±2.13        |
| Absolute gain, kg             | 45.8±1.58  | 48.6±2.03        | 50.3±2.60*       | 48.9±3.07        |
| Average daily gain, g          | 552.0±15.5 | 586.0±19.55      | 606.0±19.4*      | 589.0±39.12      |
| In % to control               | -           | 108.0            | 109.8            | 106.7            |
| Feed consumption per 1 kg of growth, EFU | 3.86        | 3.64             | 3.52             | 3.62             |

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 the consumption of feed per 1 kg of live weight decreased by 8.8% compared to the first control group. A production check showed that the economic efficiency from 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.

4. Discussion

In studies by Terré M., Calvo M.A., Adelantado C., Kocher A., Bach A., Riddell J.B., Gallegos A.J., Harmon D.L., McLeod K.R., Mohamadi P., Dabiri N., Riddell J.B., Gallegos A.J., Harmon D.L., McLeod K.R., Timmerman H.M., Mudler L., Everts H., Vanespan D.C. there were no differences in the level of productivity, health, metabolism in the presence of microorganisms from the use of mannano-oligosaccharides (MOS).

Other research results show that microbial diversity in faeces 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. The data received are consistent with the data of Mohamadi P., Dabiri N., who conducted a study to assess the effect of probiotic, prebiotic and symbiotic as a feed additive on the amount of the calf faecal microbiota. According to the results of the current research, it was found that the prebiotic "VetoKislinka" had a certain effect on the microbiocenosis of the intestinal tract in calves. The introduction of the prebiotic VetoKislinka into the diets of calves promoted an increase in the number of lactobacilli and bifidobacteria by 30.0 - 46.4 and 32.5 - 42.5%, respectively, with a decrease in Escherichia by 27.7–29.0%, as compared with the 1st control group (p<0.05).

The change in the biochemical parameters of blood serum did not exceed the physiological norms, the differences were not significant. Similar data are presented in studies by Dar A.H. et al. in which it was shown that there was no significant effect of probiotic, prebiotic and symbiotic on glucose, AST and ALT levels.

Within the physiological range, 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% (3rd experimental group) compared to the 1st control group (p<0.05). In the 4th experimental
group, where the probiotic "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 3rd experimental group by 3.2%, glucose - 15.0%, calcium - 16.0%, phosphorus - 26.7% as compared with the 1st control group. A similar pattern is confirmed in the studies by Dar, A.H., Singh, S.K., Franklin S.T., Newman M.C., Newman K.E., Meek K.I.

As a result of the research, it was found that the phagocytic reaction in the blood serum of the experimental groups was enhanced, which meant a high response of the body to the penetration of infectious agents. Thus, the excess of phagocytic activity in the group with the probiotic "VetoKislinka" in the 3rd experimental group was 5.4 abs.% than in the control. The data received are consistent with the results of studies by Indar M. et al. found that the use of a probiotic and a prebiotic in calves leads to a significant increase in the phagocytic activity of peripheral blood leukocytes.

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 3rd experimental group was significantly higher by 9.8%, while the consumption of feed per 1 kg of live weight decreased by 8.8% compared to the 1st control group. Similar results were obtained by a number of researchers: Riddell J.B., Gallegos A.J., Harmon D.L., Meleod K.R., Bayatkouhsar J., Tahmasebi A.M., Naserian A.A., Mokarram R., Valizadeh R.

5. Conclusion

According to the research results, it was found that the effective dose of the probiotic feed additive "VetoKislinka" was a dose of 1 ml per 1 liter of the drinking dose (3rd experimental group). As a result of the research, it was identified that the phagocytic reaction in the blood serum of the calves of the 3rd experimental group was enhanced, which meant a high response of the body to the probiotic feed additive VetoKislinka. Thus, the excess of phagocytic activity in the group with the probiotic VetoKislinka was found to be 5.4 abs.% higher than in the 1st control group. In addition, in the 3rd experimental group of calves, an increase in circulating immune complexes by 8.0% was found, also in comparison with the 1st 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 3rd experimental group was significantly higher by 9.8%, while reducing feed costs per 1 kg of live weight by 8.8% compared to the 1st control group. The economic efficiency from the introduction of the probiotic additive VetoKislinka into the diet of calves, at a dose of 1 ml/head per 1 liter of drinking water, per 1 calf was 561.6 rubles.

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