



INFLUENCE OF "PROBIX" FEED ADDITIVE AND ANTIBACTERIAL PREPARATIONS OF MACROLIDE GROUP ON THE AMINO-ACID COMPOSITION OF MEAT OF BROILER CHICKENS

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ABSTRACT

The article deals with the influence of "ProbiX" feed additive, and pharmazin and tilotsiklinvet antibacterial preparations on the amino acid composition of broiler chickens' meat during their use. When adding to the diet of broiler chickens "ProbiX" feed additive, one enhances the biological value of meat by increasing a content of essential amino acids in femoral muscles compared to control. It is found, that the ratio of essential to substitutable amino acids in pectoral and femoral muscles of broiler chickens at the beginning and the end of the withdrawal period reduces by 15,1 and 6,2 %, respectively, when using pharmazin. Opposite tendency is observed in muscles studied when using tilotsiklinvet (25,2 and 6 %, respectively). Also, the amino acid which limits the biological value of the protein – methionine, has been found.

1. INTRODUCTION

Amino acid composition is an important characteristic of meat proteins, as well as the criteria of its nutritional value [Yakubchak O.M., 2013]. Production of meat of a high quality is one of the main requirements of the poultry industry. Poultry meat is considered to be a dietary food, as it is balanced by the amino acid composition.

Currently, in the countries of the European Union, as well as in Ukraine, the ban on the use of fodder antibiotics and hormonal drugs, as growth stimulators for animals, including broiler chickens, was introduced [Yakubchak O. M., 2013; Antipova L.V., 2009]. It is necessary to investigate the biological value of the broiler chickens' meat in order to study the quality of this meat and its rational use. The biological value of meat is determined by the full value of proteins – the ratio and content of essential and substitutable amino acids in the proteins composition.

The body easily digests all essential amino acids. the fact is that the assimilation of protein foods and the completeness of the use of amino acids by the body can be provided only with the balanced essential amino acids conditions. Feed base and the preventive measures are important for the production of meat with high biological value, [Fotina T.I. et al., 2011; Kalnitskaya O. I., 2008].

Therefore, the search for non-harmful and effective nutraceuticals for improving the growth and maturity of broiler chickens, as well as improving the quality and safety of poultry slaughter products, is a topical issue.

However, in modern scientific literature, researchers did not study the peculiarities of the influence of the feed additive "Probix" and the antibiotics of the macrolide group on the indicators of safety and quality of the products of slaughter of broiler chickens.

According to this, the aim of this work is to study the influence of "Probix" feed additive and antibacterial preparations – pharmazin and tilotsiklinvet – on the amino acid composition of meat of broiler chickens during their use.

2. MATERIALS AND METHODS

The research was carried out on broiler chickens of the "Ross 380" cross-breeder at the age of 42 days in two stages.

At the first stage, the influence of the "Probix" feed additive on the amino acid composition of broiler chickens' meat was studied. For the experiment they have formed two groups – experimental and control (5 chickens each). the feed additive "Probix" is a mixture of probiotics and prebiotics. the probiotic component of the premix is made of the specially selected original strains of lactic acid microorganisms *Lactobacillus acidophilus* (20 %), *Lactobacillus helveticus* (5 %), *Lactobacillus bulgaricus* (5 %), *Lactobacillus lactis* (5 %), *Streptococcus thermophilus* (5 %), *Enterococcus faecium*

(20 %), which were taken from natural sources, dairy products or by other breeding methods without the use of gene modifications. the prebiotic component of the premix is made of polydextrose (10 %) and inulin (20 %). Calcium carbonate CaCO₃ (10 %) is used as a stabilizer ["Lactina", Bulgaria, 2002].

The additive was fed at a rate of 600 g/t of feed from the 5th to the 27th day, and 300 g/t – from the 28th to the 42nd day. Chickens in the control group had only the main diet. All broiler chickens, both control and experimental groups, had free access to the water

and feed. at the end of the study, the euthanasia was performed in compliance with the generally accepted principles of bioethics.

At the second stage of the study, the influence of pharmazin and tilotsiklinvet on the biological value of the pectoral and femoral muscles of broiler chickens was studied. For the experiment four groups of broiler chickens of the daily age were formed: two control and two experimental (12 broiler chickens each). the chickens of the first experimental group were fed with pharmazin containing the tylosin tartrate active substance (AS) in the amount of 500 mg/1 g; and in the second group – tilotsiklinvet containing tylosin tartrate and doxycycline glycolate as in the amount of 100 mg/g in a powder. Preparations of pharmazin and tilotsiklinvet were fed orally at a dose of 1 g per 1 dm³ of water according to the instructions for use [HUVEPHARMA AO, Bulgaria, 2013; LLC "Vetsintez", Ukraine, 2009]. Antibiotic preparations were given to broiler chickens prophylactically on the first 3 days of life, on 28–29 and 38–42 days of the experiment. at the end of the antibiotics use, 6 broiler chickens from each group were slaughtered at the beginning of the withdrawal period (in 3 hours), and after the withdrawal period (in 5–8 days), after the last pharmazin and tilotsiklinvet feeding, respectively.

The content of amino acids in pectoral and femoral muscles of broiler chickens, – those essential (valine, isoleucine, leucine, lysine, methionine, threonine, phenylalanine, tryptophan) and substitutable (alanine, arginine, aspartic acid, histidine, cystine, glycine, glutamic acid, oxyproline, serine, tyrosine), were determined with using the ion exchange chromatograph of the amino acid analyzer (AAA 339-M) according to DSTU ISO 13903:2009. They determined A/E index to assess the biological value of meat. This index represents the ratio of the content of essential amino acids (A) to their total number (E), and the amino acid score. This indicator was determined based on calculations of the percentage of each of the essential amino acids in the food protein relative to their content in the protein, named as "ideal". the amino-acid scale of the "ideal" protein was recommended by the FAO/WHO Committee [Yakubchak O. M. et al., 2012; Kryshtafovych V.I., 2012, Fotina T.I. et al., 2011]. the biological value of meat was determined by the protein-quality index – the ratio of tryptophan to oxyproline. the content of tryptophan was determined according to DSTU ISO 13904:2008, oxyproline – according to GOST R 50207-92. the analysis of the amino acid composition of broiler chickens' meat (pectoral and femoral muscles) was performed by comparing the composition of the substituted and essential amino acids.

Statistical processing of the obtained results was carried out using Student's variation statistics methods.

3. RESULTS

Based on the results of the first stage of the research, it was found that the content of essential amino acids in pectoral muscles of broiler chickens of the experimental group (have been fed with the "ProbiX" additive), does not significantly differ from the control; and in femoral muscles – by 4,78 % ($p \leq 0,001$), which is significantly higher than control.

The content of the substitutable amino acids in pectoral muscles of the poultry in control and experimental groups is higher, compared to their content in femoral muscles.

Among the essential amino acids in the meat of broiler chickens, in both control and experimental groups, the following amino acids have been identified: threonine, valine, isoleucine, leucine, lysine, tryptophan, phenylalanine and methionine. The last two amino acids are combined considering the fact, that the lack of methionine is compensated by cystine, and the lack of phenylalanine – by tyrosine [Izmailovych I. B., 2011].

Analyzing the results of the research on the content of essential amino acids in the chicken meat in the experimental group, it was found, that this content is significantly higher in pectoral muscles compared to the control group: threonine – by 3,37 % ($p \leq 0,001$), phenylalanine + tyrosine – by 2,98 % ($p \leq 0,001$), methionine + cystine – by 2,30 % ($p \leq 0,01$), lysine – by 0,53 % ($p \leq 0,05$). However, the content of valine – by 0,91 % ($p \leq 0,05$), isoleucine – by 1,92 % ($p \leq 0,01$) and tryptophan – by 7,14 % ($p \leq 0,05$) is significantly reduced, compared to control ones.

In femoral muscles of broiler chickens in the experimental group there is a significant increase of: valine – by 6,90 % ($p \leq 0,001$), isoleucine – by 8,64 % ($p \leq 0,001$), leucine – by 4,44 % ($p \leq 0,001$), phenylalanine + tyrosine – by 7,87 % ($p \leq 0,001$) and lysine – by 6,49 % ($p \leq 0,001$), compared to those femoral muscles in a control group. However, the content of threonine is significantly lower than the control by 1,33 % ($p \leq 0,05$), and tryptophan – by 9,09 % ($p \leq 0,01$) (Table 1).

Tab.1. the content of essential amino acids in a broiler chickens' meat (M±m; n=5)
(g/100 g of a product)

Amino acids	Control group		FA "ProbiX"	
	Pectoral muscles	Femoral muscles	Pectoral muscles	Femoral muscles
Threonine	0,89±0,003	0,75±0,001	0,92±0,002***	0,74±0,003*
Valine	1,10±0,002	0,87±0,003	1,09±0,003*	0,93±0,004***
Isoleucine	1,04±0,004	0,81±0,006	1,02±0,002**	0,88±0,004***
Leucine	1,67±0,003	1,35±0,002	1,67±0,003	1,41±0,005***
Phenylalanine+Tyrosine	1,68±0,003	1,27±0,002	1,73±0,005***	1,37±0,005***
Lysine	1,89±0,002	1,54±0,002	1,90±0,003*	1,64±0,005***
Methionine+Cystine	0,87±0,004	0,73±0,002	0,89±0,002**	0,73±0,002
Tryptophan	0,28±0,003	0,22±0,003	0,26±0,006*	0,20±0,004**
Total	9,42±0,024	7,54±0,021	9,48±0,026	7,90±0,032***

Note: * $p \leq 0,005$, ** $p \leq 0,01$, *** $p \leq 0,001$, compared to the control

Among the substituted amino acids of broiler chickens' meat, both in the control and experimental groups, the following amino acids were found: aspartic acid, serine, glycine, alanine, glutamic acid, proline, histidine, and arginine.

The content of the substitutable amino acids in the pectoral muscles of the poultry in experimental group is significantly lower than in the control one by 3,60 % ($p \leq 0,001$), and in the femoral muscles – by 3,20 % ($p \leq 0,001$), respectively.

In the pectoral muscles of the poultry from the experimental group, the content of the following substitutable amino acids is significantly increased (compared to control): serine – by 11,43 % ($p \leq 0,001$) and histidine – by 2,04 % ($p \leq 0,001$). There is a significant decrease in the content of such substitutable amino acids as: aspartic acid – by 1,53 % ($p \leq 0,001$), glycine – 14,82 % ($p \leq 0,001$), alanine – 4,80 % ($p \leq 0,001$), glutamic acid –

4,52 % ($p \leq 0,001$), proline – by 6,59 % ($p \leq 0,001$), arginine – by 3,52 % ($p \leq 0,001$), compared with the poultry meat in the control group.

In the femoral muscles of broiler chickens of the experimental group, the content of aspartic acid is by 2,50 % ($p \leq 0,001$), and glutamic acid – by 6,79 % ($p \leq 0,001$) higher compared to the control. it was found a significant reduction in the content of serine – by 11,94 % ($p \leq 0,001$), glycine – by 17,02 % ($p \leq 0,001$), alanine – by 0,97 % ($p \leq 0,05$), proline – by 27,17 % ($p \leq 0,001$) and arginine – by 1,77 % ($p \leq 0,01$), compared with the control. the content of oxyproline is by 13,51 % lower than in the control group of chickens, but the difference between them is not significant (Table 2).

Tab.2. the content of substitutable amino acids in a broiler chickens' meat (M+m; n=5)
(g/100 g of a product)

Amino acids	Control group		FA "Probix"	
	Pectoral muscles	Femoral muscles	Pectoral muscles	Femoral muscles
Aspartic acid	1,96±0,004	1,60±0,002	1,93±0,004***	1,64±0,003***
Serine	0,70±0,002	0,67±0,005	0,78±0,002***	0,59±0,003***
Glycine	1,08±0,002	0,94±0,005	0,92±0,001***	0,78±0,003***
Alanine	1,25±0,002	1,03±0,001	1,19±0,002***	1,02±0,004 *
Glutamic acid	3,32±0,003	2,65±0,003	3,17±0,004***	2,83±0,004 ***
Proline	0,91±0,003	0,92±0,007	0,85±0,004***	0,67±0,003***
Histidine	0,98±0,002	0,72±0,004	1,00±0,003***	0,72±0,003
Arginine	1,42±0,004	1,13±0,003	1,37±0,002***	1,11±0,004 **
Oxyproline	0,045±0,001	0,037±0,002	0,044±0,002	0,032±0,002
Total	11,67±0,023	9,70±0,032	11,25±0,024***	9,39±0,029***

Note: * $p \leq 0,005$, ** $p \leq 0,01$, *** $p \leq 0,001$, compared to the control

Analysis of the content of the substitutable amino acids in the control and experimental groups has shown, that in pectoral muscles of the poultry this indicator is higher compared with the femoral muscles.

The content of essential and substitutable amino acids in the meat of broiler chickens of experimental group is $20,73 \pm 0,050$ g/100 g in the pectoral muscles, which is by 1,71 % ($p \leq 0,001$) lower compared to the control; and in femoral muscles it is $17,28 \pm 0,061$ g/100 g, which is not significantly different from those in the control group (Fig. 1).

Total amount of amino acids in femoral and pectoral muscles of broiler chickens in the control group is $21,09 \pm 0,047$ g/100g and $17,24 \pm 0,053$ g/100g, respectively.

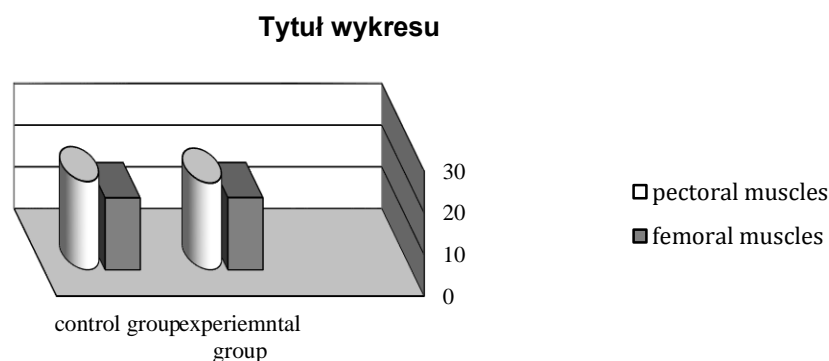


Fig. 1. Total amount of amino acids in a meat of broiler chickens, which have been fed with "Probix" additive (g/100 g of product).

The ratio of essential and substitutable amino acids in femoral and pectoral muscles of broiler chickens in the experimental group is 0,84 and 0,84, respectively. in the muscles of the poultry in the control group, this figure is 0,81 and 0,78, respectively (Fig. 2).

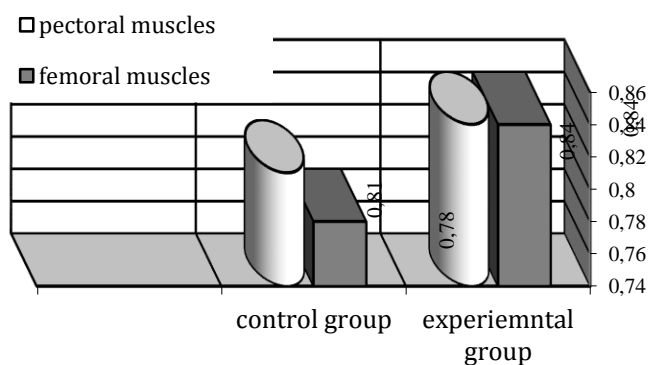


Fig. 2. the ratio of essential and substitutable amino acids in a meat of broiler chickens, which have been fed with "Probix" additive.

Therefore, the ratio of amino acids in the experimental group is slightly higher than the similar indicator in the poultry meat of the control group.

They assigned the ratio of tryptophan to oxyproline, as well as the protein amino acid score, to determine the biological value of broiler chickens' meat in control and experimental groups, according to the FAO/WHO comparative scale.

Analyzing the data of the ratio of tryptophan to oxyproline in broiler chickens' meat samples of the experimental group, one can observe a tendency to an increase of the content of tryptophan and decrease the content of oxyproline, compared with the control group (Fig. 3).

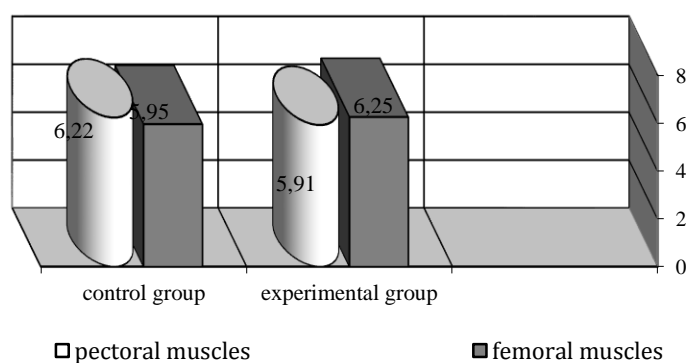


Fig. 3. the ratio of tryptophan to oxyproline in in femoral and pectoral muscles of broiler chickens, which have been fed with "Probix" additive.

It was found, that the meat of chicken broilers of the experimental group, which have been fed with "Probix" additive, contains in its composition a greater amount of muscle tissue, and therefore has a higher biological value, compared with the poultry meat from the control group.

Analyzing the results of the calculation of amino acid score, one can state that the limiting amino acids are absent in a meat of broiler chickens. This indicates that the meat of a poultry contains complete proteins. However, the amino acid content of a poultry meat in both the control and experimental groups exceeds the ideal protein in accordance with the FAO/WHO scale (Table 3).

Tab.3. Amino acid score in a meat of broiler chickens, which have been fed with "ProbiX" additive, %

Amino acids	FAO/WHO scale	Control group		Experimental group "ProbiX" feed additive	
		pectoral muscles	femoral muscles	pectoral muscles	femoral muscles
Threonine	4,0	4,6	4,6	4,6	4,2
Valine	5,0	5,7	5,4	5,4	5,2
Isoleucine	4,0	5,4	5,0	5,0	4,9
Leucine	7,0	8,6	8,4	8,3	8,0
Phenylalanine+Tyrosine	6,0	8,6	7,9	8,6	7,7
Lysine	5,5	9,7	9,5	9,4	9,2
Methionine+Cystine	3,5	4,4	4,5	4,4	4,1
Tryptophan	1,0	1,4	1,4	1,3	1,1

In pectoral muscles of the broiler chickens of the experimental group, which have been fed with "ProbiX" additive, there is a decrease in the number of such amino acids as: valine, isoleucine, lysine, compared with the control. and that brings this protein to the ideal one. in femoral muscles, the number of all amino acids is smaller compared to the control. Thus, femoral muscles' amino acid composition is much more balanced, in comparison with the pectoral muscles.

The calculation of amino acid score allows us to state that, when using "ProbiX" feed additive, the amino acid composition of broiler chickens' meat is close by its characteristics to the ideal protein, according to the FAO/WHO scale

[Kryshchak V. I., 2012].

Based on the results of the second stage of the research (Table 4), in pectoral muscles of broiler chickens of the experimental group (have been fed with pharmanin), the content of essential amino acids at the beginning of the withdrawal period was decreased by 7,0 %, compared with the first control group; and at the end of the elimination period – by 9,0 %, compared with the first control group.

Tab.4. Amino acid content in muscles of broiler chickens, which have been fed with pharmazin, g/100 g of meat, M±m; n=6

Indicator	Withdrawal period	Broiler chickens groups			
		First experimental group (pharmazin)		First control group	
		Pectoral muscles	Femoral muscles	Pectoral muscles	Femoral muscles
1	2	3	4	5	6
Essential amino acids					
Valine	3 hours	1,24±0,042	1,30±0,133	1,39±0,099	1,02±1,115
	6 days	1,19±0,035	1,27±0,170	1,44±0,101	1,07±0,138
Isoleucine	3 hours	1,30±0,053	1,43±0,092**	1,64±0,110	1,06±0,056
	6 days	1,23±0,049**	1,42±0,143	1,59±0,093	1,10±0,119
Leucine	3 hours	2,12±0,179	2,36±0,285***	2,44±0,076	1,59±0,099
	6 days	2,05±0,132***	2,29±0,329	2,49±0,079	1,56±0,143
Lysine	3 hours	2,36±0,051***	2,33±0,199	2,62±0,066	2,20±0,036
	6 days	2,34±0,059***	2,32±0,207	2,63±0,077	2,15±0,058
Methionine	3 hours	0,11±0,052**	0,38±0,075**	0,34±0,046	0,08±0,040
	6 days	0,13±0,043***	0,35±0,152	0,32±0,051	0,09±0,040
Threonine	3 hours	1,24±0,078	1,31±0,100	1,39±0,035	1,14±0,063
	6 days	1,20±0,105	1,28±0,140	1,42±0,061	1,09±0,049
Phenylalanine	3 hours	1,24±0,071	1,24±0,103	0,89±0,389	0,89±0,167
	6 days	1,26±0,062	1,21±0,137	1,01±0,126	0,86±0,143
Tryptophan	3 hours	0,59±0,078	0,25±0,127	0,27±0,127	0,51±0,114
	6 days	0,60±0,075***	0,23±0,106	0,29±0,070	0,50±0,112
Total number of essential amino acids	3 hours	10,20±0,60	10,60±1,12	10,98±0,95	8,49±1,69
	6 days	10,01±0,56	10,36±1,38	11,06±0,68	8,33±0,80
Substitutable amino acids					
Alanine	3 hours	1,47±0,041***	1,46±0,246	1,29±0,035	1,01±0,128
	6 days	1,42±0,035***	1,37±0,152	1,31±0,013	1,00±0,106
Arginine	3 hours	1,44±0,081	1,19±0,053	1,22±0,073	0,93±0,134
	6 days	1,41±0,110	1,17±0,074	1,21±0,078	0,92±0,107
Aspartic acid	3 hours	2,85±0,095	2,56±0,228	2,66±0,082	2,18±0,107
	6 days	2,80±0,062	2,47±0,137	2,65±0,071	2,17±0,088

1	2	3	4	5	6
Histidine	3 hours	1,63±0,289	1,51±0,143***	1,77±0,152	1,10±0,057
	6 days	1,58±0,316	1,47±0,130***	1,75±0,122	1,09±0,056
Glycine	3 hours	1,33±0,074	0,98±0,111***	1,15±0,094	1,30±0,048
	6 days	1,29±0,098	0,97±0,093**	1,08±0,059	1,31±0,039
Glutamic acid	3 hours	4,11±0,221	4,43±0,615***	4,37±0,133	2,57±0,254
	6 days	4,05±0,119***	4,38±0,608	4,42±0,058	2,90±0,568
Proline	3 hours	1,23±0,236	1,26±0,190	0,78±0,077	0,79±0,116
	6 days	1,19±0,224	1,21±0,196	0,79±0,055	0,81±0,093
Serine	3 hours	1,38±0,071	1,32±0,130	1,16±0,070	0,91±0,184
	6 days	1,33±0,066***	1,30±0,133	1,12±0,037	0,94±0,137
Tyrosine	3 hours	1,09±0,113	1,04±0,216	0,81±0,119	0,71±0,120
	6 days	1,05±0,099	0,99±0,260	0,82±0,082	0,73±0,129
Oxyproline	3 hours	0,07±0,005*	0,02±0,004*	0,04±0,004	0,07±0,003
	6 days	0,07±0,005*	0,02±0,004*	0,04±0,004	0,07±0,003
Total number of substitutable amino acids	3 hours	16,6±1,23	15,77±1,94	15,25±0,84	11,57±1,15
	6 days	16,29±1,13	15,36±1,80	15,20±0,58	11,95±1,33
The ratio of essential to substitutable	3 hours	0,61	0,67	0,72	0,73
	6 days	0,62	0,67	0,73	0,70
Total number of essential+ substitutable	3 hours	26,80±1,83	26,37±3,06	26,23±1,79	20,06±2,84
	6 days	26,30±1,69	25,72±3,18	26,26±1,26	20,28±2,13
Theratio of tryptophan to oxyproline	3 hours	8,4	12,5	6,7	7,3
	6 days	8,6	11,5	6,7	7,3
Total protein content	3 hours	26,32±0,75	23,37±1,65	26,49±1,37	22,33±0,69
	6 days	26,29±0,78	23,33±1,66	26,49±1,38	22,32±0,68

Note: * $p \leq 0,001$, ** $p \leq 0,01$, *** $p \leq 0,05$, compared to the control

At the beginning of the withdrawal period in femoral muscles, an opposite tendency was observed: the content of essential amino acids in the first experimental group of broiler chickens was by 24,8 % higher than their content in the first control group. and at the end of the half-withdrawal period, it was by 24,4 % higher than in the first control group.

At the beginning of the withdrawal period, the content of the substitutable amino acids in pectoral muscles of the poultry of the first experimental group was by 8,8 % higher, compared with the first control group. After the elimination period, the content of the substitutable amino acids in pectoral muscles of broiler chickens of the first experimental group exceeded the indicators of the first control group by 7,1 %. at the beginning of the withdrawal period, the content of the substitutable amino acids in femoral muscles of broiler chickens of the first experimental group was by 36,3 % higher than their content in the first control group. at the end of the withdrawal period, the content

of the substitutable amino acids in femoral muscles exceeded the one of the first control group by 28,5 %.

At the beginning of the withdrawal period, the content of essential amino acids in pectoral muscles of broiler chickens of the second experimental group (have been fed with tilotsiklinvet) was by 33,7% higher, compared to the second control group (Table 5).

Tab.5. Amino acid content in muscles of broiler chickens, which have been fed with tilotsiklinvet, g/100g of meat, M±m; n=6

Indicator	Withdrawal period	Broiler chickens groups			
		Second experimental group (tilotsiklinvet)		Second control group	
		Pectoral muscles	Femoral muscles	Pectoral muscles	Femoral muscles
1	2	3	4	5	6
Essential amino acids					
Valine	3 hours	1,08±0,085	1,16±0,062***	1,33±0,099	1,40±0,055
	9 days	0,99±0,065***	1,13±0,047***	1,31±0,095	1,37±0,069
Isoleucine	3 hours	1,57±0,220	1,06±0,043**	1,13±0,021	1,26±0,036
	9days	1,54±0,220	1,05±0,044***	1,18± 0,066	1,28±0,060
Leucine	3 hours	2,07±0,344	1,80±0,085	1,26±0,203	1,53±0,100
	9days	2,02±0,369	1,79±0,069***	1,23± 0,246	1,56±0,041
Lysine	3 hours	2,32±0,131*	2,01±0,157	1,31±0,070	2,25±0,055
	9days	2,30±0,205**	1,94±0,108***	1,29± 0,097	2,26±0,045
Methionine	3 hours	0,13±0,040*	0,22±0,040***	0,46±0,063	0,10±0,018
	9days	0,09±0,045*	0,20±0,051	0,48± 0,044	0,09±0,026
Threonine	3 hours	1,73±0,403	1,16±0,038	0,97±0,123	1,15±0,082
	9days	1,70±0,408	1,13±0,070	0,90±0,142	1,17±0,060
Phenylalanine	3 hours	1,70±0,356	1,07±0,097	1,14±0,087	1,02±0,121
	9days	1,65±0,368	1,04±0,119	1,07± 0,103	0,97±0,089
Tryptophan	3 hours	0,19±0,258	0,24±0,046**	0,38±0,084	0,51±0,025
	9days	0,17±0,288	0,22±0,093***	0,39±0,078	0,54±0,061
Total number of essential amino acids	3 hours	10,67±1,93	8,72±0,59	7,98±0,75	9,22±0,59
	9days	10,46±2,07	8,49±0,60	7,77±0,87	9,13±0,45
Substitutable amino acids					
Alanine	3 hours	2,21±0,462***	1,16±0,026	0,98±0,140	1,20±0,043
	9days	2,04±0,596	1,12±0,019***	0,96± 0,133	1,19±0,015
Arginine	3 hours	1,12±0,049	0,083±0,093**	1,29±0,063	1,18±0,025
	9days	1,09±0,037***	0,80±0,085**	1,27±0,053	1,17±0,040
Aspartic acid	3 hours	2,55±0,117***	2,19±0,084	2,17±0,064	2,25±0,078
	9days	2,50±0,168	2,15±0,078	2,19± 0,054	2,25±0,056
Histidine	3 hours	2,20±0,207***	1,09±0,156	1,57±0,100	1,30±0,107
	9days	2,18±0,280	1,07±0,153	1,54±0,147	1,27±0,131
Glycine	3 hours	1,06±0,089	1,09±0,145	1,84±0,394	1,37±0,049
	9days	1,03±0,115	1,05±0,095***	1,84±0,376	1,36±0,056

Glutamic acid	3 hours	3,39±0,331	3,31±0,162	4,16±0,309	3,66±0,071
	9days	3,34±0,342	3,28±0,145***	4,17± 0,310	3,68±0,049
Proline	3 hours	1,08±0,216	0,97±0,172	0,61±0,138	0,81±0,118
	9days	1,02±0,211	0,93±0,159	0,59± 0,142	0,80±0,111
Serine	3 hours	1,14±0,043***	0,99±0,110	1,32±0,046	1,19±0,072
	9days	1,11±0,030***	0,93±0,055***	1,30±0,056	1,18±0,063
Tyrosine	3 hours	1,24±0,201	1,01±0,090***	0,73±0,149	0,71±0,055
	9days	1,20±0,212	0,96±0,068***	0,75±0,136	0,72±0,048
Oxyproline	3 hours	0,02±0,004	0,02±0,004*	0,06±0,004	0,06±0,004
	9days	0,02±0,004***	0,01±0,004*	0,06±0,004	0,06±0,004
Total number of substitutable amino acids	3 hours	16,01±1,72	12,31±1,04	14,73±1,41	13,73±0,62
	9days	15,53±1,99	11,91±0,88	14,68±1,41	13,68±0,57
The ratio of essential to substitutable	3 hours	0,67	0,71	0,54	0,67
	9days	0,67	0,71	0,53	0,67
Total number of essential+ substitutable	3 hours	26,68±3,65	21,03±1,63	22,71±2,16	22,95±1,21
	9days	25,99±4,06	20,04±1,48	22,45±2,28	22,81±1,02
The ratio of tryptophan to oxyproline	3 hours	9,5	12	6,3	8,5
	9days	8,2	11	6,5	9
Total protein content	3 hours	25,40±0,38	21,09±0,82	25,94±1,03	23,19±0,94
	9days	25,35±0,35	21,06±0,82	25,95±1,04	23,18±0,94

Note: * p<0,001, ** p<0,01, *** p<0,05, compared to the control

At the end of the elimination period, their content in the second experimental group exceeded the second control group by 34,6 %. However, at the beginning of the withdrawal period, in femoral muscles of chicken broilers of the second experimental group, the content of essential amino acids was by 5,4 % lower than in the second control group. After the withdrawal period, the content of essential amino acids in a meat of broiler chickens of the second experimental group was by 7,0 % lower, in comparison with the second control group.

As for the content of the substitutable amino acids, at the beginning of the withdrawal period, in pectoral muscles of broiler chickens of the second experimental group it was by 8,7 % higher, than in the second control group. After the withdrawal period, in pectoral muscles of the poultry of the second experimental group, their number exceeded the second control group by 5,8 %.

At the beginning of the withdrawal period, they observed a totally different situation in femoral muscles: the content of the substitutable amino acids was by 10,4 % lower, compared to the second control group. and after the elimination period, in femoral muscles of broiler chickens of the second experimental group, their content was by 13,0 % lower, compared with the second control group.

A comparative analysis of the content of amino acids in a meat of broiler chickens in experimental and control groups suggests, that pharmazin and tilotsiklinvet have very selective influence on the amino acid metabolism in the body. Thus, pharmazin (in both femoral and pectoral muscles) and tilotsiklinvet (in pectoral muscles) improve the amino acid metabolism in the body of a poultry.

It is known, that the complete proteins are determined by the content and ratios of essential and substitutable amino acids. in our case, the ratio of the content of essential to substitutable amino acids in pectoral and femoral muscles of broiler chickens in experimental groups, at the beginning of the withdrawal period was: in the first experimental group – 0,61 and 0,67, respectively; in the second – 0,67 and 0,71. Similar indicators in the first control group of broiler chickens were 0,72 and 0,73, respectively; and in the second – 0,54 and 0,67.

After the withdrawal period, the ratio of the content of essential to substitutable amino acids in pectoral muscles of broiler chickens in both experimental groups was: in the first experimental group – 0,62 and 0,67, respectively; in the second – 0,67 and 0,71. These indicators in the first control group of broiler chickens were 0,73 and 0,70, respectively; and in the second – 0,53 and 0,67.

At this stage, the calculation of the amino acid A/E index was carried out, and it have been found that, compared to the FAO/WHO scale, there is a difference between the indicators that were determined (Table 6).

The index of sulfur-containing amino acids (methionine+cystine) in a meat of broiler chickens in the experimental groups is relatively low in relation to the FAO/WHO scale. Since these changes are due to the complete disintegration of cystine during the research process, so the calculation of the index in the experimental groups was carried out using methionine indicators.

There is an increase in the index of aromatic (phenylalanine+tyrosine) amino acids. Also, the valine index decreases, the isoleucine and leucine index increases, but these changes are negligible. we can also observe an increase in the threonine index in relation to the FAO/WHO scale. in addition, there is an increase in the amino acid index of lysine.

Tab.6. Amino acid A/E index of the essential amino acids in pectoral and femoral muscles of broiler chickens, which have been fed with pharmazin and tilotsiklinvet, mg/100 g of meat

Group		Withdrawal period	Index						
			Valine	Isoleucine	Leucine	Lysine	Methionine	Threonine	Phenylalanine+ Tyrosine
FAO/WHO scale*			139	111	194	153	97	111	167
First experimental group(pharmazin)	pectoral muscles	3 hours	121	127	208	231	11	121	228
		6–9 days	119	123	205	234	13	120	231
	femoral muscles	3 hours	123	135	223	219	36	123	215
		6–9 days	122	137	221	224	34	123	212
First control group	pectoral muscles	3 hours	126	145	222	239	31	126	155
		6–9 days	130	148	225	238	29	128	165
	femoral muscles	3 hours	120	125	187	259	9	134	188
		6–9 days	128	132	187	258	10	131	191
Second experimental group (tilotsiklinvet)	pectoral muscles	3 hours	101	147	194	217	12	162	275
		6–9 days	95	147	192	220	8	162	272
	femoral muscles	3 hours	133	121	206	230	25	133	238
		6–9 days	133	124	211	228	23	133	235
Second control group	pectoral muscles	3 hours	166	142	159	164	58	121	234
		6–9 days	168	152	158	166	62	116	234
	femoral muscles	3 hours	152	136	166	244	11	125	188
		6–9 days	150	140	171	247	9	128	185

Note. *compared to the control protein according to the FAO/WHO scale, 1974

The amino acid score of proteins in the experimental and control groups in pectoral and femoral muscles of broiler chickens is increased for isoleucine and aromatic (phenylalanine + tyrosine) amino acids, compared to the control protein, according to the FAO/WHO scale (Table 7).

The reduction of amino acid score of valine was determined in the first and second experimental groups in pectoral muscles of the poultry; also in the first control group in femoral muscles at the beginning and at the end of the withdrawal period. Leucine content decrease was determined in the second control group in both pectoral and femoral muscles of broiler chickens. In addition, lysine and threonine content decrease is observed in pectoral muscles. A decrease in amino acids score of sulfur-containing (methionine + cystine) amino acids is observed in all experimental and control groups of broiler chickens, due to the complete disintegration of cystine.

Based on our research, we believe that the amino acid that limits the biological value of the protein and the score of which is the least important, is methionine. That means, that this is the amino acid that determines the degree of the use of a certain protein in a body.

Tab.7. Amino acid score of protein in a meat of broiler chickens, which have been fed with pharmazin and tilotsiklinvet, %

Group		Withdrawal period	Indicator						
			Valine	Isoleucine	Leucine	Lysine	Methionine	Threonine	Phenylalanine+ Tyrosine
FAO/WHO scale *			5,0	4,0	7,0	5,5	3,5	4,0	6,0
First experimental group (pharmazin)	pectoral muscles	3 hours	94	123	115	163	12	118	147
		6-9 days	90	117	111	162	14	114	146
	femoral muscles	3 hours	111	153	144	181	47	140	160
		6-9 days	109	152	140	181	43	137	157
First control group	pectoral muscles	3 hours	105	155	132	180	36	131	107
		6-9 days	108	150	134	180	34	134	115
	femoral muscles	3 hours	91	119	102	179	10	128	119
		6-9 days	96	122	100	175	11	122	118
Second experimental group (tilotsiklinvet)	pectoral muscles	3 hours	85	154	116	166	14	170	193
		6-9 days	78	152	113	165	10	167	187
	femoral muscles	3 hours	110	126	122	173	30	138	165
		6-9 days	107	124	121	167	27	134	158
Second control group	pectoral muscles	3 hours	102	109	69	92	49	93	120
		6-9 days	101	114	68	90	53	87	117
	femoral muscles	3 hours	121	136	94	176	12	124	124
		6-9 days	118	138	96	177	11	126	122

Note. *relative to the control protein on the FAO/WHO scale, 1974

Also, protein value of meat was determined by the level of the content of tryptophan (the higher the level of tryptophan in meat is, the more valuable protein is in it). the protein-quality index of femoral and pectoral muscles in the first experimental group of broiler chickens, which have been fed with pharmazin, at the beginning of the withdrawal period exceeded the indicators of the first control group at 1,7g/100g and 5,2g/100g, respectively. at the end the elimination period, the protein-quality index of the pectoral and femoral muscles of broiler chickens of the first experimental group exceeded the indicators of the first control group at 1,9 g/100g and 4,2g/100g, respectively.

The protein-quality indicators in pectoral muscles of broiler chickens of the second experimental group (have been fed with tilotsiklinvet), at the beginning of the withdrawal period exceeded the indicators of the first control group, compared to the second control group, – at 3,2g/100g; and in femoral muscles – at 3,5g/100g. at the end of the half-withdrawal period, this indicator in pectoral muscles exceeded the second control group at 1,7g/100g, and in femoral muscles – at 2g/100g.

Thus, the ratio of the content of tryptophan to oxyproline in the muscles of broiler chickens in experimental groups increases compared to those indicators in the control groups, which evidences about the decrease of connective tissue and an increase of the biological value of a poultry meat.

The total content of protein in pectoral muscles of chicken broilers in the first control group exceeded its content in the experimental group (have been fed with pharmazin) at the beginning of the withdrawal period by 0,6 %, and at the end – by

0,7 %. in femoral muscles of a poultry, on the contrary, the index of the first experimental group exceeded the first control group at the beginning of the elimination period by 4,6 %, and at the end of the withdrawal period – by 4,5 %.

The total content of protein in pectoral muscles of chicken broilers in the second control group exceeded the indicator of the second experimental group at the beginning of the withdrawal period by 2,08 %, and after the elimination period – by 2,3 %. the total content of protein in femoral muscles in the second control group exceeded the indicator of the second experimental group at the beginning of the withdrawal period by 9,05 %, and upon its completion – by 9,1 %.

4. DISCUSSION

Studies on the biological value of broiler chickens' meat of the experimental group, which have been fed with "ProbiX" additive, was performed due to the need to establish the biological value of protein, which is balanced by the amino acid composition and meets the needs of the human body in amino acids for the synthesis of protein.

It was found, that when using "ProbiX" feed additive, there is a significant decrease in the content of threonine and tryptophan, compared to the control; and a tendency towards a decrease in the content of methionine+cystine in femoral muscles of broiler chickens is observed. There is a significant decrease in the content of valine, tryptophan and isoleucine in pectoral muscles of broiler chickens, compared to the control.

the content of the substitutable amino acids in a white meat of broiler chickens of the first experimental group decreases, compared to the control, at the expense of such amino acids as glycine, alanine, glutamic acid and proline.

The conducted researches have shown that the use of "ProbiX" feed additive causes a significant increase in the content of essential amino acids ($p \leq 0,001$) in femoral muscles by 4,78 %, in comparison with the control, which leads to an increase in their biological value. In pectoral muscles, the content of essential amino acids tends to increase by 0,64 %, compared to the control. Probably these processes are due to the improved digestion of feed in the intestines of chicken broilers under the influence of "ProbiX" feed additive; and, as a result, more intense assimilation of nutrients in a poultry body.

We have experimentally confirmed that the ratio of essential to substitutable amino acids, as an index of assimilation of amino acids in samples of meat of the experimental group, is higher than the control indicators. The ratio of tryptophan to oxyproline in broiler chickens' meat samples of the experimental group is characterized by a tendency to increase the content of tryptophan, and decrease the content of oxyproline, compared to the control.

Analysis of the results of amino acids score gives grounds for asserting that, when using "ProbiX" feed additive, the amino acid composition of broiler chickens' meat approaches the ideal protein indicators, according to the FAO/WHO scale. There are no limiting amino acids in a broiler chickens' meat of both control and experimental groups.

At the second stage of the study, it was found that the content of essential and substitutable amino acids, amino acids score in the muscles of broiler chickens in experimental groups (have been fed with pharbazin and tilotsiklinvet), at the beginning of the withdrawal period exceeds the indicators obtained at the end of the half-withdrawal period (6–9 days). When using pharbazin, the ratio of essential to substitutable amino acids in pectoral muscles, at the beginning and at the end of the withdrawal period, is reduced by 15,3 and 15,0 %, respectively. A similar indicator at the beginning of the withdrawal period in femoral muscles is reduced by 8,2 %; and at the end of the elimination period – by 4,3 %, compared to the first control group. When using tilotsiklinvet, the ratio of essential to substitutable amino acids in pectoral muscles, on the contrary, increases at the beginning of the withdrawal period by 24,1 %, and after the elimination period – by 26,4 %; in femoral muscles, at the beginning and at the end of the withdrawal period – by 6 %, compared to the second control group.

The calculation of the amino acid A/E index made it possible to establish that the index of sulfur-containing amino acids (methionine+cystine) in the muscles of broiler

chickens in experimental groups was rather low, and an increase of the aromatic (phenylalanine+tyrosine) amino acids index was found. The valine index decreases, the isoleucine and leucine index increases. There is also an increase of the threonine and lysine index.

The amino acid score of valine in pectoral muscles of broiler chickens of the experimental group (have been fed with pharbazin), at the beginning of the withdrawal period, decreases by 6 %; and at the end of this period, on the 6th day after the last antibiotic has been used, – by 10 %. The amino acid score of valine in pectoral muscles of broiler chickens of the experimental group (have been fed with tilotsiklinvet), at the beginning of the elimination period, decreases by 15 %, and on the 9th day after the last antibiotic has been used, – by 22 %. Based on the research carried out, the amino acid which limits the biological value of the protein – methionine, has been found. The ratio of the content of tryptophan to oxyproline in the muscles of broiler chickens in experimental groups increases compared to the indicators of control groups, which indicates a decrease in the amount of connective tissue, and an increase in the biological value of a poultry meat.

CONCLUSION

1. Use of "ProbiX" feed additive makes for an increase in the content of essential amino acids in femoral muscles by 4,78 % ($p \leq 0,001$), compared to the control. The ratio of essential to substitutable amino acids, and tryptophan to oxyproline in the experimental group is slightly higher in comparison with the control.

2. It was found that the use of pharbazin causes a decrease in the ratio of essential to substitutable amino acids in femoral and pectoral muscles of broiler chickens by 15,1 and 6,2 %, respectively, at the beginning and at the end of the withdrawal period. Use of tilotsiklinvet causes the inverse tendency: the ratio of essential to substitutable amino acids in the studied muscles increases – by 25,2 and 6 %, respectively. The amino acid which limits the biological value of the protein – methionine, has been found. The ratio of the content of tryptophan to oxyproline in the muscles of broiler chickens in experimental groups increases compared to the indicators of control groups, which indicates a decrease in the amount of connective tissue, and an increase in the biological value of poultry meat.

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