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THE EFFECT OF HERBAL SUPPLEMENTS ON DEVELOPMENT OF INTERNAL ORGANS AND CHEMICAL COMPOSITION OF BROILERS MUSCLES

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Keywords: *phytobiotics, antibiotics, essential oils, safe growth stimulants, feed additive, meat characteristic*

Abstract

The present article presents data on effectiveness of adding a phytobiotic feed additive into the diet of broiler chickens, either additionally or replacing the feed antibiotic in the chicken fodder. It has been established that the introduction of a phytobiotic feed additive into the broilers' diet, both additionally and by replacing the feed antibiotic, provided positive effect on poultry meat quality and gave no negative effect on development of internal organs. By the end of fattening the relative weight of heart, lungs, kidneys, gizzard and intestines in broilers, which consumed the antibiotics-free diet with addition of researched preparation, was higher than in the control group and in the 1st experimental group. At the same time the length of the intestine in researched group significantly exceeded the control group parameters. These changes ranged within the physiological norm, which may indicate the best detoxification capabilities of the chicken body and the activation of enhanced intestinal absorption function. Additional use of the experimental feed additive in formulation of feed for broiler chickens was accompanied by a decrease in total amount of amino acids in broilers' pectoral and leg muscles within acceptable physiological limits. At the same time, a significant decrease, compared with the control parameters, was noted in relation to content of histidine and isoleucine in pectoral muscles, and content of proline in leg muscles. The use of the researched additive as a substitute for a feed antibiotic in composition of mixed feed for broilers decreased the total amount of amino acids in broilers' breast muscle, compared with the control group, and increased level of amino acids in leg muscles. The significant decrease in content of histidine and arginine in the pectoral muscles and proline in the leg muscles was noted. The observed changes varied within acceptable physiological norm. In the experimental groups the energy value of meat was increased.

Introduction

In the 70s-80s of the last century some specific mechanisms of development of microorganisms resistance to antibiotics were shown. This is a great concern of nowadays, and this issue requires an urgent solution. The possibility of using antibiotic agents in animal husbandry has been disputed for infinitely long. Sharpness of such discussions, as well conviction of each of the parties in their right opinion, does not change. However, the focus of scientific forces is gradually shifting: about 10–15 years ago only few people opposed antibiotic growth stimulants, and nowadays there are so many opponents to antibiotics, that they can no longer be ignored.

Antibiotics used for therapeutic purposes and for stimulation of young animals growth accumulate in significant amounts in food products — meat, milk, eggs. The free concentration of antibiotics for a short period of time is excreted from the animal's body with metabolic products — feces, urine, products (milk, eggs), but the antibiotics associated with proteins and other components remains in a body for a long time. Antibiotics, excreted from the body, get to the soil as part of organic fertilizers and after that accumulate in plants [1].

Low efficiency of antibiotics in poultry farming is noted by many experts. For example, employees of the Nizhny Novgorod Research Institute of Epidemiology and Microbiology n. a. Academician I. N. Blokhin note the fact that the difficult ecological situation, imbalance in nutrition contribute to the spread of intestinal infections in poultry farms: *salmonellosis*, *colibacillosis*, *listeriosis* [2]. At the same time deaths of broilers cause significant financial losses and decrease the productivity of the poultry farm. The use of antibiotics in this case is inefficient and environmentally harmful [3].

Antimicrobial preparations operate according to general pharmacological laws; despite their high specificity they are quite effective only under strict adherence to instructions. If the conditions are not met, antibiotic agents show little effect, and in some cases they can even cause harm.

Feed additives provide positive effect on a chicken's body, but are not always necessary for diet. Innovations constantly develop. Farmers keep their eye on the innovations and they are "mentally prepared" to apply them in their farms. It is not easy for everyone to move from a state of crisis and information vacuum to a rapidly developing and turbulent modern market space. However, despite the material difficulties, which are still an integral fact of modern Russian reality, it is clear that the future of livestock and poultry farming lies with new feed additives [4]. Today the Russian poultry industry is 4th largest in the world ranking for meat production and 6th largest in egg production [5].

All over the world there is a trend to increase the share of poultry meat in total volume of meat production [6,7], which is primarily explained by lower production costs

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and, accordingly, lower selling prices, in comparison with beef or pork. To ensure high rates of meat production it is necessary to use the latest advances in breeding, feeding, compliance with growing technology and veterinary protection of animals [8, 9].

Today, in the conditions of modern industrial poultry farming, one of the leading positions is occupied by problem of protection of animals' health with minimal use of antibacterial drugs [10, 11].

In connection with all of the above specified, since July 1, 1999 the EU has prohibited several conventional antibiotics, and in Denmark, Sweden and some other countries all antibiotics used as growth stimulants were prohibited [12].

The World Health Organization in April 2014 published a report, stating that "this serious threat is no longer just a prediction for the future, as it is already manifesting itself right now in every region of the world and can negatively affect everyone in every country, regardless of age. Antibiotic resistance is a peculiar phenomenon when bacteria change so much, that antibiotics no longer have any effect on body of people who need them to fight infection, and this is now one of the most serious threats to human health" [13, 14].

The World Health Organization has concluded that inappropriate use of antibiotics in animal husbandry is a major contributor to the emergence and distribution of antibiotic-resistant microorganisms, and it is necessary to limit the use of antibiotics as growth promoters in animal feed. The International Epizootic Office has added a set of guidelines to the World Veterinary Code with recommendations to run national surveillance and monitoring programs for antimicrobial resistance, thus controlling the amount of antibiotics used in animal husbandry. They also recommend strict compliance with appropriate use of antibiotic drugs in due dosage only. Another recommendation is the implementation of methodologies to help identify factors of associated risk and assess the risk of antibiotic resistance development [15].

In recent years the development of alternative antibiotics has significantly activated. The alternative antibiotics are assigned for maintenance or improvement the health and productive rate of poultry. Alternatively, probiotics, prebiotics, synbiotics, organic acids, enzymes, phytogenics, antimicrobial peptides, hyper immune antibodies to eggs, bacteriophages, clay and metals are offered as additive. Although the beneficial effects of many developed products have been clearly demonstrated, the experts agree that these products are not consistent with each other, and the results of their application vary greatly [16,17].

One of the effective and safe remedy are phytobiotic feed additives with extended sphere of action. The included ingredients should work in collaboration, complementing each other. The result of research proved essential oils, phytoextracts and protected organic acids to be the most efficient. The mechanism of action of complex drugs of this type is very simple. Essential oils weaken the bacterial cell wall. Weak cell wall causes cell lysis. Disruption of ATP synthesis leads to a weakening of the bacterial cell itself. Hydrogen ions are less exported, the cellular environment gets acidified, and bacterial metabolism is disrupted. The bacterium spends its energy for detoxing but not for reproduction. Thus a bacteriostatic effect is achieved. Further, organic acids are included in the feed, providing bactericidal effect. In general, phytoextracts and essential oils with an antibacterial effect prevent development of many intestinal infections, which seriously affects the safety and productivity of the poultry. In addition these compounds provide complex growth-stimulating effect on animal's body and, in addition to the antibacterial effect, they increase the attractiveness of feed (enhance feed palatability), have an anti-stress effect, and increase the secretion of saliva and digestive enzymes. The aim of the research is to assess the meat qualities, development of internal organs and chemical composition of broiler chickens meat when replacing feed antibiotics in their diet with a safe growth stimulant in form of a feed additive, including phytobiotics and protected organic acids.

Objects and methods

The experimental part of the research was run in LLC "Sredneuralskaya Poultry Farm" of Sverdlovsk region. The broiler chickens of the Ross 308 cross in 2019 were exposed to experiment.

The broilers groups were formed in accordance with recommended methodology of Federal Research Center "VNITIP" of RAS[1] (Table 1).

To assess the meat qualities of broilers 3 chick carcasses were anatomically cut at the end of the growing period, each chicken was taken from each experimental group. The development of the internal organs of broiler chickens was assessed during anatomical cutting at the age of 22 and 38 days.

In the breast and leg muscles of broilers, the following parameters were determined: amino acid composition including 17 amino acids (aspartic acid, glutamic acid, serine, histidine, glycine, threonine, alanine, arginine, tyrosine, cystine, valine, methionine, phenylalanine, isoleucine, leucine, lysine and proline); mass fraction of moisture, dry matter, protein, fat; amount of ash. Based on data on chemical composition of muscle tissue, the meat quality index (the ratio of fat and protein) and the energy value of meat were calculated.

The amino acid composition of meat was determined according to the SOP (standard operating procedure) "Determination of the amino acid composition by high performance liquid chromatography (HPLC) with precolumn derivatization with OPA and FMOC agents in food" at the V. M. Gorbatov VNIIMP test center on the device Agilent 1260 Infinity II. Dansyl chloride, phenyliso-thiacyanate, and other reagents were used for derivatization.

[1] Egorov, I.A., Manukyan, V.A., Lenkova, T.N. et al. (2013). Methods of conducting scientific and industrial research on poultry feeding. Sergiev Posad: VNITIP. 2013. — 52. ISBN: 978–5–91582–047–9

Table 1. Scheme of scientific and economic experiment

Group	Number, sex	Feeding
Control	♂ 80 ♀ 80	The conventional diet (CD) is a complete feed ration, with a nutritional value according to the recommendations for the chickens cross. Feed antibiotic was included in the CD: since the 1st to the 21st day — *Albacin*, dosage: 300 g / t of compound feed and since the 22nd to the 30th day *Nosiheptide* — 250 g / t of compound feed
1 experimental	♂ 80 ♀ 80	CD + researched additive in amount of 1 kg / t of compound feed. Period of use: from the 1st day until the end of fattening
2 experimental	♂ 80 ♀ 80	Experimental diet (ED): the feed antibiotic in CD has been replaced by the researched additive in amount of 1 kg / t of compound feed. Period of use: since the 1st day until the end of fattening.

Standard research methods were used to study the physical and chemical parameters of mass fraction of protein[2], fat[3], and the content of minerals (ash)[4].

The development of the internal organs of broiler chickens was assessed during the experiment at the age of 22 and 38 days.

The data obtained were statistically processed on a personal computer, Microsoft Excel editor, using the methods of biometric analysis according to N. A. Plokhinsky. The reliability of difference was established in relation to the control group using the Student's t-test, while determining three reliability thresholds: * $P \le 0.05$; ** $P \le 0.01$; *** $P \le 0.001$.

Results and discussion

The main parameters of poultry meat qualities are: the pre-slaughter live weight, the weight of the eviscerated carcass, the yield of the eviscerated carcass.

At 38 days in order to establish the effect of the studied feed additive on the meat quality of broilers, the average chicks for the group were selected for anatomical cutting. The live weight of selected broiler chickens in the control group was 2,130 g, in 1st experimental group it was 2,131.33 g, in 2nd experimental group it was significantly higher than in the control group by 5.13% ($P \le 0.01$) and reached 2,239.33 g (Table 2).

In terms of the weight of the bloodless carcass, the 2nd experimental group was in the lead with a value of 2,162.0 g, which is significantly more than the control by 5.15% ($P \le 0.01$). This parameter in chickens of the 1st experimental group was lower in comparison to the control group by 0.13%.

One of the most important poultry products is the eviscerated broiler chicken. It was found that replacing the feed antibiotic in the diet of chickens of the 2nd experimental group with the researched phytobiotic additive contributed to increase in weight of eviscerated carcass compared to the control group by 3.3%, thus amounting to 1,513.67 g. In the 1st experimental group this parameter was 1.5% lower than in the control group.

The slaughter yield of eviscerated carcasses in the group of chickens that instead of a feed antibiotic received an additive, including phytoextracts, essential oils and protected organic acids, was slightly lower in comparison to the control group — by 1.2%. This circumstance is associated with a higher mass of internal organs in poultry of this experimental group, in particular: the mass of heart, lungs, kidneys, gizzard, intestines and spleen. These changes remained within physiological norms. The slaughter yield of eviscerated carcasses in broilers of the 1st experimental group was 1.1% lower than the control group parameters.

In composition of a carcass the amount of meat, bones and skin was analyzed, and the meat-and-bone index was calculated.

The total amount of meat in carcass of chickens in the control group was 1,146.7 g, which is 3.9% more than in the chicken of the 1st experimental group. The chickens of the 2nd experimental group featured the highest value of this parameter — 1,163 g, which exceeded the control group by 1.4%. In percentage terms, i. e. by the weight of muscle tissue apart from the carcass weight, the greatest value — 78.3% was reached in the control group, in 1st and 2nd experimental groups this ratio was less than in the control group by 1.9 and 1.4%, respectively.

The bone tissue in the carcass of chickens from the control group was 122.62 g, and relative to the weight of the entire carcass of chickens in this group, the level of bone mass was 8.4%. Chickens of the 1st experimental group had a lower bone mass than in the control group by 5.1%, while the relative value of the chicken carcass weight was 0.3% less in comparison with the control group. The cockerels of the 2nd experimental group had the greatest value in terms of bone mass, higher than similar parameter of the control group by 2.9%, while in terms of the relative bones weight to overall carcass weight this value was less than the control by 0.1%.

The amount of skin in the carcass of chickens of the 1st and 2nd experimental groups exceeded the control value by 9.4 and 29.3%, respectively.

To assess the meat qualities of the carcass, the meat-bone index (the ratio of muscles weight to bones weight) was calculated. In the control group this parameter was 9.4, in the 1st experimental group it was higher than in the control by 0.1 units, and in the 2nd experimental group it was lower by 0.2 units.

[2] GOST 25011–2017 "Meat and meat products. Protein determination methods". Moscow: Standartinform, 2018. — 14 p. (In Russian)

[3] GOST 23042–2015 "Meat and meat products. Methods of fat determination". Moscow: Standartinform, 2019. — 8 p. (In Russian)

[4] GOST 31727–2012 "Meat and meat products. Determination of total ash". Moscow: Standartinform, 2019. — 12 p. (In Russian)

Table 2. Results of anatomical butchering of chickens (M ± m), (n = 3)

Показатель	Groups		
	Control	1st experimental	2nd experimental
Live weight, g	2,130.0 ± 11.02	2,131.33 ± 2.91	2,239.33 ± 5.9 **
Weight of a bloodless carcass, g	2,056.0 ± 12.7	2,053.33 ± 5.21	2,162.0 ± 3.06 **
Eviscerated carcass weight, g	1,465.33 ± 14.62	1,443.33 ± 15.76	1,513.67 ± 9.28
Slaughter yield of eviscerated carcass, %	68.8	67.7	67.6
Total composition of the chicken carcass:			
Muscles, g	1,146.72 ± 15.10	1,102.01 ± 7.65	1,163.22 ± 12.25
% of the carcass weight	78.3	76.4	76.9
Bones, g	122,62 ± 17.93	116,40 ± 11.37	126,12 ± 16.86
% of carcass weight	8.4	8.1	8.3
Skin, g	136,10 ± 6.02	148,95 ± 12.27	175,97 ± 14.41
% of carcass weight	9.3	10.3	11.6
Bone and meat index	9.4	9.5	9.2

The results of anatomical cutting and further deboning of the chicken carcass allowed determining the development of its specific and the most valuable parts of chicken exposed to effect of the researched feed factor (Table 3).

The meat of the broiler chicken breast features special nutritional value. The total weight of the breast in the control group slightly exceeded the weight of breast of the 1st and 2nd experimental groups by 1.7 and 1.39%, respectively. The ratio of breast weight to eviscerated carcass weight was higher by 0.1 and 1.7% in the control group in comparison with this ratio of the 1st and 2nd experimental groups. The bony part of the breast in 1st and 2nd experimental groups amounted to 24.9 and 26.2 g respectively, and exceeded the weight of the control group value by 29 and 35.7%, respectively. Due to the higher content of bones in the chickens breast from the 1st and 2nd experimental groups, the meat-to-bone index (the ratio of meat to bone weight) was lower than the control by 6.5 and 7.4 units, respectively.

The 2nd experimental group (33.3 g) took in first place in breast skin yield, while the control group showed lower breast skin yield by 6.9% (31 g), in the 1st experimental group this parameter was 1.29% higher than in the control group.

According to the results of deboning of leg quarter, it was found that the greatest total weight of the leg quarter was recorded in the chickens of the 2nd experimental group — 146.67 g, which was 8.91% higher than the control value. In the 1st experimental group 1, there was a significant decrease in the weight of the leg quarter relative to the control by 15.8% ($P \leq 0.05$), amounting to 113.33 g. The ratio of the weight of the leg quarter to the weight of the eviscerated carcass was also higher in the 2nd experimental group, exceeding the control group by 0.5%. The weight of muscle tissue in the leg quarter of broiler chickens of the 2nd experimental group exceeded the control value by 3.5%, and in the 1st experimental group, this parameter was significantly decreased in comparison with the control group by 17.1% ($P \leq 0.05$). In relation to the weight of muscle tissue in leg quarter to the mass of the eviscerated carcass, the lowest value was recorded in the 1st experimental group — 6.4 units, which is 1.2% less than in the control group. This

Table 3. Results of the deboning of specific parts of carcass, (M ± m), (n = 3)

Parameter	Groups		
	Control	1st experimental	2nd experimental
Breast			
Total weight, g	557.33 ± 13.49	548.0 ± 14.0	549.67 ± 8.76
% of eviscerated carcass	38.0	37.9	36.3
muscles, g	500.0 ± 16.29	482.0 ± 17.78	484.67 ± 11.62
% of eviscerated carcass	34.10	33.4	32.0
bones, g	19.3 ± 0.66	24.9 ± 2.56	26.2 ± 2.6
meat and bones index	25.9	19.4	18.5
skin, g	31 ± 1.7	31.4 ± 1.4	33.3 ± 2.65
Quarter			
Total weight, g	134.67 ± 3.71	113.33 ± 2.4*	146.67 ± 5.21
% of eviscerated carcass	9.2	7.8	9.7
muscles, g	112.0 ± 3.46	92.82 ± 3.0*	116.0 ± 3.46
% of eviscerated carcass	7.6	6.4	7.7
bones, g	8.44 ± 0.19	8.1 ± 0.2	9.2 ± 0.84
meat and bones index	13.3	11.5	12.6
skin, g	15.5 ± 4.14	11.2 ± 2.3	18.95 ± 0.9
Drumstick			
Total weight, g	102.67 ± 1.76	103.33 ± 2.67	102.0 ± 3.06
% of eviscerated carcass	7.0	7.1	6.7
muscles, g	83.93 ± 3.23	79.54 ± 3.31	81.01 ± 2.71
% of eviscerated carcass	5.7	5.5	5.3
bones, g	12.45 ± 1	11.3 ± 0.6	10.9 ± 0.5
meat and bones index	6.7	7.0	7.4
skin, g	4.9 ± 0.34	0.3 ± 2.1	8.84 ± 3.1
Wing			
Total weight, g	152.00 ± 2.00	152.00 ± 4.16	157.33 ± 1.76
% of eviscerated carcass	10.37	10.53	10.39
muscles, g	55.6 ± 1.2	55.0 ± 2.2	55.9 ± 1.1
% of eviscerated carcass	3.8	3.8	3.7
bones, g	10.3 ± 0.25	9.8 ± 0.26	9.9 ± 0.7
meat and bones index	5.4	5.6	5.6
skin, g	9.65 ± 0.25	16.1 ± 6.7	10.6 ± 0.46
Bones structure			
Total weight, g	268.67 ± 13.33	269.33 ± 12.77	304.67 ± 11.57
% of eviscerated carcass	18.33	18.66	20.13
muscles, g	143.67 ± 1.45	165.33 ± 7.69	172.67 ± 7.69*
% of eviscerated carcass	9.80	11.45	11.41
bones, g	61.7 ± 8.8	52.9 ± 4.1	59.0 ± 2.6
meat and bones index	2.3	3.1	2.9
skin, g	45. 0 ± 6.8	42.3 ± 9.8	65.9 ± 7.35

parameter in the 2nd experimental group exceeded the control group by 0.1%. The control group showed the highest meat-bone index of the leg quarter — 13.3 points, which is 1.8 and 0.7 points higher than in the 1st and the 2nd experimental groups, respectively.

The analysis of the drumstick deboning showed no significant differences between the groups. The percentage of the eviscerated carcass weight for leg muscles was higher in the control group, accounting to 5.7 units. Despite the fact that in the 1st experimental group this parameter was lower than the control value by 0.2%, the total weight of the drumstick exceeded the control value by 0.64%.

The analysis of anatomical cutting of the wing showed that the highest total weight of the wing was achieved in the 2nd experimental group of chickens, amounting to 157.33 g, thus exceeding the control group and the 1st experimental group by 3.5%. The wings of broilers of the 1st experimental group featured the highest skin weight — 16.1 g, which exceeded the same parameter of chicken peers in the control group and in the 2nd experimental group by 66.8 and 51.8%.

The analysis of the anatomical cutting of the chicken frame showed that the highest weight of muscles was recorded in the 2nd experimental group — 172.7 g, which is significantly higher than in the control group by 20.1% ($P \leq 0.05$) and 4.47% higher than in the 1st experimental group. The number of skeleton bones was lower in the 1st and the 2nd experimental groups compared to the control by 14.2 and 4.4%, respectively. In terms of skin weight of the frame, the 2nd experimental group was in the lead, exceeding the control group by 46.4%, and the 1st experimental group by 55.6%. The meat and bone index was higher in the 1st experimental group, amounting to 3.1 points, which is 0.8 points higher than the control value.

The results of analysis of the amino acid composition in the breast and leg muscles of broiler chickens are presented below in Tables 4 and 5.

Table 4. Amino acid composition of the breast muscle of broiler chickens

Amino acids, mg / 100 g sample	Control group	1st experimental group	2nd experimental group
Aspartic acid	1,701.33 ± 118.38	1,648.00 ± 65.57	1,629.67 ± 116.65
Glutamic acid	3,963.33 ± 464.16	3,633.00 ± 181.24	3,576.67 ± 373.37
Serine	1,084.00 ± 42.59	858.00 ± 68.77	906.67 ± 129.14
Histidine	1,063.00 ± 124.50	972.00 ± 47.43*	714.33 ± 46.6*
Glycine	995.00 ± 95.77	942.33 ± 35.75	876.33 ± 96.56
Threonine	1,204.33 ± 62.86	1,070.67 ± 70.43	991.00 ± 94.52
Arginine	2,100.33 ± 132.63	1,731.00 ± 346.20	1,280.00 ± 149.84**
Alanin	1,239.00 ± 116.98	1,151.67 ± 8.67	1,133.00 ± 147.55
Tyrosine	779.33 ± 44.13	634.00 ± 47.88	658.00 ± 57.73
Cystine	227.00 ± 17.62	208.33 ± 47.56	184.67 ± 12.60
Valine	958.67 ± 111.79	962.67 ± 63.05	822.00 ± 32.05
Methionine	551.67 ± 32.20	577.00 ± 48.42	533.67 ± 38.35
Phenylalanine	830.00 ± 74.67	774.33 ± 18.85	721.67 ± 91.48
Isoleucine	1,213.00 ± 29.50	1,103.33 ± 14.19*	1,010.67 ± 90.34
Leucine	1,602.67 ± 153.53	1,560.67 ± 44.30	1,133.00 ± 271.93
Lysine	3,841.67 ± 117.14	3,715.33 ± 119.88	3,318.67 ± 309.71
Proline	274.33 ± 42.06	291.67 ± 27.23	333.00 ± 27.02
Total amount of amino acids	23,628.00 ± 1,562.34	21,834.00 ± 503.95	19,822.33 ± 1,818.29

Table 5. Composition of amino acids in the leg muscles of broiler chickens

Amino acids, mg / 100 g sample	Control group	1st experimental group	2nd experimental group
Aspartic acid	1,335.33±110.60	1,323.33±17.29	1,422.00±79.68
Glutamic acid	3,057.67±386.61	3,027.67±95.84	3,844.67±99.42
Serine	762.33±100.76	727.00±18.01	935.33±48.08
Histidine	767.00±75.41	711.00±40.51	754.00±61.26
Glycine	836.33±72.79	785.33±17.48	896.33±55.81
Threonine	829.67±95.62	719.67±28.83	969.00±22.30
Arginine	1,440.33±102.57	1,313.33±15.62	1,492.33±68.34
Alanin	1,055.33±101.99	989.67±31.83	1,118.33±43.54
Tyrosine	645.00±42.04	591.33±21.17	663.33±25.26
Cystine	135.33±20.99	125.67±8.69	162.67±14.66
Valine	826.33±52.04	739.00±37.75	882.67±50.13
Methionine	495.67±69.66	391.00±46.32	472.33±53.45
Phenylalanine	745.33±34.71	692.00±26.65	670.00±23.59
Isoleucine	823.33±106.95	816.33±19.55	994.33±52.06
Leucine	1371.67±22.00	1,256.67±56.22	1391.00±67.73
Lysine	2,295.67±450.76	1,956.67±263.61	3,110.67±142.69
Proline	383.00±11.79	357.33±34.07	284.33±9.17**
Total amount of amino acids	17,810.00±1,733.02	16,524.33±389.28	20,064.00±626.45

Based on the research it was found that the total amount of amino acids in the pectoral muscles of chickens that in addition to the main diet received a feed additive containing phytobiotics and protected organic acids, and in broilers whose diet included the researched additive instead of the feed antibiotic this value was 7.6% and 16.1% lower than the control value respectively. As for the amount of some individual amino acids in the pectoral muscles of chickens of the 1st and 2nd experimental groups, some amino acids showed decrease in comparison with the control group: aspartic acid — by 3.13% and 4.2%, glutamic acid — by 8.3% and 9.8%, serine — by 20,8% and 16.4%, histidine — by 8.6% ($P \leq 0.05$) and 32.8% ($P \leq 0.05$), glycine — by 5.3% and 11.9%, threonine — by 11.1% and 17.7%, arginine — by 17.6% and 39.0% ($P \leq 0.01$), alanine — by 7.0% and 8.6%, tyrosine — by 18.6% and 15.6%, cystine — by 8.2% and 18.6%, phenylalanine — by 6.7% and 13.1%, isoleucine — by 9.0% ($P \leq 0.05$) and 16.7%, leucine — by 2.6% and 29.3%, lysine — by 3.3% and 13.6%, respectively. With regard to the content of valine and methionine, their increase in pectoral muscle of chickens of the 1st experimental group was recorded by 0.4% and 4.6%, and decrease among the broilers of the 2nd experimental group by 14.3% and 3.3%, respectively. The amount of proline in the breast of chickens of the 1st and 2nd experimental groups exceeded the control group by 6.3% and 21.4%, respectively.

The total amount of amino acids in the leg muscles of chickens, which received the researched substance based on phytobiotics and protected organic acids added to the conventional diet, was 7.2% lower than in the control group. The introduction of the researched feed additive instead of the feed antibiotic increased the amount of amino acids by 12.7%.

The following changes were observed in content of some individual amino acids in the leg muscles of chickens exposed to the experiment. The broilers of the 1st experimental group featured the decrease in all analyzed amino acids in comparison with the level of the control group: aspartic acid — by 0.9%, glutamic acid — by 0.98%, serine — by 4.6%, histidine — by 7.3%, glycine — by 6.1%, threonine — by 13.3%, arginine — by 8.8%, alanine — by 6.2%, tyrosine — by 8.3%, cystine — by 7.1%, valine — by 10.6%, methionine — by 21.1%, phenylalanine — by 7.2%, isoleucine — by 0.85%, leucine — by 8.38%, lysine — by 14.8%, proline — by 6.7%. Among the broilers of the 2nd experimental group, an increase in their level in the leg muscle was noted for most of the amino acids in comparison with the control group: aspartic acid — by 65%, glutamic acid — by 25.7%, serine — by 22.7%, glycine — by 7.2%, threonine — by 16.8%, arginine — by 3.6%, alanine — by 5.97%, tyrosine — by 2.8%, cystine — by 20.2%, valine — by 6.8%, isoleucine — by 20.8%, leucine — by 1.4%, lysine — by 35.5%. The content of histidine, methionine, phenylalanine and proline in the leg muscles of the chickens of the 2nd experimental group was lower than the control group by 1.7%; 4.7%; 10.1% and 25.8% ($P \leq 0.01$).

Analysis of the chemical composition of the pectoral muscles in broiler chickens (Table 6) proved that the moisture content in the meat of the control group was 76.1%, in the 1st and 2nd experimental groups this parameter was lower in comparison with the control group by 2.7% ($P \leq 0.05$) and 1.3%, respectively.

Table 6. The chemical composition of the pectoral muscles of broilers, % (*M ± m*), (*n = 3*)

Parameter	Groups		
	Control	1st experimental group	2nd experimental group
Total moisture	76.1 ± 0.49	73.4 ± 0.21*	74.8 ± 0.3
Dry matter	23.9 ± 0.49	25.6 ± 0.206*	25.2 ± 0.27
Protein	20.12 ± 0.452	19.17 ± 0.483	16.64 ± 1.903
Fat	2.91 ± 0.37	5.76 ± 0.59*	7.92 ± 0.24**
Ash	1.13 ± 0.017	1.02 ± 0.015*	0.91 ± 0.103
Meat quality index (fat / protein)	0.14	0.30	0.47
Energy value, kJ / 100 g	446.0 ± 14.6	537.3 ± 14.0*	576.2 ± 12.3**

Dry matter content in the chickens pectoral muscles within the control group was 23.9%, the chickens of the 1st experimental group featured significantly higher value than the control group — by 1.7% higher ($P \leq 0.05$), and the broilers of the 2nd experimental group showed higher dry matter content by 1, 3% than in the control group.

The protein content in the muscle tissue of breast of chickens in the control group was 20.12%, in the 1st and 2nd experimental groups this parameter was lower than in the control group by 0.95 and 3.48%.

Fat content in the muscle tissue of the broilers of the 2nd experimental group was the highest — 7.92%, exceeding the control group value by 5.01% ($P \leq 0.001$). In the 1st experimental group the fat content was higher than the control level by 2.85% ($P \leq 0.05$) and amounted to 5.76%.

Crude ash content was the highest in the control group — 1.13%, which value is higher than this parameter in the 1st experimental group by 0.11% ($P \leq 0.05$). In the 2nd experimental group the amount of crude ash was minimal — 0.91%, which was 0.22% less than this parameter in the control group.

Due to the lower content of fat in the control group, the energy value of meat was the smallest and amounted to 446 KJ in 100 g. In the 1st experimental group the energy value in 100 g of breast muscle tissue was 537.3 KJ, which is 20.5% more than in the control group ($P \leq 0.05$). The highest energy value was recorded in the pectoral muscles of the chickens of the 2nd experimental group — 576.2 KJ, which exceeded the control value by 29.2% ($P \leq 0.01$).

To characterize the quality of meat and meat products, the fat / protein ratio or meat quality index (MQI) is used. In the control group the MQI was 0.14; in the 1st and 2nd experimental groups this value reached 0.3 and 0.47, respectively.

The data on the chemical composition of the leg muscles are presented below in Table 7.

The moisture content in the broilers leg muscles in the 1st and 2nd experimental groups was lower in comparison with the control group by 1.3 and 3.46%, respectively. On the contrary the amount of dry matter was higher in the 1st and 2nd experimental groups than in the control group by 1.3% and 3.46%, respectively.

Table 7. The chemical composition of the broilers leg muscles, % (*M ± m), (n = 3)*

Parameters	Groups		
	Control	1st experimental group	2nd experimental group
Total moisture	75.5 ± 0.32	74.2 ± 0.93	72.04 ± 0.31**
Dry matter	24.5 ± 0.32	25.8 ± 0.42	27.96 ± 1.31
Protein	10.49 ± 1.02	9.4 ± 0.087	6.47 ± 1.58
Fat	13.58 ± 1.28	15.88 ± 0.59	21.35 ± 2.69
Ash	0.62 ± 0.053	0.53 ± 0.0057	0.41 ± 0.95
Meat quality index (W / W)	1.30	1.69	3.30
Energy value, kJ / 100 g	687.2 ± 18.2	754.6 ± 21.5	911.5 ± 16.9**

Protein content in the leg muscles was the highest in the control group — 10.49%, which exceeded the same parameter in the 1st experimental group by 1.09%, and by 4.02% in the 2nd experimental group. The highest percentage of ash was observed in the leg muscles of the chickens in the control group — 0.62%, this value exceeded the value in the 1st experimental group by 0.09% and by 0.21% in the 2nd experimental group.

The analysis of fat amount in the leg muscles showed the increase of fat in broiler chickens of the 1st and 2nd experimental groups, compared with the control group by 2.3 and 7.77%, respectively.

During estimation of the energy value of the leg muscles, a natural relation of energy value with fat content was traced. The energy values were increased in broilers in the experimental groups. So, in the control group 100 g of the leg muscles contained 687.2 KJ, in the 1st experimental group — 754.6 KJ, in the 2nd experimental group this value reached 911.5 KJ ($P \leq 0.01$).

The meat quality index of the control group was equal to 1.3, in the 1st and 2nd experimental groups it was 1.69 and 3.3 units, respectively.

At the age of 22 days three broiler cockerels of average live weight were selected from each experimental group in order to measure the mass of some internal organs (Table 8). According to the weight of the liver, the 2nd experimental group exceeded the other compared groups, its prevalence over the control and the 1st experimental groups was 8.24% and 17.9%, respectively. The weight of broilers liver from the 1st experimental group was 1.6 g lower than the liver of chickens from the control group.

The relative weight of the kidneys and heart in the compared groups did not differ significantly and varied within 0.72–0.78% and 0.53–0.58%, respectively. The undoubted leaders in intestinal weight were broiler chickens of the 1st experimental group, outdoing the control group by 15.6%, and the 2nd experimental group by 30%. This trend was also peculiar for relative weight of the intestine and its length.

Thus, in terms of the relative intestinal weight, the 1st experimental group exceeded the control value by 1.59%, and the 2nd experimental group by 3.31%. By the length of the intestine, the 2nd experimental group was significantly lower than the control by 10.3% ($P \leq 0.05$) and less than the value of the 1st experimental group by 10.8%.

Table 8. The mass of the internal organs of broiler chickens at the age of 22 days (M ± m), (n = 3)

Parameter, UOM	Groups		
	Control	1st experimental group	2nd experimental group
Live weight, g	765.33 ± 2.91	751.33 ± 1.76*	841.0 ± 1.53***
Liver weight, %	19.52 ± 0.11	17.92 ± 1.13	21.13 ± 1.62
Relative liver weight, %	2.55	2.38	2.51
Kidney weight, g	5.72 ± 0.2	5.9 ± 0.46	6.09 ± 0.22
Relative kidney mass, %	0.75	0.78	0.72
Heart weight, g	4.46 ± 0.26	4.01 ± 0.23	4.81 ± 0.28
Relative heart weight, %	0.58	0.53	0.57
Intestine weight, g	68.62 ± 5.05	79.33 ± 4.1	61.0 ± 2.53
Relative intestinal weight, %	8.97	10.56	7.25
Intestine length, cm	184.0 ± 5.75	185.0 ± 6.21	165.0 ± 1.0*
Weight of the Fabritius bursa, g	1.7 ± 0.46	1.66 ± 0.08	1.88 ± 0.06
Relative weight of Fabritius bursa, %	0.22	0.22	0.22
Spleen weight, g	0.88 ± 0.04	0.71 ± 0.02*	1.05 ± 0.15
Relative weight of the spleen, %	0.11	0.09	0.12
Gallbladder weight, g	0.6 ± 0.13	1.18 ± 0.27	0.94 ± 0.13
The relative weight of the gallbladder, %	0.08	0.16	0.12
Stomach weight with fat without cuticle, g	15.8 ± 0.88	15.26 ± 1.59	17.39 ± 2.42
Relative weight of the stomach with fat without cuticle, %	2.06	2.03	2.07
Weight of the glandular stomach, g	5.78 ± 0.29	4.67 ± 0.3	5.02 ± 0.15
Relative weight of the glandular stomach, %	0.75	0.62	0.6

When assessing the relative weight of the Fabritius bursa, no difference was found between the groups: the values were the same in all groups and were equal to 0.22%.

There was a significant decrease in the spleen weight in the 1st experimental group in comparison with the control group by 19.3% ($P \leq 0.05$). On the contrary, in the 2nd experimental group, this value was 19.3% higher than in the control group. The relative weight of spleen in the control group, the 1st and 2nd experimental groups was 0.11; 0.09 and 0.12%, respectively.

The weight of the gallbladder in the 1st experimental group of 22-days-old broilers was the highest and reached 1.18 g, which is higher than the control group and the 2nd experimental group by 49.15% and 20.3%, respectively. The relative weight of the gallbladder was also higher in the 1st experimental group — 0.16%, this value exceeded value of the control group by 0.08% and the value of the 2nd experimental group by 0.04%.

The relative weight of the stomach with fat without cuticle in the chickens of the experimental groups did not differ significantly and ranged within 2.03 to 2.07%.

The relative weight of the glandular stomach was the highest in the control group — 0.75% and exceeded the value of the 1st and 2nd experimental groups by an average of 0.14%.

The weight of the examined internal organs of broiler chickens at the age of 38 days complied with the physiological norms, while the following differences were noted between the groups (Table 9).

The relative heart weight in the compared groups varied within the range of 0.50–0.54%. The relative lung weight was higher among the broilers of the 2nd experimental group — 0.56%, in the control group this parameter was 0.51%, in the 1st experimental group — 0.48%. The relative weight of the kidneys in the 1st and 2nd experimental groups was increased in relation to the control group by 0.11 and 0.15%.

The relative mass of the muscular stomach was higher in the chickens of the 2nd experimental group, amounting to 2.19%, which is 0.45% higher than in the control. In chickens of the 1st experimental group, this parameter was higher than the control value by 0.15%. The relative weight of the liver in the chickens of the experimental groups was within the range of 2.27–2.4%, the gallbladder weight varied within 0.08–0.11%.

As for the weight of intestine the 2nd experimental group showed significantly higher value than the control group value by 30% ($P \leq 0.05$), while the relative weight of intestine exceeded the control by 1.12%. In the 1st experimental group this parameter also exceeded the control group value in absolute weight and relative weight, respectively, by 8.1 and 0.38%.

Along with an increase in the absolute and relative intestinal weight in chickens of the 2nd experimental group, the increase in intestinal length was also observed. Intestine was significantly longer than in control group by 12.3% ($P \leq 0.05$).

The weight of the spleen was the greatest among the chickens of the 2nd experimental group, accounting to 3.09 g, which exceeded the control value by 0.39 g. In the 1st experimental group this parameter was lower in comparison with the control group by 0.24 g. The relative weight of this organ among the experimental groups of broilers varied within the range 0.115–0.138%.

The weight of the Fabritius bursa in the chickens of the experimental groups varied within the range of 0.045–0.055%.

Table 9. The weight of the internal organs of broiler chickens at the age of 38 days (M ± m), (n = 3)

Organ	Groupы		
	Control	1st experimental group	2nd experimental group
Heart mass, g	11.15 ± 0.27	10.67 ± 0.67	12.03 ± 0.33
Relative heart mass, %	0.52	0.50	0.54
Lung weight, g	11.02 ± 0.3	10.19 ± 1.0	12.54 ± 1.32
Relative lung mass, %	0.51	0.48	0.56
Kidney weight, g	12.15 ± 1.77	14.49 ± 2.12	16.07 ± 0.74
Relative kidney mass, %	0.57	0.68	0.72
Mass of the muscular stomach (with fat), g	37.13 ± 2.17	40.37 ± 2.25	49.12 ± 6.09
The relative mass of the muscular stomach, %	1.74	1.89	2.19
Liver weight, g	51.22 ± 1.12	49.09 ± 2.94	50.93 ± 2.26
Relative liver weight, %	2.4	2.3	2.27
Gallbladder weight, g	2.37 ± 0.15	1.7 ± 0.26	2.18 ± 0.12
The relative weight of the gallbladder, %	0.11	0.08	0.1
Intestine weight, g	100.44 ± 6.02	108.62 ± 7.02	130.67 ± 6.67*
Relative intestinal weight, %	4.71	5.09	5.83
Intestine length, cm	217.0 ± 4.9	208.33 ± 6.35	243.67 ± 7.13*
Spleen weight, g	2.7±0.43	2.46±0.27	3.09±0.78
Relative weight of the spleen, %	0.13	0.115	0.138
Weight of the Fabritius bursa, g	1.17±0.38	0.96±0.08	1.02±0.24
Relative weight of Fabritius bursa, %	0.055	0.045	0.046

Conclusion

The introduction of a phytobiotic feed additive into the diet of broiler chickens, both additionally and a way of replacing the feed antibiotic, provides positive effect on the meat qualities of chickens and does not negatively affect the development of internal organs. It was noted that in broilers who received antibiotics-free compound feed completed with phytoextracts, essential oils and protected organic acids, by the end of feeding those broilers showed higher relative weight of the heart, lungs, kidneys, muscle stomach, intestines than in the control group and in the 1st experimental group. At the same time the length of the intestine significantly exceeded the control group value. These changes varies within the physiological norm, which may contribute to the best detoxification capabilities of the chicken body and enhanced activation of the intestinal absorption function. During the research, the amino acid composition was studied, including 17 amino acids. It has been established that the additional use of a feed additive, including essential oils, hot pepper extract and protected organic acids in formulation of compound feed for broiler chickens, feature decrease in the total amount of amino acids in the breast and leg muscles of broiler chickens within acceptable physiological limits. At the same time, a significant decrease, compared with the control group, was noted for histidine and isoleucine in the pectoral muscle, and proline in the leg muscle.

The introduction of the tested additive to chickens mixed feed as a substitute for a feed antibiotic was characterized by a decrease in the total amount of amino acids in the breast muscle of broilers, compared with the control, and an increase in their level in the leg muscle. A significant decrease in the content of histidine and arginine in the pectoral muscle and proline in the leg muscle was noted. The observed changes varied within the physiological norm.

As for the chemical composition of the pectoral muscles and leg muscles, in the course of the research we noted an increase in mass fraction of moisture in chickens of the 1st and 2nd experimental groups. The mass fraction of fat in the pectoral muscles of broiler chickens and in the leg muscles of the 2nd experimental group exceeded the control values. At the same time, an increase in the energy value of meat was observed in both experimental groups.

The results obtained in research on the amino acid composition of the breast muscles and leg muscles of broiler chickens can be used in industrial practice to optimize the introduction of synthetic amino acids into the diet of broiler chickens simultaneously with application of alternative safe growth stimulants instead of feed antibiotics.

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