

Detoxification Effect of Soy Milk-Based Probiotic on Morphological and Biochemical Blood Parameters in Calves

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Abstract.

Perspective combined probiotics are preparations based on live and propionic acid bacteria. Bifidus bacteria are easily cultured by soy milk. Production of probiotics with pectin is based on using protective properties of the latter that allow reducing the content of heavy metals in the body. **The research aims** to study detoxification effect of the probiotic preparation based on soy milk acidified by bifidus and propionic acid bacteria with pectin additives on the analysis of morphological and biochemical blood parameters in calves. **The research methods.** The subject of research was black-pied calves up to 6 month age. By the analogue scale 30 selected calves were divided into three groups of 10 animals each. The digital data is processed statistically using information program Excel. **Research results.** Calves' diets had excess of zink, respectively 44 %. In addition in test calves' diets was found the availability of such heavy metals as lead 93,7 mg and cadmium – 7,01 mg. In the course of the experiment the content of erythrocytes and hemoglobin in calves' blood of the first and second test groups compared to their counterparts was higher respectively 0,39 and 0,55 $\times 10^{12}/l$, 3,9 and 5,0 g/l. Animal, the diets of which were enriched with probiotic preparation and pectin additives had more albumin and α -globulin indicating the increase of protective properties in their bodies. Due to pectin protective properties the probiotic preparation additives allowed to significantly ($P < 0.05$) lower in the animals' blood the zinc concentration by 62,2%, cadmium – by 63,7% and lead – by 56,7%. And the level of these heavy metals in their blood did not exceed the maximum permissible concentration (MPC).

Key words: calves, heavy metals, soy milk-based probiotic, pectin substances, detoxification, morphological and biochemical composition of blood.

Relevance of the topic. Soybean is a highly effective food means: it is rich in the complete protein, essential amino acids and energy that allows to ensure high productivity of farm animals. Moreover, it is possible to use either the whole grain or soybean meal and other soybean derivatives in animal feeding. In this case, it is more expedient to use grain of non-genetically modified soybean varieties in animal diets [1, 2, 3].

Soybean protein should be used in various cases, in particular to improve the consistency of fermented probiotic preparations produced by the reservoir method, when developing a range of lactose-free products, to increase the production of dairy products and young animal meat [4, 5, 6].

In order to maintain high consumer qualities of probiotic preparations, it is important to choose the right soybean variety in view of its functional properties, strictly follow the technology of producing soybean protein components — increase in protein concentration of the fermented milk product and decrease in caloric content [7, 8].

Currently, more promising combined probiotics are preparations based on live bifidus bacteria, propionic acid bacteria, etc. Bifidus bacteria, like other lactic acid microorganisms, are easily cultured on soy milk. At the same time, in symbiosis with other microorganisms, including propionic acid, these bacteria, when accepting in the gastrointestinal tract, secrete enzymes that increase digestibility and the utilization of feed nutrients [9].

Pectins are added to prevent the separation of the finished probiotic soy milk-based product, thus also reducing the competitive opportunity of selected potentially useful microorganisms among themselves. The production and preparation of probiotics with pectin additives is based on using the latter protective properties that allow increasing the physiological and biochemical body status and reducing the various toxicants content, including heavy metals [10].

The research aims is to study the detoxification effect of the soy milk-based probiotic preparation, fermented by bifido- and propionic acid bacteria and pectin additives on the analysis of morphological and biochemical composition of calves' blood under the excessive content of heavy metal salts in feeds.

The research methods. The experimental part of the work was carried out on a dairy farm of the collective farm "40 let Oktyabrya" in Mozdoksky District of the Republic of North Ossetia-Alania. The subject of research during the experiment was black-pied calves up to 6 month age. By the analogue scale 30 selected calves in view of their sex origin, age and live weight were divided into three groups of 10 animals each. The period of calves' rearing lasted 180 days. Scheme of scientific and production calves' tests is given in Table 1.

To study the morphological and biochemical parameters in the test calves at the age of 6 months the blood then stabilized with heparin was taken from jugular vein in the morning before feeding. Hematological studies were carried out according to generally accepted methods.

The digital material was processed statistically using the information program Excel from the current Microsoft office package.

Research results. In the course of the tests it was found that in order to more fully inactivate the antinutritive compounds of soybean products while simultaneous reducing electricity consumption the most effective is to

use the combined method of wet-heat soybean treatment unifying autoclaving and microwave treatment [11].

Soybeans treated by this method are dipped into a boiling 1,2-1,5% aqueous solution of sodium bicarbonate (Na_2CO_3) and are boiled for 4-10 minutes. Then the solution is drained and the beans are washed with hot water (75-90°C). After boiling and washing the beans are dipped into water with pH = 6,5-7, heated to 85-95°C. Soybeans are grinded in the whey to the fine state and are soaked in it for 20-30 minutes. To prepare the probiotic preparation, the soybean isolate was normalized, purified and pasteurized at 92°C with time-exposure 4-5 minutes. Samples of the soy milk-based probiotic preparation were prepared as follows: 3% starter from cultures mixture *Bifidobacterium bifidum* and *Propionibacterium shermanii* was added to the cooled milk in the ratio 1:1. At the same time while thoroughly mixing added 0,4% glucose solution, 0,01% vitamin C to the homogenate.

The process of fermentation in a thermostat at temperature of 36-37°C lasted 8-9 hours. Upon its completion, the clot was dense, without bubbles and with acidity of 80-82°T. The maturation of the probiotic preparation lasted 8 hours in the refrigerator at cooling temperature of 4-5°C. The finished product had the acidity 80-82°T. Soybean proteins, converting into soy milk, are characterized by high dispersity that is caused by their unstable system due to the large surface tension. Thereby the apple pectin additives in the amount of 0,12% by weight as a stabilizer contributed to decreasing in the volume of the precipitated dispersed phase to 2,2%, in the surface tension to 50×10^3 Pa/m and increasing in the kinematic viscosity to $5,0 \times 10^6$ m²/s.

The calves were fed 3 times a day, and their diets were balanced according to detailed feeding standards. The probiotic preparation was given to them proportionally with the mixture of concentrates during morning and evening feeding. Mainly, the calves' needs for energy and nutrients were met within physiological range. However, the calves' diets had the excess in zinc respectively by 44,0%. In addition to zinc such heavy metals as lead – 93,7 mg and cadmium — 7,01 mg were found in the test calves' diets.

Table 1 - Scheme of scientific and production tests

Groups	Feeding Features	
	Test	Main diet (MD)
1 - test	MD+2% for total food value soy milk-based probiotic preparation without pectin	
2 - test	MD+2% for total food value soy milk-based probiotic preparation with pectin additives	

n=10

Table 2 - Morphological parameters of calves' blood

Group	Parameters		
	Erythrocytes, $10^{12}/l$	Leukocytes, $10^9/l$	Hemoglobin, g/l
Control	5,14±0,23	6,47±0,10	106,3±3,7
1 test	5,53±0,33	6,50±0,17	110,2±4,9
2 test	5,69±0,32	6,38±0,31	111,3±5,2

n=3

Table 3 — Content of protein and protein fraction in calves' blood serum

n=3

Group	Total protein, g/l	Albumin,%	Globulins, %			G/R
			α	β	γ	
Control	71,2±0,61	51,1±0,21	14,9±0,58	12,7±0,48	21,3±0,55	1,05
1 test	74,9±0,52*	52,0±0,38*	13,8±0,38*	12,1±0,11	22,1±0,27	1,08
2 test	76,2±0,70*	52,5±0,36*	12,8±0,68*	11,7±0,17	23,0±0,66	1,10

*P<0,05

Blood, as a liquid body tissue, saturated with oxygen and various organic and mineral compounds with its inherent continuous movement along the animal body is an indispensable medium for the redox processes, various transformations and nutrients transportation to all organs and tissues in the body.

Erythrocytes perform an important function in providing tissues with oxygen, due to the hemoglobin presence in them, participate in the adsorption on the proper membrane of proteins, amino acids and lipids and their transportation to the body tissues.

The blood in calves of the 1 and 2 test groups contained more erythrocytes and hemoglobin by respectively 0,39 and 0,55 $\times 10^{12}/l$ and 3,9 and 5,0 g/l compared to the blood of the counterparts in the control group (P> 0,05) (Table 2).

The diets enriched with the probiotic preparation had no significant effect on the leukocytes content in the test calves' blood.

In the course of the experiment, a significant (P <0,05) increase in the total amount of protein in the blood plasma was observed in animals at the age of 6 months relative to the control in the 2nd test group by 5,0 g/l (Table 3)

It is known that blood plasma proteins and, first of all, albumins, serve as a source of protein production for various organs, as they participate in the nutrients transport. At the age of 180 days, the albumin-globulin index in the animals of the control group was 1,05. The parameters of

the protein ratio were not significantly higher in the calves of the experimental groups relative to counterparts from the control.

It was established that in the course of the experiment, test animals, diets of which were enriched with the probiotic preparation with pectin additives, at the age of 6 months had more albumins and α -globulins that indicates the increase in the protective properties of their body.

At the age of 6 months in the young cattle of the 2nd test group the total protein nitrogen significantly (P <0,05) increased, the amount of urea and ketone bodies in the blood plasma decreased relative to the control, respectively, by 1,9 and 3,2 mmol/l and 0,7 and 0,7 mmol/l (Table 4).

The dynamics of nitrogen fractions content in the blood at 6 months indicate the higher level of synthetic processes in the body of test calves. It should be noted that all morphological and biochemical blood parameters of young cattle in the compared groups were within the physiological range.

At the age of 180 days, the animals of the 2nd test group compared to the control had uncertain (P> 0.05) higher sugar index in their blood (Table 5).

The results of physiological studies in young cattle showed that their feeding with probiotic preparation at a dose of 2% for total food value with pectin improved the biochemical composition of their blood.

Table 4 - Content of nitrogen fractions in calves' blood, mmol/l

n=3

Group	Nitrogen				Urea	Ketone bodies
	Total	Nonprotein	Protein	Amine		
Control	1633±23	28,3±0,81	1597,6±19	7,1±0,44	39,8±1,1	4,8±0,44
1 test	1688±*17	26,9±0,92	1654,4±*21	6,7±0,40	38,1±1,3	4,4±0,37
2 test	1712±*27	25,1±0,77	1680,4±*28	6,5±0,38	37,9±1,4	4,1±0,32

*P<0,05

Table 5 - Biochemical parameters of calves' blood

n=3

Group	Sugar, mmol/l	Calcium, mmol/l	Phosphorus, mmol/l	Zinc, mg/kg (MPC=22)	Cadmium, mg/kg (MPC=0,05)	Lead, mg/kg (MPC=1,2)
Control	63,1±2,03	10,02±0,81	7,25±0,46	32,3 \square 0,11	0,11 \square 0,003	1,73 \square 0,04
1 test	64,3±1,87	10,12±0,79	7,37±0,42	27,5 \square 0,10*	0,08 \square 0,002*	1,57 \square 0,06*
2 test	65,1±1,93	10,21±0,83	7,43±0,51	13,2 \square 0,14*	0,04 \square 0,002*	0,75 \square 0,05*

*P<0,05

The increase in energy metabolism of ruminants depends largely on the ratio of easily soluble carbohydrates and protein in the diet. The probiotic preparation with pectin contributed to the more intensive and rapid ammonia formation, therefore, to increase the absorption of volatile fatty acids it is required the presence of easy fermented glucose formed in the rumen of the animals in the test groups by hydrolysis of starch and cellulose under the influence of cellulases, hemicellulases, amylases and pectinases produced by the rumen microflora. This process is accompanied by the increasing the sugar concentration in the blood.

For the normal skeleton formation the calves need a normal level of assimilating calcium and phosphorus in feeds. It is determined that the soy milk-based probiotic preparation both without pectin and with pectin substances had no negative effect on the blood serum saturation with these trace elements.

Of particular interest was studying the protective properties of the probiotic preparation both unstabilized and stabilized with apple pectin. Studying concentration of zinc, cadmium and lead in the calves' blood of the 1st test group indicates that the soy milk-based probiotic preparation without pectin has not high detoxification properties that are confirmed by exceeding their maximum permissible concentrations (MPC) in the liquid internal medium.

Owing to the pectin protective properties, the additives of the soy milk-based probiotic preparation allowed versus the control counterparts reliably ($P < 0.05$) to reduce in animals' blood the zinc concentration by 62,2%, cadmium - by 63,7% and lead by 56,7%. Moreover, the level of these heavy metals in the calves' blood of the 2nd experimental group did not exceed the MPC.

Discussion of the research results. Soybean proteins, converting into soy milk, are characterized by high dispersity, which is caused by their unstable system due to the high surface tension. In this regard, the apple pectin additives in the amount of 0,12% by weight as a stabilizer contributed to decrease the volume of the selected dispersed phase to 2,2% and the surface tension to 50×10^3 Pa/m as well as to increase the kinematic viscosity to $5,0 \times 10^6$ m²/s.

The concentration of volatile fatty acids (VFA) in the blood flowing off the stomach increases in proportion to its concentration in the rumen content, which in its turn is interrelated with the feed factors. The level of VFA concentration in the blood depends on the absorption rate, the metabolism intensity in the rumen wall and their concentration in the rumen content. Therefore, feeding the soy milk-based probiotic preparation with pectin substances positively affected the morphological and biochemical blood parameters.

Due to the high detoxification pectin properties, additives of the soy milk-based probiotic preparation allowed versus the control counterparts to reliably reduce in the animals' blood of the 2nd test group the concentration of zinc, cadmium and lead. Moreover, the level of these heavy metals in the calves' blood of the 2nd test group did not exceed the MPC.

CONCLUSION.

Feeding the soy milk-based probiotic preparation at a dose of 2% for total nutrition with pectin additives positively affects the morphological and biochemical parameters of calves' blood up to 6 months of age. And the heavy metals content in the animals' blood was below the maximum permissible concentrations.

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