

Preventive and Detoxicative Action of Probiotics on Metabolism and Consumer Quality of Broilers Meat

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Abstract.

Heavy metals contained in soil migrate to plants, then accumulate in the body of the poultry, and then enter the human gastrointestinal tract together with food, thereby disrupting metabolic processes. **Scope of the research** is to study the preventive and detoxifying effect of a soy milk based probiotic preparation fermented by bifido- and propionic acid bacteria with and without pectin substances additives on the organism of broiler chickens grown on rations with high content of heavy metals.

Methods.

Research objects: broiler chickens. Morphological and biochemical composition of blood in the poultry was studied using generally accepted methods. The amount of heavy metals was determined by the atomic-adsorption method. The data obtained were processed statistically using Microsoft Excel.

Results of the research.

The highest content of vitamins was observed in the II probiotic sample obtained by fermentation of soy milk with bifido- and propionic acid bacteria with apple pectin additives. This sample was 2.2 times more saturated with vitamin C than the I sample, with vitamin B1 - by 30.0%, and with vitamin PP - by 56.7%. The use of a probiotic with pectin additives in the third test group broiler diets made it possible to reduce the lead content in blood by 2.92 times, cadmium – by 1.89, and zinc - by 2.03 times. The use of apple pectin to stabilize structure of the II probiotic sample promoted an increase in the protective properties of the product, which ensured that the chickens of the 3rd test group had superiority in weight of the gutted carcass and slaughter yield. Feeding of the II probiotic sample allowed broilers of the 3rd test group to surpass the test analogs by the biological usefulness of meat. In the meat of the 3rd test group poultry there was observed a decrease in lead concentration by 3.1 times, cadmium – by 2.0, and zinc – by 2.1 times. None of these elements exceeded the maximum permitted concentrations (MPC) in the chicken meat of this group.

Keywords: probiotic, apple pectin, heavy metals, detoxification, broilers, morphological and biochemical composition of blood, ecological and consumer qualities of meat.

INTRODUCTION

The pollution of the environment by salts of heavy metals becomes one of the most acute problems of ecology and protection of human health. The greatest danger of the action of heavy metals is the gradual increase in their concentration in the food chain. Heavy metals contained in soil migrate to plants (feed), then accumulate in the poultry body, enter the human gastrointestinal tract together with

food, and gradually accumulate in organs and tissues, thereby disrupting metabolic processes.

The most dangerous heavy metals are the compounds of mercury, zinc, cadmium, lead, many of which even in small quantities are extremely toxic to poultry and humans. The main source of entry of these heavy metals into the poultry body is formulated feed (about 85%), and 2-3% enters with water. The risk of contamination of poultry products by

these toxicants can be reduced at all stages of production, including by improving the ecology of nutrition [1, 3, 4].

Proceeding from this, it is necessary to find ways and means to prevent their accumulation in the body. The removal of heavy metals from it is possible with strict control over the ecological safety of feeds and the use of technological methods that reduce the degree of their absorption and accumulation in the body of meat poultry [3].

In poultry farming, probiotic preparations are increasingly used as curative and prophylactic agents for gastrointestinal diseases to increase the secretory activity of the stomach and intestines, and in case of stresses. When creating probiotics for therapeutic and prophylactic use, it is recommended to use uniform methods of breeding production strains selection by the criterion of antagonistic activity in the conditions of mixed populations cultivation [4].

Especially promising probiotics are preparations based on living bifidobacteria, propionic acid bacteria, etc. Bifidobacteria are especially easily cultivated on soy milk. At the same time, in symbiosis with other microorganisms, including propionic acid ones, these bacteria, when ingested in the gastrointestinal tract, discharge enzymes that increase the digestibility and assimilability of feed nutrients. However, when soybean milk is fermented with various strains of bifido- and propionic acid bacteria, the final product can be separated into liquid and solid fractions. Therefore, pectins are added as a stabilizer to the finished probiotic product, which makes it possible to reduce the possibility of competition between the selected useful microorganisms among themselves. Pectins are excellent adsorbents capable of removing heavy metals from the poultry gastrointestinal tract [2, 5].

Scope of the research is to study the preventive and detoxifying effect of a soy milk based probiotic preparation fermented by bifido- and propionic acid bacteria with and without pectin substances additives on the organism of broiler chickens grown on rations with high content of heavy metals.

MATERIALS AND METHODS OF RESEARCH.

In the course of the scientific and economic test in the conditions of the Mikhailovskoye Poultry Farm in the Prigorodny district of the Republic of North Ossetia-Alania, four groups of 100 heads each were formed from the Cobb-500 day old cross chicks using the analog groups

method. The experimental poultry was fed under the scheme given in Table 1.

The content of heavy metals in feed samples, blood and broiler meat was determined by the atomic adsorption method using the AAS-115-M1 spectrophotometer.

Morphological and biochemical composition of blood in experimental poultry was studied using generally accepted methods.

After reaching the 42-day-old age, a control slaughter of the experimental chickens was performed according to the standard method. The chemical composition was studied in the samples of the pectoral and femoral muscles of broilers. Protein-quality indicator (PQI) of meat was calculated from the ratio of tryptophan to hydroxyproline.

The data obtained were subjected to statistical processing using a personal computer with the *Microsoft Excel* program.

RESULTS

Two samples of the probiotic preparation were prepared as follows: 3% of the culture ferments, including the *Bifidobacterium bifidum* and *Propionibacterium shermanii* live cultures, were introduced in the soy milk at the ratio 1: 1, with simultaneous addition of a glucose solution in the amount of 0.4%, vitamin C - 0.01% by weight, thoroughly mixing. At the same time, pectin substances were not added to the 1st sample, and apple pectin was added in an amount of 1.2% by weight to the 2nd sample as a stabilizer of the probiotic preparation structure, which contributed to a decrease in the volume of the precipitated dispersed phase to 2.2% and surface tension to 50×10^3 Pa/m.

Taking into account the need to make comparative assessment of the use of bifido- and propionic acid bacteria, both individually and in combination, it was expedient to study the microbiological and physicochemical properties of two samples of the finished probiotic (Table 2).

In terms of density, acidity, protein, fat and carbohydrate content, no differences were observed in the probiotic samples compared. However, the highest content of vitamins was observed in the II preparation sample obtained by fermentation of soy milk with bifido- and propionic acid bacteria with apple pectin additives. This sample was 2.2 times more saturated with vitamin C than the I sample, with vitamin B₁ - by 30.0%, vitamin B₂ - by 58.3%, and vitamin PP - by 56.7%.

Table 1. Scheme of scientific and economic tests

Group	Feeding features
Control	Basic diet (BD)
1st test group	BD + 2% by weight of feed - soy milk based probiotic preparation fermented with bifido- and propionic acid bacteria, not stabilized with pectin
2nd test group	BD + pectin in a dose of 0.05% by feed weight
3rd test group	BD + 2% by weight of feed - soy milk based probiotic preparation fermented with bifido- and propionic acid bacteria, and stabilized with apple pectin

Note: n=100

Table 2. The content of bifido- and propionic acid bacteria and the physicochemical parameters of the probiotic preparation

Index	I sample	II sample
Bifidobacteria, lg CFU/cm ²	10 ¹⁰	10 ¹¹
Propionic acid bacteria, lg CFU/cm ²	10 ⁹	10 ¹⁰
Clot syneresis degree, %	1.5	1.5
Acidity, T	80-82	80-82
Density after 10 days of storage, kg/m ³	42	42
Mass fraction of protein, %	5.8	5.8
Mass fraction of fat, %	1.5	1.5
Mass fraction of carbohydrates, %	4.3	4.3
Vitamins after 10 days of storage, mg/100 g:		
B ₁	0.20	0.26
B ₂	0.12	0.19
PP	0.37	0.58
C	0.18	0.40

According to the content of bifido- and propionic acid bacteria in the compared samples of the finished product, it is possible to say about the absence of competition between them. However, when they are cultivated on soy milk using apple pectin in the II preparation sample, the content of bifido- and propionic acid bacteria was an order of magnitude higher.

At the Mikhailovskoye Poultry Farm in the Republic of North Ossetia-Alania, a two-phase type of chickens feeding with dry full-fat mixed formulated feeds is used: in the I phase (age 1-28 days) according to the PK-5 formulation, and in the II phase (age 29-42 days) - according to the PK-6 formulation. But at the same time, because of the high intensity of metabolism, stricter requirements on the ecological safety of complete formulated feeds are set to the rations of broiler chickens.

Taking into account the fact that the territory of the Prigorodny District of the Republic of North Ossetia-Alania, where this enterprise is located, belongs to the zone with a high level of heavy metal contamination due to the close proximity of the large non-ferrous metallurgy enterprises, such as Electrozinc JSC and Pobedit JSC, we studied the content of zinc, cadmium and lead in the composition of mixed formulated feeds (Table. 3).

It was established that for all three elements, the MPC in the mixed formulated feeds used in the two feeding phases was exceeded: by zinc - by 49.3 and 39%, by cadmium - by 32.5 and 22.5%, and by lead - by 14.2 and 10.6%, respectively.

In general, due to the above formulations of feed in both phases of cultivation, the needs of broiler chickens were satisfied in accordance with the existing feeding standards. Biologically active feed additives have a stimulating effect on hematopoietic functions in the poultry body, as well as on the level of intermediate metabolism. Based on this, we conducted a morphological study of blood of the experimental poultry (Table. 4).

The results of the experiment indicate that a more pronounced stimulating effect on the hemo- and erythropoiesis of broiler chickens was provided by the addition of a probiotic enriched with apple pectin in an amount of 2% by feed weight. Therefore, in the poultry of the 3rd test group relative to the control analogues, the content of hemoglobin and erythrocytes in blood was 4.00 g/l ($P>0.05$) and $0.37 \cdot 10^{12}/l$ ($P>0.05$) higher.

Table 3. The content of heavy metals in the experimental poultry formulated feeds

Feeding phase	Zinc, mg/kg	Cadmium, mg/kg	Lead, mg/kg
MPC	100.0	0.4	5.0
I (age of chickens - 1-28 days)	149.3	0.53	5.71
In % of MPC	149.3	132.5	114.2
II (age of chickens - 29-42 days)	139.0	0.49	5.53
In % of MPC	139.0	122.5	110.6

Table 4. Morphological composition of experimental poultry blood

Indicators	Group			
	Control group	1st test group	2nd test group	3rd test group
Hemoglobin, g/l	80.9±0.34	84.2±0.31*	82.2±0.28	84.9±0.43*
Erythrocytes, 10 ¹² /l	3.51±0.18	3.81±0.16*	3.62±0.21	3.88±0.22*
Leukocytes, 10 ⁹ /l	8.92±0.48	9.04±0.52	8.78±0.44	9.12±0.39

Note: n=5

* $P<0.05$

The amount of leukocytes in the blood of the poultry of the compared groups during all scientific and economic experiments was almost identical and corresponded to physiological parameters.

In conditions of feed contamination with heavy metals, their toxic effect impacts primarily the biochemical indicators of blood. To reduce the risk of intoxication of the body of broiler chickens by heavy metal salts, the use of feed additives with high adsorption properties is required. With the removal of these toxic substances from the body, the indicators of intermediate metabolism in meat poultry stabilize.

Proceeding from this, it was of interest to study the effect of a probiotic soy milk based preparation, both with and without pectin additives, on certain biochemical indicators of the blood of broiler chickens of the groups being compared (Table 5).

According to the chemical analysis of blood serum, it was found that under the influence of the additives of the probiotic preparation stabilized with pectin, the content of

total protein, calcium and inorganic phosphorus in the blood serum was the highest in the chicks of the 3rd test group, exceeding by these indicators the control by 4.7 g/l, 2.39 and 0.48 mmol/l, respectively.

The level of heavy metals in the blood of the control group of poultry was higher than the MPC. Exceeding the MPC by the lead concentration was by 1.29 times, cadmium – by 1.36 and zinc – by 1.19 times. The use of a probiotic with the apple pectin additives in the 3rd test group broiler diets made it possible to reliably ($P<0.05$) reduce the lead content in their blood by 2.92 times, cadmium – by 1.89 and zinc – by 2.03 times relative to control analogues. Moreover, their concentration in the blood of the 3rd test group poultry blood was significantly lower than the MPC in all cases.

After reaching the age of 42 days, a control slaughter was conducted, the results of which were used to study the slaughter performance of the experimental broilers, for which 5 heads have been selected from each group (Table 6).

Table 5. Some biochemical indicators of the blood of broiler chickens

Index	Group			
	Control group	1st test group	2nd test group	3rd test group
Total protein, g/l	74.4 ± 0.22	78.3 ± 0.22	75.7 ± 0.22	79.1 ± 0.22
Calcium, mmol/l	12.73 ± 0.07	14.77 ± 0.13*	13.81 ± 0.04*	15.12 ± 0.15*
Phosphorus, mmol/l	5.35 ± 0.04	5.76 ± 0.04*	5.47 ± 0.06	5.83 ± 0.04*
Lead, mg/kg	1.55 ± 0.04	1.44 ± 0.07	0.74 ± 0.06*	0.53 ± 0.05*
Cadmium, mg/kg	0.068 ± 0.005	0.060 ± 0.004	0.047±0.004*	0.036±0.008*
Zinc, mg/kg	26.10 ± 0.28	25.12 ± 0.33	15.12 ± 0.17*	12.88 ± 0.28*

Note: n=5

* $P<0.05$

Table 6. Slaughter performance for the experimental poultry

Index	Group			
	Control group	1st test group	2nd test group	3rd test group
Pre-slaughter weight, g	1,907±11.4	2,158±10.2*	2,076±10.0*	2,201±13.3*
Weight of half-gutted carcass, g	1,569±10.9	1,840±13.1*	1,741±12.0*	1,903±11.2*
In % to live weight	82.3±0.18	85.3±0.14*	83.9±0.17*	86.5±0.26*
Weight of gutted carcass, g	1,239±8.8	1,441±9.5*	1,362±7.8*	1,486±9.9*
Slaughter yield, %	65.0±0.15	66.8±0.11*	65.6±0.11*	67.5±0.19*

Note: n=5

* $P<0.05$

Table 7. Chemical composition of the femoral and pectoral muscles of broiler chickens

Index	Group			
	Control group	1st test group	2nd test group	3rd test group
Femoral muscle				
Dry matter, %	23.52±0.04	23.98±0.02*	23.78±0.02*	24.00±0.05*
Protein, %	19.99±0.04	20.48±0.03*	20.36±0.03*	20.52±0.06*
Fat, %	2.52±0.03	2.27±0.02*	2.37±0.01*	2.25±0.02*
Pectoral muscle				
Dry matter, %	26.22±0.03	27.62±0.02*	26.83±0.02*	27.60±0.04*
Protein, %	22.63±0.02	23.25±0.03*	22.84±0.01*	23.32±0.04*
Fat, %	2.30±0.03	2.01±0.02*	2.20±0.01*	2.03±0.03*
Tryptophan, %	1.52 ± 0.002	1.70 ± 0.004*	1.61 ± 0.002*	1.74± 0.003*
Oxyproline, %	0.37 ± 0.008	0.34 ± 0.003	0.35 ± 0.004	0.33 ± 0.010
PQI	4.11 ± 0.04	5.00 ± 0.07*	4.60 ± 0.012*	5.30 ± 0.03*

Note: n=5

* $P<0.05$

Table 8. The content of heavy metals in the pectoral muscle of chickens, mg/kg

Index	Group			
	Control group	1st test group	2nd test group	3rd test group
Lead	1.50 ± 0.02	1.47 ± 0.03	0.68 ± 0.05*	0.49 ± 0.03*
Cadmium	0.077 ± 0.003	0.071 ± 0.004	0.048 ± 0.003*	0.039 ± 0.002*
Zinc	23.79 ± 0.40	23.63 ± 0.22	13.70 ± 0.27*	11.44 ± 0.19*

Note: n=5 *P<0.05

The use of the apple pectin to stabilize the structure of the II sample of the probiotic preparation promoted an increase in the protective properties of the product, which ensured a significant (P<0.05) superiority in the weight of the half-gutted carcass by 21.3%, the gutted one - by 19.9%, and slaughter yield - by 2.5% in chickens of the 3rd test group against the control group.

The consumer quality of poultry meat was assessed by the chemical composition of the femoral and pectoral muscles (Table 7).

The use of a sample of a probiotic obtained by fermentation with bifido- and propionic acid bacteria and stabilized with apple pectin, in the diet of chickens in an amount of 2% by feed weight, considerably improved the chemical composition of the poultry meat compared to the use of these cultures alone. Therefore, the poultry of the 3rd experimental group had a significant (P<0.05) superiority over the control by concentration of dry matter in the pectoral and femoral muscles by 1.38 and 0.48%, and protein by 0.69 and 0.53%.

To study the consumer qualities of the poultry meat, the PQI of the pectoral muscle was calculated as the most biologically valuable compared to the remaining muscles. Feeding of the probiotic stabilized with apple pectin in an amount of 2% by weight of the feed allowed the broilers of the 3rd test group to reliably (P<0.05) exceed the control analogs by 14.5% in tryptophan content and by 28.9% in the PQI value.

In the context of a disturbance in the nutrition ecology, it was important to assess the detoxification potential of the studied probiotic specimens, which are determined by the level of heavy metals in the pectoral muscle of the experimental poultry (Table 8).

The most favorable effect on the ecological safety of the experimental poultry meat was provided by the additives of the probiotic preparation sample obtained by fermentation with bifido- and propionic acid bacteria and stabilized with apple pectin. Due to this, in the course of relatively control analogues in the meat poultry of the 3rd experimental group, a significant (P<0.05) decrease in the lead concentration by 3.1, cadmium – by 2.0 and zinc – by 2.1 times was observed. None of these elements exceeded the MPC in chicken meat of this group.

DISCUSSION

Bifido- and propionic acid bacteria differ in good intestinal engraftability, therefore, the antagonistic activity of *Bifidobacterium bifidum* and *Propionibacterium shermanii* strains, which are part of a probiotic stabilized with apple pectin, was studied by the perpendicular strokes method. The results of the studies indicate that there is no competition between them. In addition, these strains enrich

the probiotic with a wide range of enzymes, vitamins, essential amino acids and organic acids that improve the processes of digestive metabolism. Pectin is used as a stabilizer for the protection and separation of various types of soy protein.

In the course of the experiment it has been found that feeding of probiotic preparation, both with pectin and without pectin, has a beneficial effect on the hematopoietic function of the broiler chicken organism and on the biological usefulness of poultry meat. However, the preparation enriched with pectin improves the consumer properties of broiler meat. We believe that the increase in the PQI of the meat was achieved due to the intensification of tryptophan deposition in the pectoral muscle of broilers, since propionic acids, and especially bifidobacteria, are active producers of tryptophan.

The probiotic preparation without pectin additives has almost no detoxification properties. With the addition of pectin as a stabilizer, the probiotic preparation removes heavy metal ions from the digestive channel by adsorbing them on the pectin surface. The carbon chain of pectin ends with D-galacturonic acid, which forms strong compounds from heavy metals that are not absorbed through the mucous membrane.

CONCLUSION.

On the basis of the experimental data obtained, we believe that in order to intensify the metabolism and to increase the ecological and consumer properties of the poultry meat in broiler diets with an increased background of heavy metals, the soy milk based probiotic preparation obtained by fermentation with bifido- and propionic acid bacteria and stabilized with apple pectin, in an amount of 2% by feed weight, should be included as a prophylactic and detoxifying feed additive.

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