



FORMATION OF MUSCLE FIBERS IN BROILER CHICKENS WHEN ADDING A BETULIN-BASED PHYTOBIOTIC AND PROBIOTIC INTO THE DIET

U.I. Kundryukova¹, M.V. Novikova^{2*}, L.I. Drozdova¹, I.A. Lebedeva², A.V. Buhler²

¹ Department of Morphology and Expertise, Ural State Agrarian University, Ekaterinburg, RUSSIA.

² Industrial Poultry Department, Ural Federal Agrarian Research Center of the Ural Branch of the Russian Academy of Sciences, Ekaterinburg, RUSSIA.

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ABSTRACT

This scientific study presents the introduction of a feed additive based on betulin in various concentrations and together with a probiotic-based on *Bacillus subtilis* bacteria into the diet of broiler chickens. The introduction of natural-like technologies with the use of new-generation phytobiotics into the technological cycle of broiler cultivation is very important for science and practice. Production tests were carried out on cross-308 broiler chickens. The positive effect of betulin-based supplements on the growth of body weight due to muscle fiber, and not due to fat deposition, was obtained. The indicators of the biological value of meat products and their technological properties have also improved. Detailed studies of the chest and leg muscles under a microscope were performed. Histological studies of muscle fiber samples revealed significant differences between the control group and the experimental groups. In the samples of experimental groups, where a phytobiotic based on betulin was used, the muscle tissue was more compact, with the completed process of maturation of the tissue and slightly formed fine-drop fat. In contrast to the control group samples, where the process of formation and maturation of muscle tissue (in the thoracic and femoral muscle groups) tended to incomplete maturation with large-drop obesity, which is confirmed by production indicators for the set of live weight with high indicators for fat deposition in the carcass. The use of betulin-based phytobiotics obtained by extraction from birch bark, which have a wide range of effects on the body, makes it possible for the poultry industry to abandon several feed antibiotics and introduce feed additives of domestic production for farm animals and birds.

Disciplinary: Biological Science, Poultry Science, Animal Nutrition, Food Science, Biotechnology.

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1. INTRODUCTION

Nowadays various ways to maintain health in the population of farm animals and birds are becoming increasingly important. People are looking for ways to prevent metabolic pathologies, stabilize the immune system and reduce the load from technological and feed stresses. The most common way to solve this problem is to use feed additives – adaptogens: probiotics, enzymes and antioxidants. In recent years there has been an active search for tools that would correct the negative effects of intensive technologies and at the same time would be biological elements of the natural environment, or as they are called – nature-like technologies (Byuler, 2017). These products include probiotics, natural sorbents and phytobiotics (betulin). The use of such tools does not violate the established technological processes and operations, fits well into the technological cycle and generally has a biological and economic effect (Motovilov, 2016; Kislitsyn, 1994).

Betulin can be found quite widely in nature, in the bark of birch (*Betula* is "birch" in Latin); betulin belongs to the group of triterpene alcohols.

Considering the multi-sided and high activity of betulin and its derivatives, the ability to transform into compounds involved in many major biochemical processes, it was found that betulin has several properties that can be effective in preventive and therapeutic veterinary measures (Byuler, 2017; Zadorozhnaya, 2011; Novikova, 2018).

Betulin has antiseptic, wound healing and anti-inflammatory properties in inflammatory processes of the gastrointestinal tract (anti-ulcer activity). The choleric and hepatoprotective activity of betulin makes it very popular for the prevention of liver diseases associated with alimentary factors (Lysko, 2010). Hepatoses are quite common in broilers and adult hens. The hypocholesterolemic and hypolipidemic activity of betulin and its compounds allows it to be used for the normalization of lipid metabolism (Byuler, 2019).

It is believed that some viral diseases of farm animals and birds are similar to cancer in their nature. Betulin and its derivatives have anti-tumor and anti-cancer properties. Betulinic acid is a selective inhibitor of cancer cell growth against a wide range of malignancies (Ignatiev, 2016; Byuler, 2017).

An interesting fact is that betulin has been described as having antibacterial activity against some streptococcal bacteria, but the antibacterial activity is low relative to the *Bacillus subtilis* bacteria. This fact suggests that betulin and its derivatives can be used together with probiotic drugs and probiotic feed additives containing *Bacillus subtilis* in their composition (Motovilov K., 2016, Byuler, 2017).

The purpose of this work is to study the effect of feed additives based on betulin and *Bacillus subtilis* on the live weight of broiler chickens of the Ross cross and on the process of muscle fiber formation.

2. RESEARCH MATERIAL AND METHODS

Experimental studies on the effect of betulin on the productivity and quality of meat were carried out on broiler roosters of the "Ross-308" cross. Groups were formed based on analogues (the control group and 1, 2, 3, 4 experimental groups). Dry betulin was added into the feed from 21-35 days of growth. A probiotic based on *Bacillus subtilis* together with betulin was given to broiler chickens of the four experimental groups. Other feeding and maintenance conditions were the same in all groups (Table 1).

Table 1: Research Scheme.

Group	Diet	Dosage of Betulin, mg/kg of live weight	Probiotic dosages, kg/ton of feed
Control	Basic diet (BD)	-	-
1	BD+Betulin	2.5	-
2	BD+Betulin	25	-
3	BD+Betulin	250	-
4	BD + Betulin+Probiotic	25	2

3. RESEARCH RESULTS

At the beginning and the end of the experiment, birds were weighed. The results for live weight and preservation are presented in Table 2.

Table 2: Live weight of chickens before and after the experiment (grams).

Age. days	Control groups	Group 1	Group 2	Group 3	Group 4
21	520.0±18.6	522.8±26.3	518.00±27.65	519.60±12.14	521.80±20.94
35	1924.8±44.9	1942.80±57.70	1911.40±38.10	1834.60±41.40	1767.60±36.75*
Weight gain	1404.8	1420.0	1393.4	1315.0	1246.8

* The difference with the control group is significant ($P \leq 0.01$).

The regularity of reducing live weight from the amount of betulin input was established: the more betulin there is, the less is the live weight. This is explained by its hepatoprotective properties. The live weight at the level of control values was noted in the 1st and 2nd experimental groups. However, a significant difference in live weight was found in the 4th experimental group, where a probiotic was given along with betulin, which increases the effect and reduces the deposition of fat in the body.

Livestock conservation indicators in all groups were at the level of 100%.

At the end of the experiment, a controlled slaughter of broiler chickens of 5 heads from each group was carried out by random sampling (following the recommendations of all-Russian research and Technological Institute of Poultry Farming, 2010) and samples of pectoral muscles were selected for the study of biological value, chemical analysis (Table 3) and histological studies.

Table 3: Biological value and chemical composition of the pectoral muscle.

Group	Tryptophan	Oxyproline	The tryptophan/oxyproline	Protein	Ash
Control	0.281±0.048	0.369±0.066	0.762	22.88 nitrogen 3.66	1.08±0.16
1	0.276±0.047	0.368±0.066	0.750	21.38 nitrogen 3.42	1.86±0.25
2	0.285±0.049	0.384±0.069	0.742	22.81 nitrogen 3.65	1.22±0.17
3	0.314±0.053	0.426±0.077	0.737	22.94 nitrogen 3.67	1.26±0.18
4	0.283±0.05	0.379±0.068	0.746	22.62 nitrogen 3.62	1.09±0.16

Analysis of the results obtained a direct correlation revealed: increasing the amount of betulin in the diet led to an increase in the content of tryptophan and oxyproline, where tryptophan characterizes the level of essential amino acids, and oxyproline characterizes the level of connective tissue. In terms of protein content, a positive trend was also detected when increasing the dosage of betulin: the

protein content increases, which is an important technological indicator of its value. The content of ash and protein in meat characterizes the number of macronutrients and nutrients, thus, betulin contributed to a greater accumulation of useful components compared to the control group. It should also be noted that the more fat accumulates between the muscle fibers, the lower the "ash" index is, which is confirmed by histological studies (Figure 1). The muscle cells of the pectoral muscles are united in separate groups with a clearly defined pattern of striated striation, and the longitudinal musculature is clearly expressed. Between groups of muscle cells, groups of adipose tissue with blood vessels are well expressed.

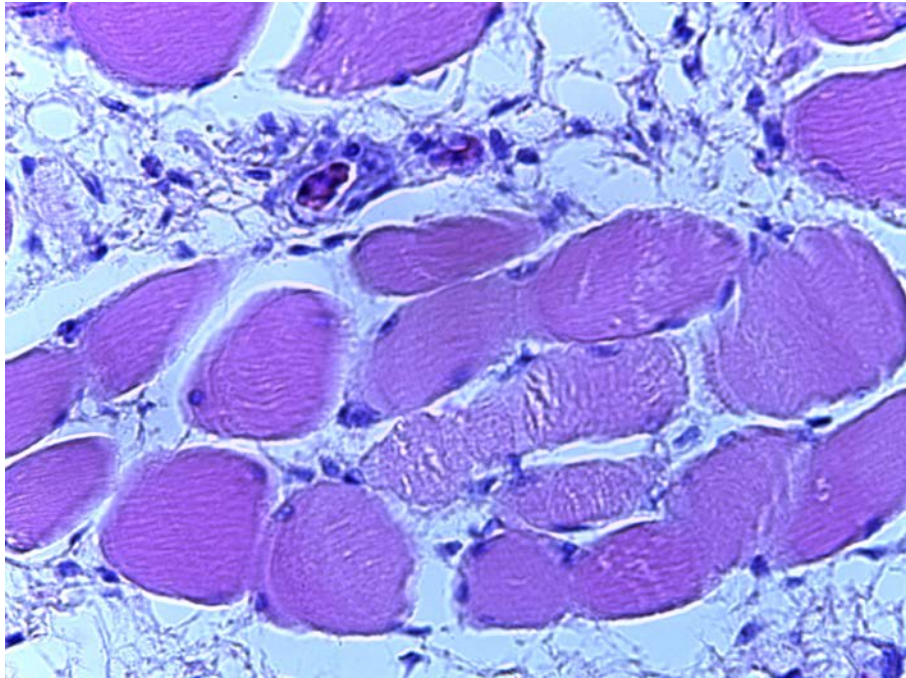


Figure 1: Pectoral muscles. Control group. Between groups of muscle cells, there are well-defined accumulations of fat (630X).

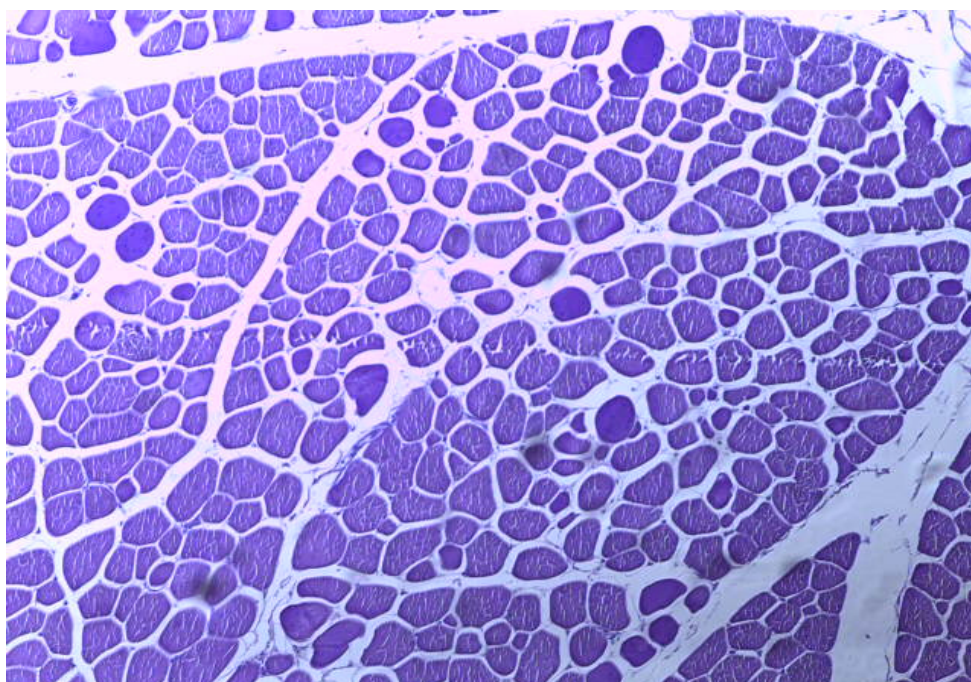


Figure 2: Compact formed bundles of muscle fibers of the femoral broiler muscle group with single immature muscle fibers. Experimental group. Stained with hematoxylin and eosin. (100X).

Histological studies of muscle fibers revealed significant differences between the control group and the experimental groups. The structure of the muscle tissue and interstitial connective tissue of chickens from the experimental groups, in contrast to the control group, was represented by a compact, with almost absolutely completed maturation process, muscle tissue, with small-drop fat, located perivascular (Figure 2). While in the control group, the process of formation and maturation of muscle tissue, both in the pectoral and femoral muscle groups, tended to incomplete maturation (Figure 3) with large-drop obesity of the interstitial connective tissue (Figure 4).

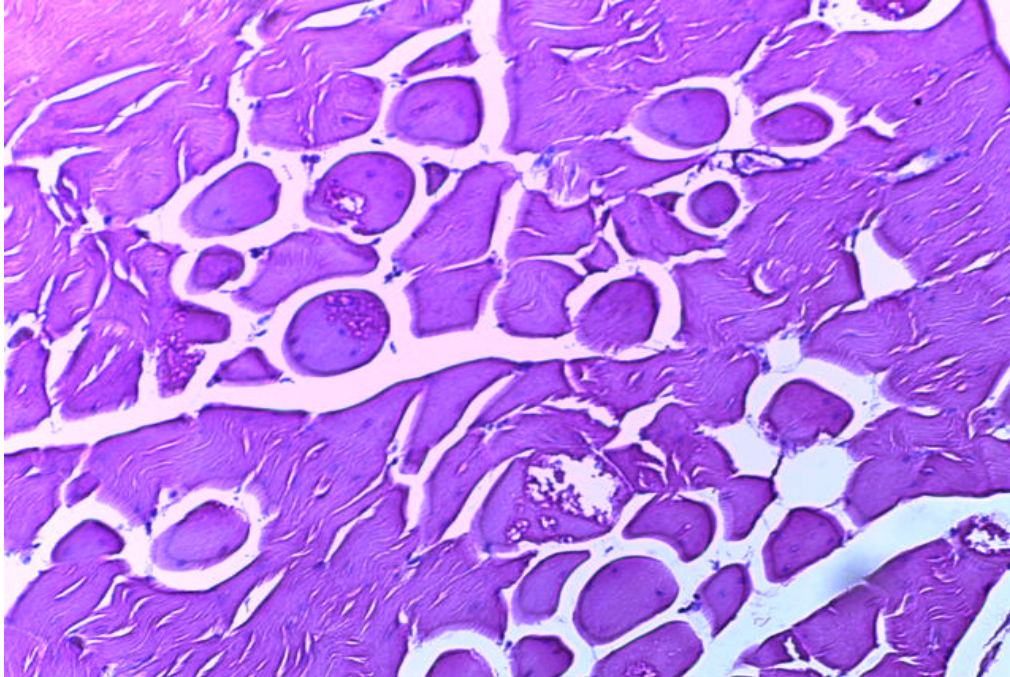


Figure 3: The femoral muscle group of a broiler chicken with a significant amount of immature muscle fibers with the breakdown of some of them. Control group. Stained with hematoxylin and eosin. (400X).

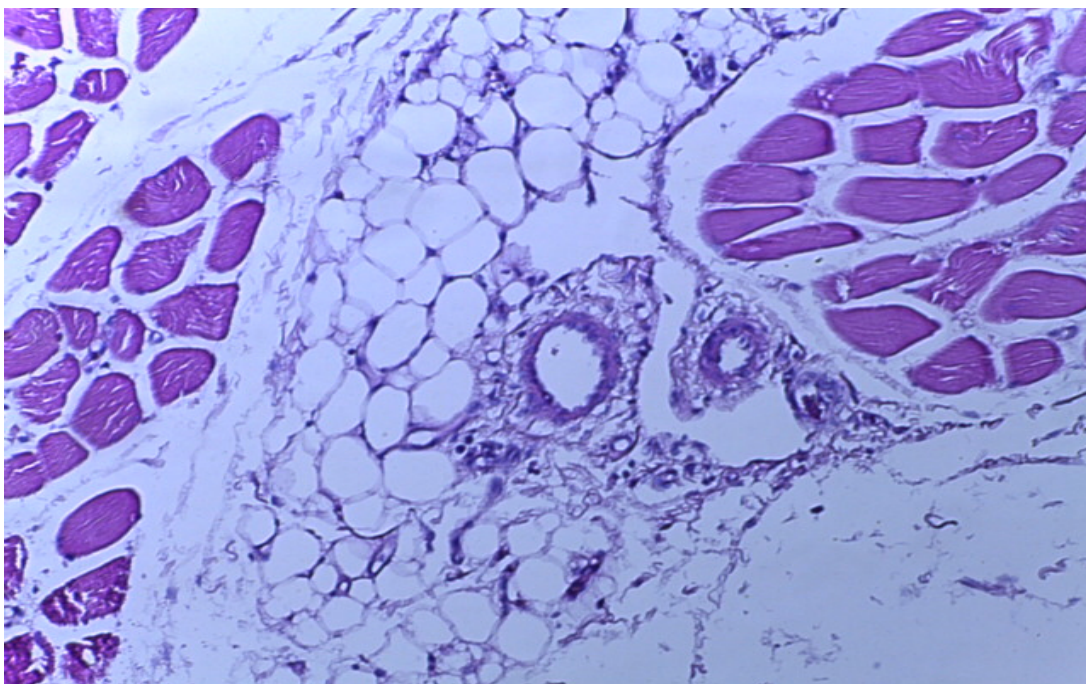


Figure 4: Large-drop obesity of the connective tissue of the pectoral muscle group of the broiler. Control group. Stained with hematoxylin and eosin. (200X).

It should also be noted that given betulin had a positive effect on the moisture-retaining capacity of the muscle fibers of the pectoral muscles, as an important technological indicator. When betulin was added to the diet, the water retention capacity increased by 5.1%, 7.4%, 8.6%, and 5.5% in relation to the control group.

4. CONCLUSION

As a result of the conducted studies, it was found that when the dose of betulin (phytobiotic) increases, there is a tendency to reduction of body weight due to less deposition of subcutaneous and abdominal fat (hepatoprotective properties). Also, this study found incensement of the biological value of meat by increasing the content of tryptophan, oxyproline, protein and ash elements. Botulin also helps to improve the technological properties of meat by increasing the moisture-retaining capacity of muscle fiber and the accumulation of proteins and minerals in the meat. It was found beneficial effects of betulin on the formation and maturation of the muscle fiber of broiler chickens.

5. AVAILABILITY OF DATA AND MATERIAL

All relevant data are already included in this article.

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Novikova Maria Vladimirovna is a senior researcher at the industrial poultry department. She is a candidate of biological sciences. Her research interests are Veterinary Genetics, Biology, Virology, Laboratory Diagnostics.



Dr. Lebedeva Irina Anatolyevna is an Associate Professor and Head of the Industrial Poultry Division. She holds a Doctor of Biological Sciences. Her research interests are Veterinary Genetics, Biology, Virology, Laboratory Diagnostics.



Kundryukova Ulyana Ivanovna is an associate professor at the Department of Morphology and Expertise. She is a candidate of veterinary sciences. Her research interests include Veterinary Sanitary Examination.



Professor Dr. Drozdova Lyudmila Ivanovna is Professor and Head of the Department of Morphology and Expertise. Her research interests include a study of the Morphology of Histoematological Barriers and their Permeability for Various Pathogens in the Mother-Placenta-Fetus System in Various Technogenic Zones.



Buhler Alexey Vladimirovich is a Candidate of Chemical Sciences. His research interests include Veterinary Genetics, Biology, Chemistry, Virology, Laboratory Diagnostics.

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