The effect of the new probiotic feed additive rhodofen on blood indicators of gobies during fattening

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Abstract. This article is devoted to the study of the effect of the new probiotic feed additive rhodofen, containing live spore-forming microorganisms of the genus Bacillus – Bacillus subtilis and Bacillus licheniformis, on the morphological and biochemical blood parameters of Hereford bulls fed. The introduction of a probiotic feed additive rhodofen into the diet at a dose of 1.5 kg per ton of feed does not lead to pathological and inflammatory processes, and contributes to a more intense metabolism in the body.

1. Introduction

Currently, in veterinary practice and animal husbandry, antibiotic substances of various groups are widely used. The feasibility of using antibiotics as therapeutic and prophylactic agents in animal husbandry has been proven. When antibiotics are introduced into the diet, there is a significant reduction in the waste of animals and birds as a result of the prevention of various diseases, as well as the acceleration of their growth and development as a result of a more complete use of the nutrients of the diet [4, 13].

The unsystematic use of antibiotics and chemotherapeutic agents leads to disruption of the normal microflora and a decrease in the immune status of farm animals and poultry. This leads primarily to diseases of the digestive and respiratory organs, and the accumulation of residual amounts of drugs in meat and animal products and poultry. Currently, the most promising group of pharmacological agents are probiotic drugs, which are highly effective therapeutic and prophylactic drugs in farms where the microflora of animals is characterized by dysbiosis [2, 5, 9, 12, 15].

A sufficiently large number of probiotics have been developed, including both monoculture and associations of various strains. Probiotic preparations based on various symbiotic microorganisms have different pharmacological activity and are highly effective therapeutic and prophylactic agents [10, 16, 17].

A group of probiotics based on microorganisms of the genus Bacillus was formed not so long ago and has more than 20 drugs. Representatives of this genus are characterized by high adaptability to environmental conditions and the ability to form spores.

The advantages of creating drugs using bacteria of the genus Bacillus, it is harmless to the macroorganism even in high concentrations; ability to increase non-specific resistance of animals; antagonistic activity to a wide range of pathogenic microorganisms; high enzymatic activity; resistance to lytic enzymes and, accordingly, high viability throughout the gastrointestinal tract; storage stability and environmental safety [11, 14].
The duration of stay of spores of microorganisms in the gastrointestinal tract depends on the genetic characteristics of the organism, as well as on the stage of the pathological process in the host organism. After stopping the drug, the bacteria are excreted naturally for 2–5 days.

For veterinary medicine, several preparations have been created, which include bacteria of the genus *Bacillus* – vetom-1.1, vetom-3, subaline, bactocellolactin, endobacterin, sporitis, sporobacterin and others. Some of the above drugs have already been registered, and are being used as therapeutic and prophylactic agents in veterinary practice, and some are at the testing stage of experimental industrial series [1, 3, 7].

The predominant formulations are dry, packaged in bags, ampoules, vials or capsules.

The main disadvantage of probiotic preparations based on bacteria of the genus *Bacillus* is their low biological effectiveness in relation to the strains of *Pseudomonas*, *Streptococcus* and the inability to use it in complex therapy with antibiotics for severe infections.

A new class of drugs are probiotics obtained on the basis of recombinant strains that will be resistant to a number of antibiotics and antagonistic activity against pathogenic microorganisms.

The task of new developments is to create a dry complex probiotic feed additive, which is characterized by high antagonistic activity to pathogens and is resistant to antibiotics.

In the limited liability company “Basis” (Russia, Ufa) under the guidance of Doctor of Technical Sciences B.P. Strunin obtained a dry probiotic feed additive rhodafen, which contains live spore-forming microorganisms of the genus *Bacillus* – *Bacillus subtilis* TB-26 (collection "Basis") and *Bacillus licheniformis* B-020 (collection "Basis"), in equal proportions. 1 g of rhodafen contains at least 1 x 10⁹ CFU of live spore-forming bacteria. Rhodafen does not contain genetically modified microorganisms. The synergistic action of the strains provides a more complete protection of the body from colonization by opportunistic microorganisms with increased virulent properties.

From the results of studies on livestock of various species of birds, it was found that the strains included in the probiotic supplement rhodafen have antagonistic activity against opportunistic microflora, take part in microbial digestion, produce enzymes and biologically active substances that increase the nonspecific resistance of the body [6].

Drinking a rhodafen probiotic in case of dyspeptic phenomena helped normalize the microflora of the gastrointestinal tract in young cattle [8].

The objective of our research was to study the effect of the probiotic additive of rhodafen on the body of bulls of Hereford breed during the entire feeding period.

2. Methods and materials

The experiments on testing a new probiotic feed additive rhodafen were carried out on the basis of the enterprise of OAO “Zirgan MTS” of the Meleuzovskoye branch of the Yuldash section of the Meleuzovsk region of the Republic of Bashkortostan, the animal husbandry department of the Bashkir Agricultural Research Institute, Russian Academy of Sciences, and a limited liability company.

Scientific and production tests were conducted on bulls of Hereford breed, put on fattening at the age of 6 months.

Three groups of 25 goals were formed according to the principle of analogues: Group 1 – control, without drugs; Group 2 – experimental (with a probiotic feed additive rhodafen at a dose of 1 kg per ton of feed); Group 3 – experimental (with a probiotic feed additive of rhodafen in a dose of 1.5 kg per ton of feed).

The animals were in the same conditions, the premises were equipped with automatic drinkers, mechanisms for distributing feed and cleaning manure.

The diets of the experimental animals were balanced and compiled taking into account the detailed norms and the technological scheme of feeding for intensive rearing and fattening of young cattle. Accounting for animal feed intake was determined on a monthly basis, and the chemical composition of the feed and its residues was studied according to generally accepted methods of zootechnical analysis in the analytical laboratory of the Bashkir Agricultural Research Institute.
The growth and development of experimental gobies was determined by monthly weighing, as well as taking basic measurements. The clinical condition of animals was determined according to methods generally accepted in veterinary medicine. The physiological state and metabolic processes of the experimental animals were monitored by taking blood from the venom vein in the morning hours before feeding. Complex hematological analysis of peripheral blood was performed on an automated analyzer Abacus junior vet (manufactured by DIATRON, Austria). The level of protein fractions was determined nephelometrically (according to Bessey, as modified by Anisova). Laboratory biochemical studies were carried out on a ChemWell Combo biochemical and enzyme-linked immunosorbent analyzer.

The effectiveness of the drug was judged by changes in the clinical status of animals and the dynamics of biochemical parameters of blood homeostasis, which were compared with control studies. The results of the digital data obtained were processed statistically using the method of variation statistics and verification of reliability by Student's criterion (P).

3. Results

Of great importance is the study of morphological and biochemical parameters of blood, not only in the diagnosis of diseases of various etiologies, but also with the introduction of new drugs and additives. In this regard, every month when fattening the gobies of the studied groups for fattening, blood was taken to study its parameters after using various doses of the probiotic rhodafen. The results of morphological blood parameters of gobies are presented in Table 1.

The study of individual indicators of the general analysis of blood of bulls showed that when using the probiotic feed additive rhodafen at the age of 15 months in the 2nd and 3rd experimental groups, the number of red blood cells was significantly higher than in the control by 3.6 and 5.8 % (P ˂0.5; P ˂ 0.05) and amounted to 7.73 ± 0.18 x 10¹²/l; 8.01 ± 0.24 x 10¹²/l and 8.18 ± 0.27 x 10¹²/l, respectively.

| Table 1. Dynamics of the morphological composition of blood of experienced bulls (n = 25) |
|----------------------------------------|----------------|----------------|
| Indicator                              | Age, month     | Group of animals |
|                                       | 1              | 2              | 3              |
| Red blood cells 10¹²/l                 | 6              | 7.83±0.17      | 8.27±0.16*     | 8.73±0.14**  |
|                                       | 9              | 7.98±0.11      | 8.14±0.29      | 8.55±0.12*   |
|                                       | 15             | 7.73±0.18      | 8.01±0.24*     | 8.18±0.27**  |
| Hemoglobin, g/l                        | 6              | 119.13±0.18    | 121.12±0,15    | 126.08±0.14  |
|                                       | 9              | 117.29±0.19    | 119.07±0,18*   | 122.11±0.33  |
|                                       | 15             | 115.48±0.29    | 117.69±0,31    | 120.72±0.42* |
| White blood cells, 10⁹/l               | 6              | 8.39±0.20      | 8.59±0.23      | 8.87±0.42    |
|                                       | 9              | 7.21±0.22      | 7.59±0.41      | 7.98±0.23    |
|                                       | 15             | 7.96±0.29      | 8.02±0.17*     | 8.01±0.21*   |

Note: * – reliably with respect to control (P<0.5); ** – significant in relation to control (P<0.05).

A similar trend was observed in the study of hemoglobin in the blood of experimental animals. So, in the 2nd and 3rd experimental groups, the hemoglobin content was higher than in the control group by 2.0 and 4.5 % (P<0.5) or 115.48 ± 0.29 g/l; 117.69 ± 0.31 g/l and 120.72 ± 0.42 g/l, respectively, in the control and experimental groups. In the study of leukocytes, we found that at the beginning of the experiment, their content did not differ between the control and experimental groups. At the end of the experiment, the leukocyte level in the control group was 7.96 ± 0.29 x 10⁹/l, and in the 2nd and 3rd experimental groups, respectively, 8.02 ± 0.17 x 10⁹/l and 8.01 ± 0.21 x 10⁹/l. A slight increase in the level of leukocytes by 0.7 and 0.6 % in the 2nd and 3rd experimental groups was within the physiological limits for this animal species.

A study of the biochemical parameters of blood serum of experimental gobies testified to the positive effect of the probiotic supplement rhodafen on metabolic processes in the body (table 2). The
The total protein content in blood serum was higher in the experimental groups during the entire experiment, and at the age of 15 months this indicator was significantly higher than the control values by 1.4 and 62 % ($P<0.5$; $P<0.05$). The higher total protein content in the 2nd and 3rd experimental groups indicates a better use of the nitrogenous part of the diet, and, accordingly, a greater absorption of nitrogen in the body.

**Table 2.** Biochemical composition of blood of experienced bulls (n = 25)

| Indicator          | Age, month | Group of animals |
|--------------------|------------|------------------|
|                    | 1          | 2                | 3                |
| Total protein, g/l | 6          | 63.59±1.57       | 66.73±1.29*      | 69.42±0.89**       |
|                    | 9          | 68.24±0.98       | 71.43±1.08       | 72.51±1.14*        |
|                    | 15         | 72.88±1.27       | 73.91±0.69*      | 77.39±1.19**       |
| Albumin, g/l       | 6          | 36.45±0.84       | 37.61±0.73       | 38.86±0.78*        |
|                    | 9          | 34.62±0.56       | 35.27±0.81       | 37.04±0.43*        |
|                    | 15         | 33.17±0.58       | 34.41±0.77       | 36.29±0.47*        |
| Globulin, g/l      | 6          | 27.14±0.30       | 35.08±0.42**     | 33.51±0.16***      |
|                    | 9          | 33.24±0.79       | 38.11±0.28*      | 39.92±0.26**       |
|                    | 15         | 37.29±0.98       | 42.76±0.82       | 43.29±0.74         |
| Albumin-Globulin ratio | 6       | 1.34             | 1.07             | 1.16               |
|                   | 9          | 1.04             | 0.93             | 0.93               |
|                   | 15         | 0.88             | 0.80             | 0.84               |

Note: * – reliably with respect to control ($P<0.5$); ** – significant in relation to control ($P<0.05$); *** – significantly with respect to control ($P<0.005$).

An indicator characterizing the metabolism of proteins in the body is the protein coefficient (Albumin-Globulin Ratio), a significant difference of which was not detected in the control and experimental groups. So, at the beginning of the experiment (6 months), this indicator was 1.34; 1.07 and 1.16, and by the end of the experiment (15 months, 0 was 0.88; 0.80 and 0.84, respectively, in the control and 2nd and 3rd experimental groups.

By studying the dynamics of protein fractions, it is possible to establish the intensity of protein metabolism in the body. The research results (Table 3) show that in all groups there was a gradual increase in protein fractions with age. So, at the age of 6 months, the content of $\alpha$-globulin fractions in the control and 2nd and 3rd experimental groups was 6.13 ± 0.28 g/l; 7.53 ± 0.33 g/l and 8.57 ± 0.22 g/l ($P<0.5$; $P<0.05$) The number of $\alpha$-globulin fractions in the 2nd and 3rd experimental groups increased by 11.0 and 12.3 % relative to the control group by the end of the experiment, respectively.

**Table 3.** Content of globulin fractions of experienced bulls (n = 25)

| Indicator          | Age, month | Group of animals |
|--------------------|------------|------------------|
|                    | 1          | 2                | 3                |
| $\alpha$-globulins, g/l | 6          | 6.13±0.28        | 7.53±0.33*       | 8.57±0.22**        |
|                    | 9          | 8.54±0.49        | 8.81±0.47        | 9.27±0.44*         |
|                    | 15         | 10.61±0.39       | 11.78±0.29       | 11.92±0.58         |
| $\beta$-globulins, g/l | 6          | 9.64±0.41        | 12.31±0.47*      | 12.97±0.27**       |
|                    | 9          | 8.27±0.49        | 9.92±0.61        | 10.91±0.52*        |
|                    | 15         | 8.53±0.24        | 9.03±0.42        | 9.58±0.11*         |
| $\gamma$-globulins, g/l | 6          | 11.37±0.27       | 11.44±0.61       | 11.97±0.94         |
|                    | 9          | 16.43±0.73       | 19.38±0.32*      | 19.74±0.72**       |
|                    | 15         | 18.15±0.51       | 21.05±0.68*      | 21.79±0.39**       |

Note: * – reliably with respect to control ($P<0.5$); ** – significant in relation to control ($P<0.05$).

The content of $\beta$-globulins at the beginning of the experiment was 9.64 ± 0.41 g/l in the control and 2nd and 3rd experimental groups; 12.31 ± 0.47 g/l and 12.97 ± 0.27 g/l, by the end of the experiment – 8.53 ± 0.24 g/l; 9.03 ± 0.42 g/l and 9.58 ± 0.11 g/l, respectively.
By the end of the experiment, a significant difference ($P < 0.5; P < 0.05$) was found for the content of γ-globulin fractions, which were 16.0 and 20.0 % higher than the control indices in the 2nd and 3rd experimental groups or. 18.15 ± 0.51 g/l; 21.05 ± 0.68 g/l and 21.79 ± 0.39 g/l, respectively, in the control and the 2nd and 3rd experimental groups.

The physiological work of internal organs, in particular the heart and liver, is evidenced by the activity data in the blood serum of experimental animals of aspartate aminotransferase and alanine aminotransferase (Table 4). The degree of increase in the activity of aminotransferases indicates the severity of the cytolytic syndrome and indirectly indicates impaired organ function. The level of AST activity at the beginning of the experiment was 1.01 ± 0.09 mmol/tsp; 1.21 ± 0.13 mmol/tsp and 1.27 ± 0.16 mmol/tsp, and ALT 0.27 ± 0.03 mmol/tsp; 0.38 ± 0.15 mmol/tsp and 0.39 ± 0.09 mmol/tsp, respectively, in the control and 2nd and 3rd experimental groups. At the age of 15 months, there was a slight downward trend in the experimental groups of AST levels in relation to the control group by 1.5 and 0.7 %, and ALT by an average of 2.1 % in the 2nd and 3rd experimental groups, respectively. It should be noted that in all age periods, the level of AST and ALT activity in all groups increased within the physiological norm, which also indicates that at this time there was an intensive growth of muscle and bone tissue, which was accompanied by a high intensity of biochemical processes in the body.

| Table 4. Activity of blood serum aminotransferases of experimental gables (n = 25) |
|-----------------------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Indicator  | Age, months | Group of animals | Group of animals | Group of animals |
| AST, mmol/tsp | 6 | 1.01±0.09 | 1.21±0.13 | 1.27±0.16 |
| | 9 | 1.26±0.14 | 1.23±0.04* | 1.48±0.08** |
| | 15 | 1.35±0.06 | 1.33±0.17* | 1.34±0.08* |
| ALT, mmol/tsp | 6 | 0.27±0.03 | 0.38±0.15 | 0.39±0.09 |
| | 9 | 0.43±0.08 | 0.42±0.27 | 0.41±0.26 |
| | 15 | 0.47±0.13 | 0.46±0.31 | 0.46±0.42 |

Note: * – reliably with respect to control ($P < 0.5$); ** – significant in relation to control ($P < 0.05$).

4. Conclusion

Thus, our results indicate that the use of the probiotic feed additive rhodafen at a dose of 1.5 kg per ton of feed helps stimulate hematopoiesis, increase hemoglobin, as a result of which the oxygen content in the animal is normalized and, accordingly, oxidation-reduction processes increase. The introduction of the probiotic feed additive rhodafen does not lead to pathological and inflammatory processes in the body of experimental gables, and contributes to a more intense protein metabolism in the body.

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