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Metabolic processes of young goats when using probiotic bacillus Amyloliquefaciens

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Abstract. The effect of the probiotic Bacillus Amyloliquefaciens VKPM V-11475 on the morphological and biochemical parameters of the serum of young goats was studied. Prescribing probiotic to goats at a dose of 4×10^9 significantly increased nitrogenous metabolism: total protein by 5.8% ($p\leq0.05$), albumin by 13.9% ($p\leq0.01$), creatinine by 11.5% p ≤0.01) and reduced the amount of urea by 32% ($p\leq0.05$); at a dose of 4×10^7 - increased total protein by 6.1% ($p\leq0.01$), albumin by 14.7% ($p\leq0.01$), creatinine by 10.6% ($p\leq0.01$) and decreased the amount of urea by 35% ($p\leq0.05$), relative to the control. The use of a probiotic in animals at a dose of 4×10^9 had a positive effect on the parameters of carbohydrate-lipid metabolism - it reduced the amount of bilirubin by 19% ($p\leq0.05$), cholesterol by 15% ($p\leq0.05$); increased the content of triglycerides by 15.7% ($p\leq0.05$), at a dose of 4×10^7 - the amount of bilirubin decreased by 21.8% ($p\leq0.05$), cholesterol by 13.7% ($p\leq0.05$); the number of triglycerides increased by 17.5% ($p\leq0.05$), relative to the control. The probiotic Bacillus Amyloliquefaciens VKPM V-11475 can be recommended to increase the adaptation parameters of kids at the studied doses.

1 Introduction

The article was written within the framework of the scientific project: "Development and introduction into industrial production of the Samara region of a method for increasing the adaptive and productive indicators of small cattle by prescribing a domestic biological product" № 23-26-1000.

Goat breeding is one of the oldest branches of animal husbandry. Goat's milk ranks second in the global milk production structure (2.4%). Goats have a number of features, because of which many livestock breeders give them respect over other types of agricultural herbivores: they have a high adaptive ability to various conditions of maintenance, a wide range of plant species being grafted, perfectly use low-yielding pastures, digest feed with a high (up to 60%) fiber content [1].

Considering that from 2003 to the present, the indicators of the development of goat breeding have almost doubled, as well as the growing interest of consumers primarily in healthy and proper nutrition, favorable conditions are emerging for the development of this market segment. With an annual increase in the number of goats in the country amounting to

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7.1%, by 2050 the number will reach 6 million 278 thousand goats. With the increase in livestock, it is expected to achieve the production of goat meat of 17.1 thousand tons [2].

In order to increase the productivity of animals and the opportunity to fully realize the genetic potential inherent in their breed, it is necessary to organize full-fledged animal feeding based the use of biologically active substances to stimulate metabolism.

It is known from literary sources that metabolism is a natural order of transformation of substances and energy in living systems, aimed at maintaining and self-renewal of the body. Metabolism includes all chemical reactions that occur in the body. [3].

Metabolism does not proceed in direct ways, but occurs completely freely. The body draws the substances it needs – fats, proteins, carbohydrates from the general resources of the body – the "metabolic boiler" - carbon skeletons formed during metabolism and necessary for its existence [4].

N.V. Bogolyubova and colleagues note that anaerobic conditions are created in the rumen, its contents have an acidic reaction and buffer properties that determine the development of symbiotic microflora depending on the composition of the feed ration, pH conditions, temperature, lack of oxygen, and etc. Synergism and antagonism among the microorganisms of the scar content is complex and diverse, therefore it is difficult to establish the role of a specific group. As a result, their body receives up to 70% of energy from microbial fermentation of feed and the most valuable microbial protein [5].

Scientists have proven that the use of probiotics in addition to the basic diet leads to an increase in metabolism in the body of farm birds and animals, increased absorption of nutrients and reduced feed costs per unit of body weight gain [6-9].

Scientists AlGburi A., Volski, A. and colleagues studied the probiotic properties of Bacillus amyloliquefaciens B-1895 and Bacillus subtilis KATMIRA1933. These two strains interacted with Escherichia coli and Pseudomonas aeruginosa, and cell-free supernatants suppressed the growth of Streptococcus intermedius and Porphyromonas ginivalis. The endospores B. amyloliquefaciens B-1895 and B. subtilis KATMIRA-1933 were resistant to 0.3% bile salts and incubated for 4 hours in MRS broth at pH 2.0-3.0. The ability of the two strains to produce antimicrobial compounds potentiates their application in health care formulations, personal care products, food and animal feed [10].

Scientists studied the prothelitic ability of Bacillus amyloliquefaciens subsp. plantarum UKM B-5139 and UKM B-5140, which are part of the probiotic Endosporin. The isolated proteases are capable of lysing living cells of Staphylococcus aureus and Candida albicans. The presence of strains of B. amyloliquefaciens subsp. plantarum UKM B-5140 and UKM B-5139 proteases capable of cleaving native insoluble proteins (elastin, fibrin, collagen) has been shown. These enzymes have lytic activity against yeast and Gram-positive bacteria and can be used in biotechnology [11].

This study's findings Jubair Al-Rashid and his colleagues provide compelling evidence for the biocontrol potential of Bacillus amyloliquefaciens FH1-RS1 and Bacillus cereus R19, demonstrating significant volumedependent antifungal effects against Verticillium dahliae V991. The inhibition of fungal growth by FH1-RS1 not only establishes a clear dose-response relationship but also identifies a saturation point beyond which further increases in bacterial volume do not translate into increased fungal inhibition. This phenomenon is crucial for determining the optimal concentrations necessary for maximum biocontrol efficacy and is consistent with other reports of dose-dependent responses in biocontrol agents [12].

Scientists Liu H. and his colleagues studied 96 strains of B. amyloliquefaciens and proved that the probiotic has an extensive pangenome and represents a large number of unique genes. The population size and the corresponding versatility of the ecological niche of B. amyloliquefaciens are considered the most influential factors determining the size of the pangenome. For example, group 1 includes 23 strains of B. amyloliquefaciens, of which 56% were associated with food, 17.39% with soil and 8.69% with the rhizosphere. [13].

The effectiveness of a feed additive based on the Bacillus amyloliquefaciens strain B-20/VKPM B 12168 was tested on newborn piglets diagnosed with diarrhea. Piglets of the experimental groups were given a feed additive of 20 ml three times a day for three days. The piglets stopped having diarrhea, there was no case, and after two days all the animals were practically healthy. Tests were carried out on chickens of a broiler poultry farm. As a result, the chickens in the experimental group gained weight more intensively. When giving probiotics to fish, the growth rate of juveniles in the experimental group compared to the control group was 18%. The water in the aquariums did not spoil, there was no turbidity. The conducted tests showed an increase in the productivity of animals, poultry and fish when using a feed additive based on the proposed strain of Bacillus amyloliquefaciens B-20/VKPM B 12168 [14].

Medvedeva P.I. and colleagues have created a probiotic drug based on Bacillus amyloliquefaciens, a unique active ingredient that is an antagonist of pathogenic microorganisms such as Escherichia coli, Salmonella and others. The authors used a probiotic drug based on Bacillus amyloliquefaciens for poultry. The drug showed high efficacy in experimental studies with a concentration of lyophilized mass of spore-forming bacteria Bacillus amyloliquefaciens in an amount of at least 1×10^7 CFU/g. It is recommended for use in poultry farming in order to increase the productivity and quality of poultry meat [15].

In connection with the above, the study of the use of a microbiological preparation based on B. Amyloliquefaciens in goat breeding to improve the metabolism of productive animals is an urgent topic.

2 Material and methods

A probiotic based on Bacillus Amyloliquefaciens VKPM B-11475 (B. amyloliquefaciens) was produced in the research laboratory of an individual entrepreneur, the head of a peasant farm (PF) "Tsirulev E.P.". The drug is a light brown liquid. For the scientific experiment, a freeze-dried form of the drug was used in the form of capsules at a dose of 4×10^9 and 4×10^7 CFU/g (4×10^9 and 4×10^7).

Scientific and production experience was carried out on a farm for the production and processing of goat's milk of the farm "Semkina O.V." of the Privolzhsky district of the Samara region. The goats were selected according to the principle of pairs of analogues of 10 heads in the group of 2 and 3 months of age. The animals of the control group were kept on a basic diet (BD). The goats of the experimental group I took probiotic at a dose of 4×10^9, experimental group II - 4×10^7 30 minutes before feeding, 1 capsule per head 1 time per day for a month using a bolus dispenser.

Blood was taken from the jugular vein on an empty stomach at the age of 120 days of the animals. The obtained blood samples were examined at the L.K. Ernst FITZVIZH Federal State Medical University in the Department of Physiology and Biochemistry of agricultural animals. Determination of the content of biochemical parameters in blood serum was carried out on an automatic biochemical analyzer ChemWell. Analyticon Biotechnologies AG and Spinreact reagents were used for the determination. The data obtained during the research were processed by biometrics with the calculation of generally accepted constants and using the STADIA program.

3 Results and discussion

Zoohygienic indicators in the livestock room PF "Semkina O.V." meet the standards of keeping small cattle. The indoor microclimate was characterized by the following indicators: the average air temperature was 18.60 ± 0.300 C, the relative humidity was 73.80 ± 1.50%. The chemical composition and nutritional content of the feed used in the farm for feeding goats corresponds to all-Russian data. Felucene (salt for small catle with macro- and microelements), as well as tricalcium phosphate, are freely available in feeders [7].

Table 2 presents the results of a morphology analysis of the blood of young goats after the use of a probiotic B. amyloliquefaciens daily for a month.

Table 1. Blood morphology of young goats

Indicators	group control	group experienced I	group experienced II
1	2	3	4
Red blood cells, 10^{12}/l	3.24±0.09	2.31±0.07**	2.9±0.11*
hemoglobin, g/l	109±3.06	119±3.18*	121±2.55**
hematocrit, %	12.67±0.32	13.54±0.24*	13.88±0.37*
white blood cells, 10^{9}/l	10.61±0.23	10.45±0.16	11.08±0.29
leukoformula, %			
Basophils	0.50±0.10	0.30±0.06	0.21±0.04
eosinophils	0.60±0.35	0.68±0.12	0.61±0.13
neutrophils, incl.			
juvenile	1.2±0.02	0.5±0.02	0.4±0.03
rod-shaped	5.20±0.09	5.46±0.15	5.02±0.12
segmented	38.80±0.09	39.96±1.06	41.07±1.08*
lymphocytes	51.40±1.13	51.90±0.85	51.25±1.05
monocytes	2.30±0.07	1.20±0.04**	1.44±0.06**

Here and further note: * – $p \leq 0.05$, ** – $p \leq 0.01$, *** – $p \leq 0.001$ – relative to the control data.

All morphological parameters were within the physiological norm and corresponded to the age of the animals. The number of red blood cells in experimental groups I and II was 0.1 and 0.6×10^{12}/l higher ($p \leq 0.05$) compared to control data (Table 1). The hemoglobin content in the blood of goats of the control group was 109±3.06 g/l, in the experimental groups the parameters were higher by 10 and 12 g/l ($p \leq 0.05$), respectively. Hematocrit values in the blood of goats treated with 4×10^{9} and 4×10^{7} daily probiotic B. amyloliquefaciens in addition to BD for a month were 0.9% and 1.2% higher than in control animals. Hematocrit values in the blood of goats treated with 4×10^{9} and 4×10^{7} daily probiotic B. amyloliquefaciens in addition to BD for a month were 0.9% and 1.2% higher than in control animals. The number of leukocytes in the control group was at the level of 10.61×10^{9}/l, in the I experimental group it was 1.5% less, in the II experimental group it was 4.4% higher than in the control. Analyzing the leukoformula, it should be noted that the number of rod-shaped neutrophiles in the I experimental group was 5% higher, in the II experimental group - 3% lower; the number of segmented neutrophils in group I was 1.2% higher, in group II - 2.3% ($p \leq 0.05$), compared with the control group. Lymphocyte counts ranged from 51.25±1.05 to 51.90±0.85%.

Monocytes in the blood of animals treated with probiotic B. amyloliquefaciens were 1.1% less in experimental group I ($p \leq 0.01$), 0.9% less in experimental group II ($p \leq 0.01$) than in the control. All parameters were within the physiological norm and corresponded to the age of the animals. The introduction of probiotic B. amyloliquefaciens into the diet of goats contributed to increased hematopoiesis in the animals of experimental groups: the content of red blood cells increased by 3.1% ($p \leq 0.05$) and 9.4% ($p \leq 0.01$); the amount of hemoglobin - in 9.2% ($p \leq 0.05$) and 9.4% ($p \leq 0.01$) compared with the parameters of the control group.

There were no significant differences between indicators I and II of the experimental groups. In group I, the number of red blood cells and hematocrit were lower by 0.5×10^{12}/l and 0.3 g/l; hemoglobin and white blood cells counts were 2 g/l higher and 0.63×10^{9}/l higher than controls. Thus, the use of a probiotic B. amyloliquefaciens at a dose of 4×10^9 and 4×10^7 daily for a month had a positive effect on the morphological component of the blood of goats.

Table 2 presents the results of a biochemical analysis of the blood of young goats after the use of a probiotic B. amyloliquefaciens daily for a month.

Table 2. Biochemical blood parameters of young goats

Indicators	group control	group experienced I	group experienced II
nitrogen metabolism			
Total protein, g/L	66.27±1.25	70.12±1.21*	70.33±1.12*
Albumins, g/L	27.12±1,07	30.91±1.15**	31.12±1.31**
Globulins, g/L	39.15±1.16	39.21±1.18	39.21±1.21
A/G	0.69	0.78	0.79
AST, IU/L	86.10±3.31	81.46±3.55	79.14±3.96
ALT, IU/L	18.12±1.01	17.65±0.98	18.55±1.03
Urea, mmol/L	4.24±0.21	5.60±0.27*	5.76±0.39*
Creatinine, µmol/L	62.88±2.08	70.14±1.62**	69.56±1.88**
carbohydrate-lipid metabolism			
Total bilirubin,, µmol/L	0.87±0.04	0.70±0.06*	0.68±0.04 *
Cholesterol, mmol/L	2.77±0.12	2.35±0.13*	2.39±0.12 *
Triglycerides, mmol/L	0.57±0.03	0.66±0.02*	0.67±0.03 *
Glucose, mmol/L	2.60±0.11	2.80±0.07	2.74±0.09
mineral metabolism			
Phosphatase, alkaline U/L	387.06±13.35	340.12±10.25*	328.67±11.43 *
Calcium, mmol/L	2.65±0.06	2.68±0.11	2.69±0.08
Phosphorus, mmol/L	3.23±0.11	3.12±0.21	3.10±0.19
Magnesium, mg/dL	0.84±0.04	0.96±0.04	1.03±0.05*
Chloride, mmol/L	102.98±4.63	100.73±7.17	103.61±5.48

All biochemical parameters were within the physiological norm and corresponded to the age of the animals. Serum total protein in the control group was 66.27±1.25 g/l, which is 3.9 g/l ($p \leq 0.05$) and 4.1 g/l ($p \leq 0.05$) less than in the experimental groups I and II (Table 2). The amount of albumin in goats receiving a probiotic B. amyloliquefaciens at a dose of 4×10^9 and 4×10^7 was higher by 3.8 g/l ($p \leq 0.01$) and 4 g/l ($p \leq 0.01$), which indicates a more intense protein metabolism in the body of experimental animals. The parameters of AsAT and AlAT enzymes content in the control were 86.10±3.31 and 18.12±1.01 IU/ l, in goats of experimental group I the parameters were less by 6.6 and 0.5 IU/ l; Test group II - on 6.9 and 0.4 IU/l, respectively. The amount of urea in the blood serum of experimental groups of kids increased 1.36 mM/l ($p \leq 0.05$) and 1.52 mM/l ($p \leq 0.05$), compared with the indicator in the control. The creatinine content increased by 7.3 µM/l ($p \leq 0.01$) in comparison with the data of the control group in experiment I, and by 6.7 µM/l ($p \leq 0.01$) in experiment II. Our studies on the use of a probiotic B. amyloliquefaciens in addition to BD during the period of intensive growth of young animals have demonstrated its positive effect on nitrogen metabolism in animals.

The amount of bilirubin in the blood serum of goats was less in the I and II experimental groups by 0.2 μM/L ($p \leq 0.05$), compared with control indicators. The cholesterol content in the control was 2.77±0.12 μM/l, which was higher by 0.42 μM/l ($p \leq 0.05$) compared to animals taking a probiotic B. amyloliquefaciens at a dose of 4×10^9, and by 0.38 μM/l ($p \leq 0.05$) - at a dose of 4×10^7. The parameters of the scientific study indicate an improvement in the liver function of animals receiving a probiotic in addition to the BD. Triglyceride concentration increased in the test groups by 0.09 and 0.1 mM/l ($p \leq 0.05$), relative to the data in the control. The glucose content in test group I was higher by 0.2 mM/l, in group II - by 0.1 mM/l, than in the control. The use of a probiotic B. amyloliquefaciens had a positive effect on the carbohydrate-lipid metabolism in the body of experimental animals.

The total calcium content in the young MRS control group was 2.65±0.06 mM/l, in the experimental groups the indicator was higher by 0.03 and 0.04 mM/l, respectively. The amount of inorganic phosphorus in the serum of experimental caprines was lower by 0.11 and 0.13 mM/l, compared with the control. Alkaline phosphatase in test group I of kids was lower by 47 IU/l ($p \leq 0.05$), in group II - lower by 58 IU/l ($p \leq 0.05$), relative to the data in the control group. The use of a probiotic does not significantly affect the mineral metabolism in the body of young goats.

4 Conclusion

Prescribing a probiotic B. amyloliquefaciens at a dose of 4×10^9 in freeze-dried form daily for a month significantly increased nitrogenous metabolism: total protein by 5.8% ($p \leq 0.05$), albumin by 13.9% ($p \leq 0.01$), creatinine by 11.5% ($p \leq 0.01$) and decreased the amount of urea by 32% ($p \leq 0.05$), compared with control data. The use of a probiotic B. amyloliquefaciens animals at a dose of 4×10^9 had a positive effect on the parameters of carbohydrate-lipid metabolism - it reduced the amount of bilirubin by 19% ($p \leq 0.05$), cholesterol by 15% ($p \leq 0.05$); increased the content of triglycerides by 15.7% ($p \leq 0.05$), relative to the control.

The use of a probiotic B. amyloliquefaciens at a dose of 4×10^7 significantly increased protein metabolism: total protein by 6.1% ($p \leq 0.01$), albumin by 14.7% ($p \leq 0.01$), creatinine by 10.6% ($p \leq 0.01$) and reduced the amount of urea by 35% ($p \leq 0.05$), relative to the control. Administration of the probiotic B. amyloliquefaciens at a dose of 4×10^7 goats improved the indicators of carbohydrate-lipid metabolism - the amount of bilirubin decreased by 21.8% ($p \leq 0.05$), cholesterol by 13.7% ($p \leq 0.05$); the number of triglycerides increased by 17.5% ($p \leq 0.05$), relative to the control. The probiotic Bacillus Amyloliquefaciens VKPM V-11475 can be recommended to increase the adaptation parameters of young goats at the studied doses.

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