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RESEARCH ARTICLE

Growth Performance and Haematological Indices in Calves Fed with Probiotic Supplement 'Bacticor'

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Abstract

The paper presents the study results on the effect of a feed probiotic supplement 'Bacticor' on biochemical and morphological parameters of blood, absolute and average daily live weight gains in calves and economic efficiency of raising the animals. The farm experiments conducted before showed that 20 g per head was the optimal daily dose of the supplement. The established dose of the feed probiotic supplement increased the final live weight by 1.77 % and absolute live weight gain rose by 8.3 %. The viability of the study calves for all groups was reported 100 % throughout the experiment. The study feed supplement had a positive effect on the formation of specific immunity in calves. A noticeable decrease in the concentration of lymphocytes was observed in the middle of the experiment, where the concentration of these specific cells was $3.6*10^9$/l compared to $8.9*10^9$/l in the control group. The feed supplement 'Bacticor' slightly increased the concentration of total protein by 1.73-3.22%, glucose by 7.0-14.5% in the blood serum of calves. Rearing one calf for three months of the experiment required 3.89-3.93 EFU (Energy Feed Unit); the costs for 1 kg of live weight gain were by 7.2 % lower in the test group than in the control group.

Keywords: *Probiotic supplement, Bacillus subtilus, Strains, Microorganisms, Lactobacilli, Pre-weaning calves, Haematological parameters, Immune resistance, Feed conversion.*

Introduction

Currently, the use of new biological products containing probiotics has become particularly relevant in rearing young animals. The need to obtain environmentally friendly animal products is one of the reasons behind the process [1, 8]. However, the wide range of probiotic-based products and their compositional characteristics resulted in certain difficulties while considering the effectiveness and economic efficiency of the products. The use of microbial feed supplements depends largely on their composition and biological nature of the strains.

A number of studies have proved that products containing spore forming Bacillus bacteria have high efficiency [9, 11]. The effect is achieved due to an increased antagonistic activity of these bacteria to pathogens of the digestive tract, and activation of immune protection as a result of the produced biological metabolites.

Studies indicate that Bacillus subtilis are able to secrete more than 70 active biological substances. The Bacillus subtilis bacteria feature the ability to reduce the acidity of the environment and produce antibiotics. As a result, probiotics containing the microbial strains are able to restrain the development and suppress the spread of potential pathogenic and pathogenic agents in the animal body [12, 15].Dosage form and substrate carrier are important aspects when choosing an effective probiotic based product.

Simple dosing and introduction of the supplement are essential in practical application too. In particular, conventional farms face the problem to accurately weigh or measure small doses of the supplement, and then evenly distribute the dose in the feed. In this regard, the search for the most effective ways to introduce probiotic bacteria and their metabolites containing the necessary elements

of protection and nutrition has a special practical value and relevance. Dry probiotic products are much more convenient in use. The probiotic supplement 'Bacticor' is based on Bacillus subtilis bacteria with concentration of 10^9 CFU and strains of lactobacilli and yeast of at least 10^7 CFU in 1 g of beet pulp. The supplement is produced by OOO NVP [LLC] 'BASHINKOM' (Ufa, Russia).

Research Methods

Pre-weaning calves of the black-and-white Holstein breed were used in the experiment. The experiment lasted three months. The study animals were kept in identical conditions. The calves were selected into groups on an analogue-group basis (sex, date of birth, live weight). The age of the heifers was 2-2.5 months; the groups were made up of 10 calves each.

The calves of the control group received a basic diet without adding the study supplement. The calves of the test group were fed the supplement at 20 g per head added to the basic diet. The calves were fed based on the conventional feeding scheme and in line with the planned weight gain parameters. Feed nutritional values and feeding standards related to the age of animals were taken to develop the feeding scheme.

Methods used for the Analysis of Haematological Indices and Growth Performance in Calves

The calves were weighed once a month at the same time two hours before feeding. Blood samples were taken before the experiment, in the middle of the experiment (45 days after the starting point) and at the end of the experiment after morning feeding, and delivered to the laboratory of the University (Bashkir state agrarian university). Blood samples were collected from four calves in each group before weighing. The aim of the sampling was to determine the metabolism level and assess protective properties of the body in the experimental calves. Blood was taken from the jugular vein into separate vacuum test tubes for whole blood and blood serum for biochemical analysis. Blood serum sedimentation was conducted at room temperature (+18-25°C), the serum was then centrifuged to separate it from the formed elements at 2000 rpm for 15 minutes. The automatic haematological analyzer Abacus Junior B was used to analyse morphological indices of the blood; the semi-automatic analyzer Stat Fax combined with 'Olvex' test kits was used to perform a biochemical analysis of the blood serum. Individual test kits were used for each analysed indicator and in compliance with the techniques accepted for analyzers.

Feed Costs Considered

Consumption of feed was controlled through weighing (of hay, silage, haylage and grain feed) monthly on two days following each other.

Incidence Rates Considered

Clinical state of the animals was regularly monitored for signs of disturbance or disorders in digestion (diarrhoea) and the skin state.

Statistical Analysis

The software package Statistica 10 was used to perform a statistical analysis. Quantitative evidence is written in the arithmetic average and its standard error (x±Sx). The student's *t* test was used to determine the reliability of intergroup differences. Differences in the compared groups were reported significant when the error level of the first range (p) was lower than 0.1.

Results of the Study

The study results of morphological and biochemical composition of the whole blood in the calves are presented in Table 1.

Table 1: Morphological indices of the blood in the calves for the experimental period

Name	Physiological standard value	Control group	Test group
The starting point of the experiment			
Red blood cells, 10^{12} / l	7.5-8.0	9.52±0.12	9.36±1.02
Haemoglobin, g / l	100-129	93.5±2.5	102.0±1.0
Hematocrit (RBC plasma ratio), %	38-40	29.72±0.70	31.16±1.08
White blood cells, 10^9 / l	9.0-12.0	14.72±1.06	10.47±0.71

Differential white blood cell count, 10^9/l	Lymphocytes	2.5-7.5	9.37±0.98	6.50±1.03
	Monocytes	0-0.85	0.15±0.01	0.44±0.35
	Neutrophils,	0.6-6.5	5.31±0.04	3.53±0.16
	Eosinophils	0.1-1.0	0.09±0.01	0.05±0.00
Platelets, 10^9 / l		100-800	549.0±52.1	413.3±32.95
on day 45 of the experiment				
Red blood cells, 10^{12} / l		7.5-8.0	9.46±0.72	9.4±0.89
Haemoglobin, g / l		99-129	99.0±4.74	97.0±4.04
Hematocrit (RBC plasma ratio), %		25-40	30.93±0.83	31.6 ±1.03
White blood cells, 10^9 / l		9.0-12.0	14.2±2.6 x	8.3±1.06*
Differential white blood cell count, 10^9/l	Lymphocytes	2.5-7.5	8.9±1.3	3.6±1.81*
	Monocytes	0-0.85	0.56±0.45	0.16±0.01*
	Neutrophils,	0.6-6.5	4.72±1.41	4.6±0.77
	Eosinophils	0.1-1.0	0.06±0.01	0.05±0.01
Platelets, 10^9 / l		100-800	305.5±52.7	320.7±71.3
The end of the experiment				
Red blood cells, 10^{12} / l		7.5-8.0	10.55±0.56	9.34±0.44
Haemoglobin, g / l		100-129	105.6±4.92	104.1±3.37
Hematocrit (RBC plasma ratio), %		38-40	33.93±1.38	31.75±0.88
White blood cells, 10^9 / l		9.0-12.0	10.29±1.25	10.27±0.95
Differential white blood cell count, 10^9/l	Lymphocytes	2.5-7.5	6.43±0.56	4.53±0.56
	Monocytes	0-0.85	0.43±0.15	0.29±0.07
	Neutrophils,	0.6-6.5	3.42±0.99	5.45±0.38
	Eosinophils	0.1-1.0	0.02±0.00	0.02±0.00
Platelets, 10^9 / l		100-800	300.2±68.81	423.8±55.9

*Note: here and elsewhere the value is significant at p≤ 0.1

Our study demonstrated that the probiotic supplement 'Bacticor' had a positive effect on forming the specific immunity in calves. The concentration of lymphocytes and segmented neutrophils was reported lower in the test group when fed the supplement. A noticeable decrease in the concentration of lymphocytes was observed in the middle of the experiment when the number of species-specific cells dropped to 29.5% and monocytes fell 3.5-fold. By the end of the experiment we observed a trend of some deviations from the average values of the species-specific cells in the blood of the control calves. We observed a similar pattern in the white blood cells of the test group animals, which indicated a lower immunity level in the animals. The immunity stimulating probiotic bacteria in the 'Bacticor' supplement might have helped balance the intestinal microflora. As a result, this had a positive effect on the growth and development of the calves. The red blood cells exceeded the recommended physiological standard values in all of the groups. The deviation might be a typical feature of the Holstein breed or due to the period when the analysis was performed, though no noticeable changes in the clinical state of the animals were observed. The analysis of the biochemical blood composition showed that the principal serum indices of total protein and albumin, glucose, urea, calcium and phosphorus were in line with the physiological standard values throughout the experiment. Table 2 presents values of the biochemical blood composition in the calves.

Table 2: Biochemical composition of blood in the calves

Blood composition index	**Physiological standard value**	**Group**	
		Control group	Test group
The starting point of the experiment			
Total protein, g / l	62-85	66.33±2.21	66.03±0.75

Albumin, g / l	28-45	33.55±1.31	33.17±0.78
Urea, mmol / l	3.3-6.7	2.75±0.15	2.94±0.12
Glucose, mmol / l	2.2-3.3	2.09±0.12	2.38±0.54
Calcium, mmol / l	2.38-3.13	2.82±0.10	2.78±0.10
Phosphorus, mmol / l	1.45-1.94	1.60±0.04	1.58±0.10
Carotene, mg%	0.4-0.9	0.18±0.01	0.18±0.01
The end of the experiment			
Total protein, g / l	62-85	67.62±1.85	68.79±1.66
Albumin, g / l	28-45	35.09±0.99	35.33±1.38
Urea, mmol / l	3.3-6.7	3.29±0.24	3.33±0.27
Glucose, mmol / l	2.2-3.3	2.13±0.26	2.28±0.41
Calcium, mmol / l	2.38-3.13	2.99±0.08	2.88±0.14
Phosphorus, mmol / l	1.45-1.94	1.62±0.06	1.68±0.09
Carotene, mg%	0.4-0.9	0.15±0.01	0.19±0.01

We found that the feed supplement 'Bacticor' slightly increased the concentration of total protein by 1.73-3.22%, glucose by 7.0-14.5% in the blood serum of the calves. The point should be made that glucose values were within the lower range of the physiological standard values, and carotene values were not in line with the standard values. Table 3 demonstrates the study results on the live weight of the calves.

Table 3: Growth performance and viability indices of the calves when fed the probiotic supplement 'Bacticor'

Indices	**Group**	
	Control group	Test group
Live weight at the starting point of the experiment, kg	79.6±3.81	74.3±3.16
Live weight at the end of the experiment, kg	175.6±9.20	178.7±7.07
Absolute live weight gain, kg	96.0±6.75	104.4±4.62
Average daily weight gain throughout the experiment, g	800.0±56.25	866.7±38.5
Relative live weight gain, %	54.4±1.54	58.48±1.03*
Viability of the calves, %	100.0	

The findings showed that the probiotic feed supplement 'Bacticor' had an effect on growth intensity in the experimental calves. Adding the product at 20 g per head to the diet increased the live weight of the animals by 3.1 kg or 1.77 %. The average daily and relative live weight gain was by 66.7 g (8.3 %) and 4.08 %, respectively, higher in the test group than in the control group. The viability index was 100 % in all of the groups throughout the experiment. Few cases of diarrhoea were observed. The cases were not infectious, in fact. The test calves were more disease-resistant. This is probably due to the additional body protection formed by active biological substances that probiont bacteria secreted. To determine the cost of raising the calves on a dairy and pedigree stock farm (SEC collective farm) 'Hero' we based our calculations on the main costs associated with the consumption of feed and additives accounting for about 52% of the total costs.

Table 4: Average daily feed consumed and energy feed units (EFU) per 1 kg of live weight gain in calves

Indices	Group	
	Control group	Test group
Feed consumed per day per group for 1 month of the experiment, kg		
Hay	6.3	6.1
Grain haylage	9.0	10.0
Concentrated feed	12.5	12.5
Total EFU consumed	24.05	24.45
Feed consumed per day per one group for 2 months of the experiment, kg		
Hay	10	10
Grain haylage	16.0	16.0
Concentrated feed	23.0	23.0
Total EFU consumed	42.88	42.88
Feed consumed per day per group for 3 months of the experiment, kg		
Hay	11.5	12.0
Grain haylage	36	36.5
Concentrated feed	30	30
Total EFU consumed	62.90	63.51
Total feed consumed for 3 months per 1 head, kg		
Hay	83.4	84.3
Grain haylage	183	189
Concentrated feed	196.5	196.5
Total EFU consumed per head/ including per 1 kg weight gain	389.4/4.06	393.3/3.77

The findings demonstrated that the control calves consumed 389.4 EFU of feed while the test calves consumed 393.3 EFU of feed. The running costs for 1 kg of live weight gain were by 7.2 % lower in the test group than in the control group. The cost of 1 kg live weight gain was by 6.39 % lower in the test group than in the control group. An increased profit of 1320.04 roubles (20.7 %) per head was obtained through the improved feed conversion. Thus, increased economic efficiency obtained from the feed supplement 'Bacticor' was 12.64 roubles per 1 kg of live weight gain

Discussion

The use of probiotics has no significant effect on haematological indices of animals. Ulger [16] reported that probiotic supplementation did not affect blood biochemical parameters significantly ($P<0.05$), except ALT, triglyceride and iron levels ($P<0.05$). Experiments performed by Al-Saiady showed that serum total proteins, albumin and total globulins were comparable in all three groups as were the serum concentrations of urea, BUN and triglycerides. Serum inorganic constituents also showed no significant differences between the control and treated groups. Our study demonstrated that total protein and albumin, glucose, urea, calcium and phosphorus were in line with physiological standard values throughout the experiment and had no significant deviations.

The experimental findings are consistent with the results obtained by other researchers. Despite the slight effect of probiotic supplement products on the biochemical composition of the blood, Soto et al [17].Pointed out that the probiotic administration increased the systemic immune response specific in calves. A lot of Russian and foreign researchers report that many probiotics based on exogenous bacteria B. subtilis act to correct non-specific immunity by secreting a number of metabolic substances that affect the general body resistance to infections.

It was found that B. subtilis synthesized vitamins, thiamine (B1), pyridoxine (B6) and menaquinone (K2), in particular. Various strains of B. subtilis secrete different amino acids, some are considered as the key amino acids, such as valine [18, 19].The experiments performed by Noori et al [20].Demonstrated that partial substitution of milk with probiotic yogurt increased lymphocytes ($P<0.01$) and decreased serum triglycerides ($P<0.01$) and cholesterol.

Their findings proved that the partial substitution of milk with probiotic yogurt might improve growth performance and modulate the immune status in calves. Punetha et al [21].Obtained similar results: the probiotic supplement improved the postpartum health, antioxidant status and immune function, of transition dairy cows.

Our study demonstrated that the probiotic supplement 'Bacticor' had a positive effect on forming the specific immunity in calves. This was proved by a decrease in the concentration of lymphocytes and segmented neutrophils in the test groups. A marked decrease in the concentration of lymphocytes was observed in the middle of the experiment, especially in the 2nd test group, where the number of protective cells was $3.6*10^9$/l compared to $8.9*10^9$/l in the control group.

Agazzi et al [22].Found lower blood eosinophils in animals during the first week of probiotic treatment, (0.05% compared to 0.22%; P <0.01). In addition, they revealed that supplementation improved the intestinal microflora and growth performance and increased some biometric indices of calves. Probiotic supplementation reduces diarrhoea cases in young animals. Timmerman et al [23].Found that microorganisms administered lowered mortality rates.

The ingestion of probiotics reduced the percentage of calves that required therapy and the amount of treatments needed against digestive or respiratory diseases. Renaud et al [24].Showed that a multispecies probiotic bolus administered to calves at the onset of diarrhoea reduced the duration of diarrhoea. Lopez-Valencia et al [25].Concluded that daily provision of 5 g of a standardized product containing probiotics, ß-glucans, and glyconutrients to diarrheic calves under antibiotic therapy will promote health and enhance early growth performance However, Riddell et al [26].

Found that calves housed indoors with little added stressor may not benefit from probiotic feeding. Our study recorded few cases of diarrhoea among the calves. The cases were not infectious. The viability was 100 % in all groups throughout the experiment. Our findings are consistent with the results obtained by Dias et al [27].Dar et al [28].Proved experimentally that addinga lactic acid-based probiotic to the diet changed some aspects of feeding calves. Microbial preparations increased the duration of time spent on the consumption of food. Probiotics can increase the live weight of calves. Shokaiyan et al [29].Reported in their study that the live weight of calves fed probiotics with mixed lactobacilli increased within 7-9 weeks, and up to 10-12 weeks when fed probiotics with L. acidophilus 27SC. The study by Laxmi et al [30].Recorded that supplementation improved growth performance in calves with low body weight. The calves fed probiotics had 5.25% higher live weight and 11.4 % higher daily live weight gain (P<0.05) then the control calves. We found that raising one calf for three months of the experiment required 3.89-3.93 EFU (Energy Feed Unit); the running costs for 1 kg of live weight gain were by 7.2 % lower in the test group than in the control group. Sun et al [31, 32].

Found that Bacillus subtilis natto increased general performance by improving the average daily gain and feed efficiency. Our findings are in line with the results of other researchers: the use of the probiotic feed supplement 'Bacticor' has a positive effect on growth performance and immune stimulation in pre-weaning calves.

Conclusions

Thus, the probiotic feed supplement 'Bacticor' improved growth performance and health of the calves. The supplement added at 20 g per head increased absolute and daily weight gain values by 8.3 %. The results of haematological tests indicated improved protective properties of the blood in the test group heifers. No cases of infectious diarrhoea and death reported in the study support the findings. The test group had the most significant changes in the morphological structural elements of the white body cells; the changes indicated a significant improvement in cellular immunity of the body.

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