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The Effects of a Probiotic Dietary Supplementation on the Amino Acid and Mineral Composition of Broilers Meat

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AS, DK and MR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SK, VK, ZY and MK managed the analyses of the study. Authors AA, EO and OB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Commercially available probiotic "Vetosporin Active" was used in chicken broilers feed as an additive. The study was carried out to evaluate the effect of a dietary probiotic supplementation on the amino acid and mineral composition of broilers meat. One control and three experimental groups of chicken broilers were fed for 42 days where for II, III and IV experimental group the "Vetosporin

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Active” probiotic additive has been added at the level of 0.5, 1.0 and 1.5 g/kg to feed for broilers. The broiler meat from III experimental group was more nutritious being richer in protein (21.2%), while similar protein content was detected in I and II groups (20.2% and 20.3%, respectively) and III and IV groups (21.2% and 21.0%, respectively). Significant reducing of fat amount was in III group (up to 3.9% comparing with 4.7% in the meat of I group). The analysis revealed differences in the amino acid composition of broilers meat. The sum of essential amino acids was highest in III group. Thus, the amount of leucine and isoleucine in III group was higher to 1.8% comparing to I control group. However, the methionine content was lower in III group than in other groups. The mineral content is higher in samples of meat from II, III and IV groups comparing with I control group. The concentration of calcium in the samples from III group was higher to 4.6 mg, 1.9 mg and 1.4 mg of those samples from I, II and IV group, respectively.

On the contrary summarized evaluation was done to find out whether the amino acid or other mineral contents are significant in the broiler meat or not, rather if it is not sufficient enough to assure the values, then further processing of the meat in needed to be done with much professional attitude. Diets of the animals also have to be improved enough so that all the animals could ensure the required dietary need.

Keywords: Chicken broilers; meat; probiotic additive; amino acid; mineral.

1. INTRODUCTION

Poultry industry has a strong market position and is a fast developing and science-based industry. This industry provides population with highly nutritional food products rich in highly soluble proteins, fats, including polyunsaturated fatty acids and mineral elements.

Under existing conditions of increasing poultry meat products consumption the demands on manufacturers to the quality of poultry meat are growing. However, violation of poultry sanitation standards, cleaning and disinfecting of poultry facilities, process flow disruption and disturbances of process conditions lead to the lowering of the quality of broiler meat [1,2]. Also, the quality of the feed has large impact on the meat quality [3].

Feed quality is a major measure for evaluating the broiler performance and the nutritional value of feed should be sufficient for animal feeding. Vegetable raw materials, food by-products, grain crops, probiotics, prebiotics etc. have been cited as potential approaches for enhancing the nutritional value [4-9].

In recent time much attention in agriculture is given on use of probiotics in animal and bird diet. Probiotics are the biopreparations which represent an established culture of microorganisms or enzymes [10]. The main effects of probiotic using are restoration or improvement of digestive processes, gastrointestinal tract disease prevention, and strength immune system [11].

Use of probiotics in poultry nutrition helps in growth of beneficial microflora. In gastroenteric tract probiotics bind with epithelial cells of stomach and bowels and help to synthesize the vitamins, amino acids which result in improving the digestibility of feed and increasing the live weight of chicken broilers [12]. Moreover the probiotics participate in decontamination of food and water from the toxin compounds [13].

The purpose of this study was to determine the amino acid and mineral composition of meat from chicken broilers fed with a probiotic dietary supplementation.

2. MATERIALS AND METHODS

Four hundred one-day old broilers were allocated into four groups, each with 100 animals. The first group (I) was the control, fed with a diet without the addition of the probiotic. For II, III and IV groups the “Vetosporin Active” probiotic additive was incorporated in the diet at the level of 0.5, 1 and 1.5 g/kg, respectively.” Each group was divided to four subgroups with 25 broilers and we detected the chick livability in each subgroup then calculated the average rate for one group.

The “Vetosporin Active” probiotic additive was developed by “BashInkom” Company (Ufa, Russia). It consists of *Bacillus subtilis* (*Bacillus subtilis 11 B* and *Bacillus subtilis 12B*) live microorganisms which were incorporated into the activated carbon particles and is an odor-free flowing powder of black color. One gram of probiotic additive contains at least 10^9 CFU of each bacterial species.

The experiment lasted 42 days. The “Vetosporin Active” probiotic additive was gradually added and mixed with the animal diet. The live condition and the management of broilers were similar among the experimental groups.

2.1 Amino Acid Determination

Liquid chromatography was used to quantify amino acids. The instrument used was a “Shimadzu LC-20 Prominence” liquid chromatography system (Shimadzu, Japan) equipped with fluorometric and spectrophotometric detectors. The chromatographic column used was SUPELCO C18, 5 µm (Sigma-Aldrich, USA) offering a surface area of 200 $m^{2/}$g. The chromatographic analysis was performed under a linear gradient with an eluent flow rate of 1.2 mL/min, and the column was heated in an oven at 400℃. Amino acids were detected using fluorometric and spectrophotometric detectors at wavelengths of 246 nm and 260 nm following acidic hydrolysis and treatment with a phenylisothiocyanate solution in isopropyl alcohol to give phenylthiohydantoins [14]. Identification and estimation has been performed with comparing to amino acid standard solution (AAS18 Sigma-Aldrich Denmark A/S, Brondby, Denmark) and plotting the calibration curve.

2.2 Mineral Elements Determination

The content of elements in muscle samples was determined with the inductively coupled plasma–mass spectrometric method (ICP-MS, Varian-820 MS, Varian Company, Australia). The method was validated with certified reference materials. Calibration standards Var-TS-MS, IV-ICPMS-71A (Inorganic Ventures Company, USA) were used for calibrating the mass-spectrometer. The sensitivity of the mass-spectrometer was tuned up using a diluted calibration solution Var-TS-MS with concentration of Ba, Be, Ce, Co, B, Pb, Mg, Tl, Th of 10 µg/L. Three calibration solutions were used for the detector calibration. They were IV-ICPMS-71A of Cd, Pb, Cu, Zn elements diluted to 10, 50 and 100 µg/L. Discrepancies between the certified values and concentrations quantified were below 10%. The operating parameters of the inductively coupled plasma mass spectrometer Varian ICP 820–MS were as follows: plasma flow 17.5 L/min; auxiliary flow 1.7 L/min; sheath gas 0.2 L/min; nebulizer flow 1.0 L/min; sampling depth 6.5 mm; RF power 1.4 kW; pump rate 5.0 rpm; stabilization delay 10.0s [15]. All analyses were performed in triplicate, and the results are presented in Table 3 as the means of measurements expressed in mg/kg wet weight.

2.3 Statistical Analysis

Statistical analysis was performed using Statistica 12.0 (STATISTICA, 2014; StatSoft Inc., Tulsa, OK, USA). The differences between samples were evaluated using ANOVA method. The differences were considered to be statistically significant at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

The chemical composition of feed with a dietary probiotic supplementation was 23.0%, 6.5% and 2.54% respectively for crude protein, fat and crude fiber. From our previous study [7] the chicken-broilers from the experimental group that was fed with this feed had higher live weight than those from the control group. The live weight of broilers at the 7th day of age was 176.8, 177.8 and 175.9 g for IV, III and II groups against 150.7 g in control group. With advancing age of chicken-broilers (on day 28 of age) the weight gain in III, IV and II groups were higher to 118.9 g, 96.8 g and 83.8 g comparing with control group. The superiority of the live weight of the chickens of III test group, compared to the control group, at the age of 42 days was 277.1 g, in IV group - 236.1 g, in II group - 197.5 g. Thus, the addition of “Vetosporin Active” probiotic additive at the level of 1 g/kg resulted in the highest live weight of broilers. Increase of probiotic additive level to 1.5 g/kg did not have an additional positive effect [7].

The nutritional value of meat is defined by its chemical composition. The chemical composition of 42-day broilers meat was presented in Table 1.

It is evident from Table 1 that meat from III group of broilers was richer in protein. The moisture content had been decreased gradually in II, III and IV groups comparing with I control group and there was a negative quadratic effect ($y = 0,4x^2 - 1,08x + 74,6$, $R^2 = 0,993$). Thus, the moisture content in I group was 74.6% while in III and IV groups – 73.9%. Similar protein content was detected in I and II groups (20.2% and 20.3%, respectively) and III and IV groups (21.2% and 21.0%, respectively). Addition of different amount of probiotic supplementation to feed formulation had positive linear effect on protein content ($y = -0,3x^2 + 1,11x + 20,10$, $R^2 = 0,758$).

Table 1. Chemical composition of broiler meat, %

Index	Group*			
	I – control	**II**	**III**	**IV**
Moisture	74,6±0,11	74,2±0,60	73,9±0,96	73,9±0,11
Protein	20,2±0,21	20,3±0,30	21,2±0,58	21,0±0,23
Fat	4,7±0,10	4,6±0,12	3,9±0,08	4,1±0,10
Ash	0,97±0,013	0,94±0,009	0,98±0,014	0,95±0,017

***** *I control group – feed without probiotic additive; II, III and IV experimental groups – feed with the probiotic additive at the level of 0.5, 1.0 and 1.5 g/kg, respectively*

Significant reducing of fat amount was in III group (up to 3.9% comparing with 4.7% in the meat of I group) ($y = 0,3x^2 - 0,95x + 4,775$, $R^2 = 0,748$).

Similar research has been performed by others. For example, Hidayat et al. [16] studied the effect of liquid turmeric extract (LTE) on chemical composition of broiler meat, where the addition of 2% to 10% of LTE did not significantly change the protein content with range of 21.49% to 23.94% and fat content with the range of 1.41% to 2.21%. Ignatyev et al. [17] studied the effect of probiotic preparation “Monosporin” to the biochemical characteristics of broiler meat. Use of this preparation in the diet of chicken results in an increase of protein content up to 21.3% comparing with control group (20.93%). Abdullabekov [18] found that the content of protein in the breast meat of broilers fed with feed containing grape squeeze powder (up to 4%) varied from 21.03% to 22.89 within the experimental group and fat content – from 3.32% to 4.02%. Burayev et al. [19] reported that the content of protein in breast meat was 19.6%, fat – 6.59% and ash was 0.99% in poultry which fed with a silicon containing feed additive.

It is known, that the nutritional value of the broiler meat is characterized not only by the protein content but its amino acid composition. The amino acid composition of the broiler meat is presented in Table 2. Herewith, the high amounts of amino acid were lysine, leucine, isoleucine

Table 2. Amino acid composition of broiler meat, %

Amino acid	Group+			
	I - control	**II**	**III**	**IV**
Essential:				
Lysine	4,5 ± 0,06	5,0 ± 0,08*	5,1 ± 0,10*	5,2 ± 0,07**
Phenylalanine	3,3 ± 0,02	3,4 ± 0,06	3,9 ± 0,05**	3,5 ± 0,03
Leucine+Isoleucine	7,8 ± 0,12	8,0 ± 0,15	9,6 ± 0,12***	9,2 ± 0,14*
Methionine	2,3 ± 0,04	2,2 ± 0,02	2,1 ± 0,04*	2,4 ± 0,05
Valine	5,8 ± 0,14	5,9 ± 0,11	5,9 ± 0,15	5,9 ± 0,17
Threonine	3,2 ± 0,07	3,2 ± 0,09	3,3 ± 0,06	3,2 ± 0, 05
Tryptophan	1,12±0,011	1,28±0,017**	1,35±0,012**	1,33±0,016***
Sum of essential	28,02	28,98	31,25	30,73
Non-essential:				
Arginine	5,9 ± 0,16	5,7 ± 0,15	5,2 ± 0,16**	5,5 ± 0,19
Tyrosine	2,6 ± 0,06	2,3 ± 0,02**	2,0 ± 0,04***	2,2 ± 0,02**
Histidine	4,4 ± 0,13	4,3 ± 0,08	4,5 ± 0,09	4,5 ± 0, 11
Proline	3,4 ± 0,09	3,3 ± 0,05	3,1 ± 0,07*	3,2 ± 0,08
Serine	4,7 ± 0,07	4,4 ± 0,06	4,1 ± 0,08**	4,1 ± 0,05**
Alanine	5,5 ± 0,09	5,3 ± 0,05	5,2 ± 0,07	5,2 ± 0,03
Glycine	7,5± 0,18	7,6± 0,19	7,4 ± 0,14	7,7± 0,17
Cystine	1,7 ± 0,05	1,6 ± 0,03	1,6 ± 0,04	1,6 ± 0,02
Oxyproline	0,29±0,006	0,28±0,008	0,26±0,004	0,27±0, 007
Sum of non-essential	35,99	34,78	33,36	34,27
Total amino acids	64,01	63,76	64,61	65
Tryptophan to oxyproline ratio	3,86	4,57	5,19	4,92

+ *I control group – feed without probiotic additive; II, III and IV experimental groups – feed with the probiotic additive at the level of 0.5, 1.0 and 1.5 g/kg, respectively*

** - P< 0,05; ** - P< 0,02; *** - P< 0,001*

Table 3. Mineral composition of broiler meat

Mineral	Group+			
	I-control	II	III	IV
Macroelement, mg:				
Potassium	206,4±4,02	231,5±4,36*	259,0±4,54***	247,0±4,48**
Calcium	11,1±0,15	13,8±0,18***	15,7±0,12***	14,3±0,16***
Magnesium	17,9±0,14	17,4±0,12	16,5±0,16	16,7±0,15
Sodium	78,3±2,12	80,0±2,20	83,9±2,46	82,3±2,32
Phosphorous	134,4±3,96	142,6±3,82	143,0±3,92	142,8±3,78
Microelement, µg:				
Iron	1356,2±5,74	1394,0±5,80	1472,8±5,96	1416,3±5,76
Cobalt	7,0±0,14	7,6±0,17	7,9±0,18*	7,8±0,17**
Manganese	12,1±0,17	11,6±0,11	11,0±0,15*	11,1±0,18
Copper	64,2±1,54	62,2±1,57	58,0±1,53**	59,8±1,51
Zinc	1125,2±5,24	1115,3±5,17	1109,1±5,03	1118,4±5,21

+ I control group – feed without probiotic additive; II, III and IV experimental groups – feed with the probiotic additive at the level of 0.5, 1.0 and 1.5 g/kg, respectively.
** - P< 0,05; ** - P< 0,02; *** - P< 0,001*

and valine. Protein quantity and quality was determined by the ratio of tryptophan to oxyproline, the high ratio means high biological value of the protein.

In general, the sum of essential amino acids was highest in III group. Thus, the amount of leucine and isoleucine in III group was higher to 1.8% comparing to I control group. However, the methionine content was lower in III group than in other groups.

The sum of essential amino acids were higher in I control group than in others. Thus, arginine, tyrosine, proline, serine, alanine, cystine and oxyproline contents were higher in I control group than in others. However, the content of histidine was 4.5% in III and IV groups higher than in the meat samples from I and II groups. The same situation was observed for glycine amino acid. The ratio of tryptophan to oxyproline was 5.19 in III group which was highest value between all other groups.

The nutritional quality of meat also includes the mineral composition. Table 3 shows the mineral composition of broiler meat.

These data demonstrate that mineral content is higher in samples of meat from II, III and IV groups comparing with I control group. Among the studied group the highest concentration of mineral elements was observed in III group, where the chicken broilers diet included the probiotic feed supplement “Vetosporin-Activ” of 1 kg per 1000 kg of total feed. Thus, the concentration of calcium in the samples from III group was higher to 4.6 mg, 1.9 mg and 1.4 mg of those samples from I, II and IV group, respectively.

However the concentration of magnesium in control group was highest (17.9 mg) comparing with studied groups, where the magnesium content in II group was 17.4 mg, III group – 16.5 mg, IV group – 16.7 mg.

The feed with probiotic supplement in chicken broilers nutrition had a positive effect to iron content in the meat. The iron content was more in meat from experimental group than from the control group, where the highest concentration was detected in the meat of III group (1472.8 µg), which was higher to 9% than in control group (1356.2 µg).

These mineral elements play a vital role in chicken broilers body. Thus, iron incorporated in hemoglobin synthesis, oxidation-reduction process, zinc participates in the processes of bone formation, metabolism of nucleic acids and synthesis of proteins, shaping of eggshell [20]. Potassium controls the water-salt balance in chicken body, while manganese assists to oxidation processes and participates in fat splitting [21].

4. CONCLUSION

Therefore, based on the obtained results stated above the “Vetosporin-Active” probiotic feed supplements at the amount of 1 g/kg had a positive effect to the nutritional value of the broiler meat which depends on its chemical, amino acid and mineral compositions. Besides a significant increase in the number of essential amino acids, there was a significant increase in the content of calcium, potassium, iron, sodium in the meat of chicken broilers from III experimental group.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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