

# Changes in the biochemical composition of broiler body tissues when feeding with *Bacillus cereus* probiotic and coumarin

Galimzhan Duskaev, Baer Nurzhanov, and Shamil Rakhmatullin \*

Federal Research Centre of Biological Systems and Agrotechnologies of the RAS, 29, 9 Yanvarya St., Orenburg 460000, Russia

**Abstract.** The article presents the study results on the effect of probiotic (*Bacillus cereus* IP 5832) and coumarin on the chemical and amino acid composition of body tissues when fed to broiler chickens together with the main diet. According to the experimental results, an increase in the concentration of dry matter by 0.27% and crude fat by 0.75% was found in the liver of young animals from the I experimental group compared to the control. At the end of the experiment, the broilers from the control group were inferior in the protein amount in the liver of the experimental poultry by 0.46%, 2.69 ( $P \leq 0.05$ ) and 1.16%. A similar pattern was observed when studying pectoral muscles' chemical composition on the 16 and 29 day study period; the accumulation of dry matter and protein in the poultry's pectoral muscles from the III experimental group by 0.7 and 0.71%; 0.89 and 1.21% relative to the control was revealed. Feeding broilers with a basic diet plus coumarin contributed to the accumulation of essential amino acids in their liver: lysine – by 1.05%, phenylalanine – by 0.41% and leucine-isoleucine by 0.63% relative to the control.

## 1 Introduction

In most countries of the world, food security is based on the production of poultry meat as this process is much faster compared to other industries. An aid in stimulating the pace of meat production, as well as to preserve the physical condition of animal, was previously performed by feed antibiotics given in the preventive daily dose. However, public health began to record the negative effect of their application since their long-term use contributed to addiction and a decrease in the effect [1, 2].

To this day, the search for new products with a similar effect and their application methods remain one of the pressing problems for the entire poultry industry. Therefore, much attention should be paid to probiotics and active substances isolated from medicinal plants [3, 4]. It should be noted that medicinal plants contain a significant amount of various

---

\* Corresponding author: [baer.nurzhanov@mail.ru](mailto:baer.nurzhanov@mail.ru)

biologically active substances including coumarins [5]. The potential role of coumarins as agents positively affecting muscle tissue is emphasized [6, 7].

It is essential to consider the synergism of the active components isolated from phytobiotics in combination with other additives in more detail, particularly probiotics. There are probiotic strains of *Bacillus* used as animal feed additives [8], although the mechanism of individual strains' action has not been fully studied. The action mechanism of complex additives when administered orally to ruminants and poultry is based not only on the suppression of pathogenic microflora, but also on the overall positive effect on the digestive system as a whole. Together with plant extracts, probiotic preparations belong to natural growth stimulants and can become a large-scale replacement of antibiotic growth stimulants in poultry farming [9].

## 2 Materials and Methods

The object of the study was 7-day-old broiler chickens (Arbor Acres cross); the experiment period - 42 days (n=200, 4 groups). The *Bacillus cereus* IP 5832 probiotic and 7-hydroxycoumarin were used in the experiment. The keeping and procedure for poultry keeping - in the implementation of instructions and recommendations of Russian regulatory documents (Order of the Ministry of Health of the USSR No. 755 dated 12.08.1977) and the "Guide for the Care and Use of Laboratory Animals" (National Academy Press, Washington, DC, 1996).

The young poultry were raised in group sections with unlimited access to feed and water. Young broilers from the base group (control) were kept on the main diet - MD, I experimental group - MD+*Bacillus cereus* (dose of  $12.6 \cdot 10^3$  microbial bodies/kg of feed/day), II experimental - MD+coumarin (dose of 2 mg/kg of feed per day), III experimental - MD+*Bacillus cereus*+coumarin.

The content of the main nutrients was determined in tissue samples (liver, muscle tissue) after slaughter, namely: dry matter, protein, fat, fiber, ash, amino acid composition of tissues - according to standardized methods in the Center for Collective Use of Biological Systems and Agrotechnologies of the Russian Academy of Sciences. The analysis of the elemental tissues' composition was determined by mass spectrometry with inductively coupled plasma and atomic emission spectrometry with inductively coupled plasma on the Nexion 300D quadrupole mass spectrometer and Optima 2000 DV atomic emission spectrometer.

Statistical analysis of the obtained data was carried out using the Statistica 10RU package.

## 3 Results and Discussion

When analyzing the obtained digital material, a decrease in the concentration of dry matter and fat in the liver tissue was found in the second experimental group by 5.34 and 0.97% compared with the poultry receiving the main one (Table 1).

**Table 1.** Chemical substances content (43 days), %

Indicator	Moisture	Dry matter	Fat	Protein	Ash
Liver					
control	73,99± 0,18	26,01± 0,30	3,63± 0,49	21,42± 0,41	0,96± 0,01
I experimental	75,74± 0,37*	24,26± 0,46	3,78± 0,51	19,52± 0,66	0,96± 0,01
II experimental	79,33±	20,67±	2,66±	17,04±	0,97±

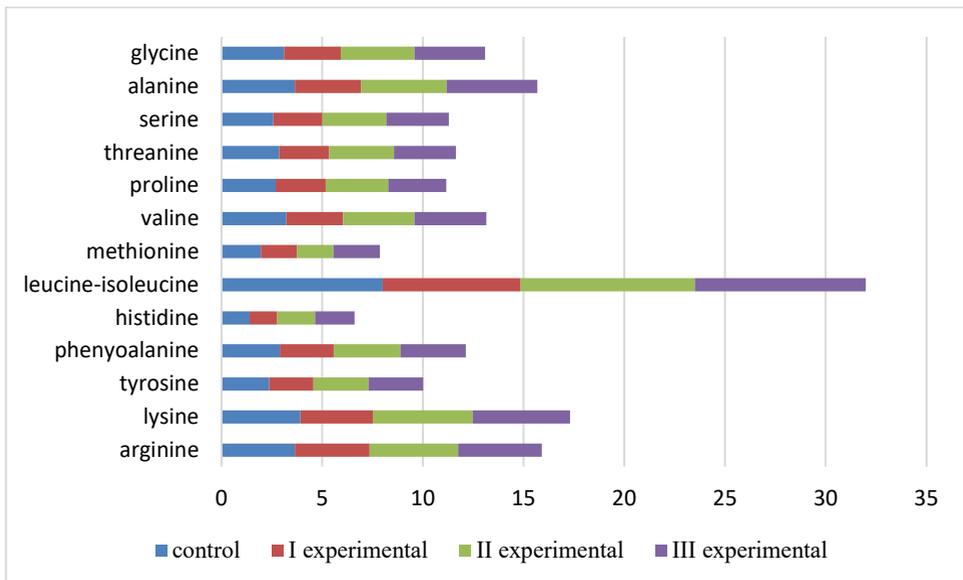
	0,46**	0,58	0,77	0,74	0,01
III experimental	76,29± 0,40*	23,71± 0,42	3,1± 0,73	19,64± 0,60	0,97± 0,01
Pectoral muscles					
control	77,21± 0,24	22,79± 0,18	1,01± 0,60	20,79± 0,40	0,99± 0,01
I experimental	76,68± 0,33	23,32± 0,56	0,83± 0,88	21,5± 0,37	0,99± 0,02
II experimental	77,11± 0,29	22,89± 0,51	1,01± 0,97	20,89± 0,72	0,99± 0,02
III experimental	77,26± 0,36	22,74± 0,28	0,99± 0,63	20,76± 0,44	0,99± 0,01

On average, all the main indicators in the liver of experimental broilers decreased relative to the control at the end of the experiment, and the amount of moisture significantly increased by 1.75%, 5.34%, and 2.30%.

Analyzing the data on the pectoral muscles' chemical composition at the beginning of the experiment and its middle, an increase in the amount of dry matter and protein in the poultry's pectoral muscles from the III experimental group was established by 0.7 and 0.71%; 0.89 and 1.21% relative to the control.

Over the entire experimental period, the pectoral muscles of broilers from the experimental groups contained less fat relative to the control. By the 43rd day of the experiment, the best parameters of dry matter and protein in the muscles were shown by young poultry receiving the *Bacillus cereus* probiotic (group I) with the main diet by 0.53 and 0.71%, respectively, in comparison with the control poultry.

The analysis of the amino acid profile of broiler chickens' liver revealed accumulation features of interchangeable and essential acids (Fig. 1).



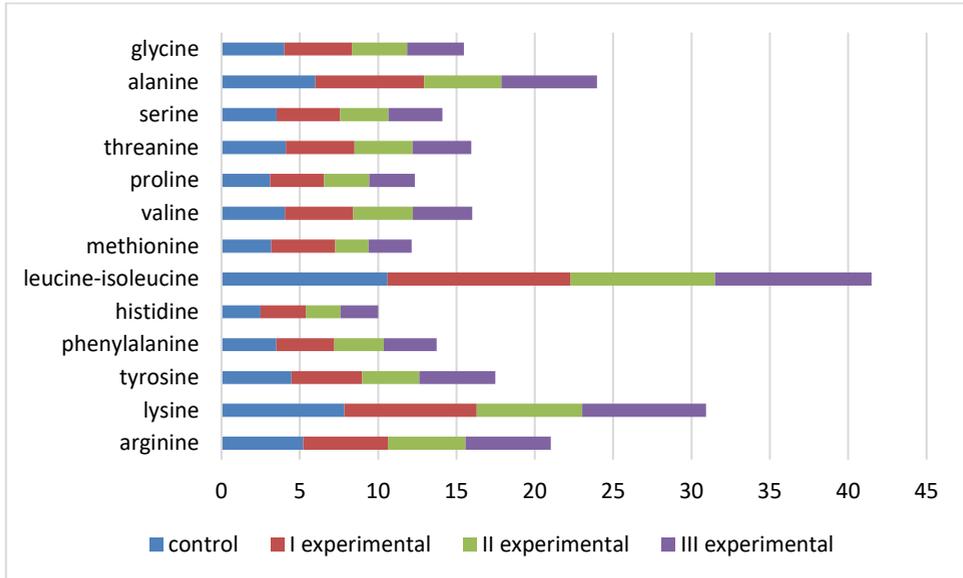
**Fig. 1.** Amino acid composition of the liver (day 43), %

Thus, in terms of the content of lysine, phenyoalanine and leucine-isoleucine in the liver, broilers from group II exceeded their peers from the control group by 1.05%, 0.41, and 0.63%, respectively. In terms of interchangeable amino acids' amount in the liver, the

advantage was also on the side of the young poultry from group II; for arginine - by 0.73%, tyrosine - by 0.36%, proline - by 0.41%, serine - by 0.59%, and alanine - by 0.56% relative to the control.

The liver of young poultry from the III experimental group contained 0.56% more histidine and 0.86% more alanine compared to the control group. The lowest concentration of histidine and alanine was found in the liver of the first group poultry compared to the control, II and III groups, respectively, by 2.18%, 36.49 and 43.06%; 10.97%, 29.87 and 37.19%.

An important indicator of broiler meat's full-value is the amino acid composition (Fig. 2).



**Fig. 2.** Amino acid composition of the pectoral muscles (day 43), %

According to the remaining interchangeable amino acids in the muscle, the advantage was for the poultry in group I: histidine - by 0.39%, proline - by 0.34%, serine - by 0.55%, alanine - by 0.93%, and glycine - by 0.34% relative to the control.

Broiler chickens in group I had the highest concentration of lysine in the pectoral muscles – 0.61%, phenylalanine – 0.21%, leucine-isoleucine – 1.08%, methionine – 1.03%, valine – 0.32% and threonine - 0.28% compared to analogues from the control group.

According to the study results, a favorable effect of *Bacillus cereus* on the protein content in the pectoral muscle was noted, which is consistent with previous studies [10]. There was also a concentration increase of dry matter, crude protein, and essential extract in chicken thighs and breast when feeding black cumin seeds (*Nigella sativa*) containing coumarins [11]. In the conducted experiment, this effect was less noticeable.

There were no changes in the amount of protein and the composition of amino acids in the coumarin groups, although there is evidence of a beneficial effect of this substance on the muscle protein balance. It is assumed that a possible action mechanism in this case may be a lower level of oxidative stress and better mitochondrial function [12-14].

There is data [15, 16] on coumarins' ability to suppress oxidative stress in the liver, which probably had a positive effect on the amino acid composition of this organ's tissues in the second and third groups. In addition, coumarin and coumarin-related compounds have also been found to be superior to ascorbic acid in terms of their antioxidant activity.

## 4 Conclusion

As a result of the study, it was found that in the liver and pectoral muscles of broilers, the concentration of essential and interchangeable amino acids increases when feeding probiotic and coumarin compared to the control group.

## Acknowledgments

The studies were performed in accordance with the plan of research works Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences 0761-2019-0005.

## References

1. World Health Organization, Antimicrobial Resistance: Global Report on Surveillance Geneva: World Health Organization (2014)
2. F. Aarestrup, *Nature* **486** (7404), 465–466 (2012)
3. A.S. Vasilchenko, A.V. Vasilchenko, T.M. Pashkova, M.P. Smirnova, N.I. Kolodkin, I.V. Manukhov, G.B. Zavilgelsky, E.A. Sizova, O.L. Kartashova, A.S. Simbirtsev, E.A. Rogozhin, G.K. Duskaev, M.V. Sycheva, *J Pept Sci. Dec* **23(12)**, 855-863 (2017) doi: 10.1002/psc.3049. PMID: 29193518.
4. I. Karimov, K. Kondrashova, G. Duskaev, O. Kvan, *E3S Web of Conferences* **143**, 02034 (2020) doi: 10.1051/e3sconf/202014302034.
5. W. Kchaou, F. Abbès, R.B. Mansour, C. Blecker, H. Attia, and S. Besbes, *Food Chemistry* **194**, 1048–1055 (2016)
6. L. Peng, X. Huang, X. Jin, Z. Jing, L. Yang, Y. Zhou, J. Ren, Y. Zhao, *Exp Gerontol. Oct 1* **96**, 73-81 (2017) doi: 10.1016/j.exger.2017.06.011.
7. S.H. Park, D.S. Kim, J. Oh, J.H. Geum, J.E. Kim, S.Y. Choi, J.H. Kim, J.Y. Cho, *Am J Chin Med.* **49 (6)**, 1493-1514 (2021) doi: 10.1142/S0192415X21500701
8. X. Tang, X. Liu, H. Liu, *Front Vet Sci. Nov 22* **8**, 767802 (2021) doi: 10.3389/fvets.2021.767802.
9. P.J. Delaquis, K. Stanich, B. Girard, G. Mazza, *Int. J. Food Microbiol.* **74(1-2)**, 101-109 (2002) doi: 10.1016/S0168-1605(01)00734-6
10. F.J. Reen, J.A.Gutiérrez-Barranquero, M.L. Parages and F. O’Gara, *Appl Microbiol Biotechnol* **102(5)**, 2063–2073 (2018)
11. P. Kumar, A.K. Patra, G.P. Mandal, B.C. Debnath, *J Anim Physiol Anim Nutr (Berl) Jun* **102(3)**, 769-779 (2018) doi: 10.1111/jpn.12880.
12. M. Francaux, L. Deldicque *Curr Opin Clin Nutr Metab Care.* May **21(3)**, 159-163 (2018) doi: 10.1097/MCO.0000000000000455. PMID: 29356695.
13. J. Rodriguez, N. Pierre, D. Naslain, F. Bontemps, D. Ferreira, F. Priem, L. Deldicque, M. Francaux, *J Cachexia Sarcopenia Muscle.* Aug **8(4)**, 583-597 (2017) doi: 10.1002/jcsm.12190.
14. J. Wang, W. Zhang, M. Li, X. Li *Biomed Pharmacother.* May **137**, 111293 (2021) doi: 10.1016/j.biopha.2021.111293.
15. A. Özkaya, K.Türkan, *Acta Chim Slov. Mar* **68(1)**, 222-228 (2021)
16. A.A. Al-Amiery, Y.K. Al-Majedy, A.A.H. Kadhum and A.B. Mohamad *Scientific Reports*, vol. **5**, Article ID 11825 (2015)