Assessment of Lipid Metabolism in Broilers against Plant Extract and Probiotic Substance or Their Combined Use

G. Duskaev¹, O. Kvan¹*, I. Vershinina¹ and S. Rakhmatullin¹

¹ Federal Research Centre of Biological Systems and Agro-Technologies RAS, Orenburg, Russia

Received on: 18 Feb 2020  
Revised on: 24 May 2020  
Accepted on: 31 May 2020  
Online Published on: Mar 2021

*Correspondence E-mail: kwan111@yandex.ru

© 2020 Copyright by Islamic Azad University, Rasht Branch, Rasht, Iran

Online version is available on: www.ijas.ir

ABSTRACT

In course of our research, we studied content of chemical elements in body of broiler chickens, lipid content in body and blood serum and change composition of fatty acid of muscles under influence of probiotic (Lactobifadol) and oak bark (Quercus cortex) extract introduced into the diet, both separately and combined. Studies were carried out in vivarium of Federal Research Centre of Biological Systems and Agrotechnologies of Russian Academy of Sciences on broiler crosses Smena 8 (n=120). For experiment, 7-day broiler chickens were selected, they were divided into 4 groups by method of analogues (n=30). According to experimental design, control group received basal diet (BD), group I - BD + Quercus cortex extract (1 mL/kg live weight), group II - BD + probiotic Lactobifadol (0.3 g/kg), and group III - BD + oak bark extract (2.5 mL/kg body weight) + Lactobifadol (0.3 g/kg of live weight). Thus, combined introduction of oak bark extract and Lactobifadol into diet of studied birds led to decrease content of dry matter by 13.5% relative to control. Oak bark extract contributed to increase in protein content by 7.3% (P≤0.05), relative to control. Level of fat decreased by 27.9% (P≤0.05) in comparison to control after the combined use of the extract and the probiotic. After the use of oak bark extract, chemical composition parameters significantly exceeded dry matter content of the control group by 10.8% (P≤0.05) and fat by 45.7% (P≤0.05), the protein level decreased by 2.4% (P≤0.05), relative to the control. The combined introduction of oak bark extract and Lactobifadol into diet of the studied birds contributed to a significant decrease in cholesterol on the 42nd day by 20.3% (P≤0.05), relative to the control. The level of triglycerides in the III experimental group also significantly decreased on the 21st day by 22.4% (P≤0.05), relative to the control group. Thus, we have shown that the use of oak bark extract in the diet of broiler chickens, both separately and together with the introduction of Lactobifadol probiotic, had a positive effect on blood serum lipid content of broilers and a decrease fat content in the carcass of broiler chickens. It was the most pronounced after the combined use of extract and probiotic.

KEY WORDS: broiler chickens, fatty acid composition, lipids, oak bark extract, probiotic.

INTRODUCTION

The poultry industry is developing at a faster pace, as this industry is able to provide the population with an inexpensive, high-quality and high-grade food product. In terms of cost recovery, poultry farming occupies a leading position among other areas of agriculture that produce meat products, since broiler poultry farming has a rapid turnover of funds and high profitability of enterprises (Nkukwana, 2018; Wilson, 2018; Tonsor and Wolf, 2019). A necessary condition for these indicators is a constant increase in poultry productivity and an improvement in the quality of poul-
ty products. For this, a wide range of various feed additives has been developed and is currently used (Sizova et al. 2016; Duskaev et al. 2018a).

However, today it is important to use additives based on biologically active substances and probiotic preparations, since they do not have side effects, do not pollute the environment and have great potential for practical use, allowing you to adjust the state of intestinal digestion (Alagawany et al. 2018; Wang et al. 2018).

At the same time, it is known that by feeding with a certain fodder it is possible to change the concentration of fatty acids in broiler carcasses and their diet, it affects the productive qualities of carcass and makes this line of research challenging today (Bagirov et al. 2018; Kumar et al. 2018).

It is worth noting that lipids are the most important components of body chemical composition. There is a high need of these substances in the body to ensure vital activity, but in excess, they can cause serious harm to it. From year to year, the level of fat in the carcasses of broilers of Russian and many foreign crosses is growing or remains extremely high, i.e. 16-20%. It is due to poultry breeding is carried out only for the growth rate, without taking into account the determination of level of fatty acids in muscle tissue.

The content of phospholipids in the composition of total lipids of broiler carcasses is 17-18%, turkeys-56-58%, while in waterfowl and adult chickens-from 2 to 9%. The amount of polyunsaturated fatty acids (PUFA) in the muscles of different species of birds ranges from 20 to 38% of the total lipid content, in adipose tissue-up to 80%. The amount of these acids in the body is directly affected by their amount in the diet (Wang et al. 2015). Excess lipid content promotes the development of obesity and many life-threatening diseases (Heymsfield and Wadden, 2017).

It is possible to maintain the normal lipid content in the body using probiotic preparations based on Lactobacilli (Drissi et al. 2017). Lactobacilli help to reduce lipid absorption in the small intestine. It is based on deconjugation of bile acids under the influence of hydrolases produced by probiotic strains. As a result of this process, the lipophilicity of bile acids and, as a consequence, the solubility of lipids entering the small intestine are reduced (Alonso et al. 2016).

However, it seems interesting to look for new ways to control the processes occurring in the gastrointestinal tract that directly affect the productive qualities of broilers, for example, the use of biologically active substances with an antibiotic effect (Wagle et al. 2017). A well-known example of such a substance is oak bark. Oak bark is a natural product that contains at least 8% tannins, gallic and ellagic acids and many biologically active substances (Deryabin, Tolmacheva, 2015; Fisinin et al. 2018), and these compounds can inhibit quorum sensing (QS), a system of bacterial pathogens (Das and Mehta, 2018).

However, as it was shown earlier in our previous studies, oak bark extract had a serious inhibitory effect on the intestinal biota of poultry (Duskaev et al. 2018b). So, it can be used as an alternative to antibiotics that have been banned from growing poultry since 2006 (EU). At the same time, there is a need to maintain the optimal composition of microflora, for which probiotic preparations, in particular, Lactobifadol, mentioned above, are successfully used (Iskhakova, 2016). There are studies that suggest that the combined use of plants or their extracts has positive properties, for example, chicory food powder maintained growth rates and improved intestinal microbiota in broiler chickens (Khoobani et al. 2020). Similar results were also obtained using cow parsnip (Heracleum persicum), flavophospholipol and probiotics (Javandel et al. 2019), Thymus vulgaris and Astragalus membranaceus (Tayeri et al. 2018).

Based on the foregoing, we suggested that the introduction of oak bark extract and probiotic (Lactobifadol) can increase the protein content in the body of broiler chickens, reduce the content of lipid in the blood and body, and improve fatty acid composition in the tissues of broiler chickens. The aim of our study was to study the content of chemical elements in the body of broiler chickens, lipid content in blood serum, as well as changes in fatty acid composition of muscles under the influence of the probiotic Lactobifadol and oak bark extract introduced in the diet, both separately and used together.

MATERIALS AND METHODS

Research object

The studies were carried out in a vivarium of the Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences on a broiler crosses Smena 8 (n=120). For the experiment, 7-day broiler chickens were selected (Fisinin et al. 2018), they were divided into 4 groups by the method of analogues (n=30). During the experiment, all birds were in the same conditions. Basic diets (BD) were composed taking into account the recommendations of the All-Russian Research and Technological Poultry Institute (Fisinin et al. 2009) (Table 1).

Experimental design

According to the experimental design, the control group received BD, group I - BD + Quercus cortex extract (1 mL/kg live weight), group II - BD + probiotic Lactobifadol (0.3 g/kg), and group III - BD + oak bark extract (2.5 mL/kg body weight) + probiotic Lactobifadol (0.3 g/kg of live weight).
The choice of dosage was determined based on previously conducted experimental studies of Deryabin and Tolmacheva, (2015). Lactobifadol (LLC "BF Komponent, Buguruslan, Orenburg Region) contains a microbial mass of lactic acid bacteria *Lactobacillus acidophilus* LG1-DEP-VGNKI, dried by a sorption method and *Bifidobacterium adolescentis* B-1-DEP-VGNKI.

Feeding of experimental poultry
The birds were fed twice a day; feed intake was taken into account daily. The poultry keeping and procedures during the experiments were in accordance with the instructions and recommendations of the Russian regulations (Order of the Ministry of Health of the USSR No. 755 as of 08/12/1977) and “The Guide for Care and Use of Laboratory Animals (National Academy Press, Washington, D.C., 1996”).

Every effort has been made to minimize animal suffering and reduce the number of samples used. The decapitation of the bird under nembutal ether was carried out on the 42nd day.

### Table 1

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Starter (7-28 days)</th>
<th>Finisher (29-42 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient composition (%)</td>
<td>Control, I, II, III</td>
<td>Control, I, II, III</td>
</tr>
<tr>
<td>Wheat</td>
<td>49.0</td>
<td>43.0</td>
</tr>
<tr>
<td>Barley</td>
<td>3.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Corn</td>
<td>8.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Soybean meal (46% CP)</td>
<td>23.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Sunflower meal (38% CP)</td>
<td>5.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Di-calcium phosphate</td>
<td>1.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Mel stem</td>
<td>0.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Salt</td>
<td>0.32</td>
<td>0.22</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.18</td>
<td>0.16</td>
</tr>
<tr>
<td>L-lysine</td>
<td>0.35</td>
<td>0.17</td>
</tr>
<tr>
<td>Vitamin-mineral premixa2</td>
<td>2.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

### Calculated nutrients

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Starter (7-28 days)</th>
<th>Finisher (29-42 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolizable energy (kкал/100 г)</td>
<td>296.0</td>
<td>302.0</td>
</tr>
<tr>
<td>Crude protein</td>
<td>24.0</td>
<td>19.1</td>
</tr>
<tr>
<td>Methionine + cysteine</td>
<td>0.87</td>
<td>0.79</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.35</td>
<td>0.96</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.95</td>
<td>1.0</td>
</tr>
<tr>
<td>Available phosphorus</td>
<td>0.54</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Control: basal diet (BD); I: BD + *Quercus cortex* extract (1 mL/kg live weight); II: BD + probiotic Lactobifadol (0.3 g/kg), and III: BD + oak bark extract (2.5 mL/kg body weight) + Lactobifadol (0.3 g/kg of live weight).

The preparation of oak bark extract
To prepare the *Quercus cortex* extract, 50 g of crushed bark (dosage form) was put into 500 mL of hot (70 °C) distilled water, heated in a boiling water bath for 30 minutes, filtered (anesthetized filters “White tape”, d 70 mm, “ApexLab”, Russia). The filtered extract was analyzed by gas chromatography-mass spectrometry using a GCMS Solutions, GCMS PostRun Analysis software (Shimadzu, Japan), a set of CAS spectrum libraries ([https://www.cas.org](https://www.cas.org)), NIST08 ([https://www.nist.gov](https://www.nist.gov)), Mainlib ([http://catalog.mainlib.org](http://catalog.mainlib.org)), Wiley ([http://www.sisweb.com](http://www.sisweb.com)) were used. The number of the identified components was estimated by a relative value (%) correlating the component peak area of the total peak area of the extract.
Chemical composition of broiler tissues

The experiment was completed with balance experiments to determine the digestibility of feed and the use of nutrients in poultry (28). Ammonia in an average sample of poultry litter was fixed by 0.1 N. oxalic acid solution (4 mL per 100 g of litter). At the end of the balance experiment, the samples were dried at a temperature of 60-70 °C and stored in a container with a ground lid. According to the daily data on the mass of poultry litter and its composition, the loss of substances and the assimilated amount of feed was calculated.

The chemical composition of broiler tissues after slaughtering was determined by standardized techniques (GOST 13496.15-97, GOST 51479-99, GOST23042-86, GOST 25011-81, GOST R 53642-2009), fatty acid composition of muscle tissue - using a Crystal-4000 Lux gas chromatograph (LLC NPF Meta-Chrom, Russia) and a Lumachrome liquid chromatograph (Lum-ex, Russia) (GOST 51486-99).

Hematological parameters of broiler chickens

Blood samples were collected for biochemical studies in vacuum tubes with a coagulation activator (thrombin). Blood biochemical analysis was carried out on a CS-T240 automated biochemical analyzer (DIRUI Industrial Co., Ltd, China) using commercial biochemical kits for veterinary medicine DiaVetTest (Russia).

Statistical analysis

Means of three estimations were presented with their standard deviation and analysis of variance (ANOVA) was used to detect significant differences among means. Treatment means were separated using Duncan's Multiple-Range Test (DMRT). Correlation coefficient (r) values were estimated by using treatment means. P ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

According to the results of our studies, data on the content of chemical elements in the body of broiler chickens were obtained; it made it possible to obtain material on the composition of the empty body of experimental broilers (Table 2). The combined introduction of oak bark extract and Lactobifadol into the diet of the studied birds led to a significant decrease in dry matter as relating to the control and experimental groups, by 13.5%, 16.8% and 12.5%, respectively.

The additional inclusion of oak bark extract in the diet of broiler chickens contributed to increase in protein content by 7.3% in group I, relative to the control, its decrease was registered in the II and III experimental groups by 4.8% and by 4.3%, respectively.

Compared with the experimental groups, there was a decrease in protein in the II and III group by 11.8% and 11.3%, respectively, relative to Group I.

Fat level in Group III significantly decreased by 27.9% (P≤0.05), relative to control group and by 24.7%, and 30.2%, respectively, relative to Group I and II.

Content of minerals in Group I were significantly higher by 14.4% (P≤0.05), relative to control, after the comparing the experimental groups with each other.

It should be noted that in experimental groups II and III there was a significant decrease of the latter by 12.9% and 18.3% (P≤0.05), relative to the experimental group I.

Considering the chemical composition of separate tissues, namely the muscle tissue of an experimental bird, the following picture might be seen. So, in group II, the indicators of chemical composition significantly exceeded those of the control group: in terms of dry matter by 10.8% (P≤0.05) and fat by 45.7% (P≤0.05), the protein level decreased by 2.4% (P≤0.05), relative to control. In the experimental group I, fat content decreased relative to control group by 24.5% (P≤0.05) (Table 3).

Lipids are energy sources, components of bioorganic structure, able to regulate the thermal conductivity of cell membranes. It is possible to maintain the lipid concentration within normal limits using probiotic preparations based on Lactobacilli, due to their ability to produce hydrolases that break down the intramolecular complex of bile acids with taurine and glycine. As a result of this process, the degree of lipid sorption in the digestive tract is reduced. However, the combined administration of a probiotic preparation and oak bark extract can ambiguously affect the concentration of lipid metabolites in blood of broiler chickens. Our studies have established that under the influence of oak bark extract there was a significant increase by 7.7% (P≤0.05) in the level of triglycerides at the end of the experiment, comparing to control. On the 21st day of the experiment, there was a significant increase 2.8 times (P≤0.05) in the latter, compared with 7 days of the experiment; there has already been a significant decrease in triglycerides 2.3 times (P≤0.05) on the 42nd day compared with 21 days of the experimental study (Figure 1).

The additional inclusion of a probiotic preparation in the diet of group II showed a similar picture: so, on the 21st day of life it led to a significant increase in triglycerides 2.8 times (P≤0.05), relative to the 7th day of life; in the final period of study the level of triglycerides in blood significantly decreased 2.7 times (P≤0.05), comparing to the 21st day of the experiment (Figure 2). The combined introduction of oak bark extract and Lactobifadol into the diet of the studied bird contributed to a significant decrease in cholesterol on the 21st day of life by 16.7% (P≤0.05) and on the 42nd day by 20.3% (P≤0.05), regarding control.
The level of triglycerides in group III also significantly decreased on the 21st day of life by 22.4% (P ≤ 0.05), relative to the control group. It should be noted that on the 21st day, in comparison with the 7 days of life, there was also a significant increase in the latter 2.9 times (P ≤ 0.05) and on the 42nd day, in comparison with 21 days, a significant decrease 2.3 times (P ≤ 0.05).

Triglycerides are esters of glycerol and higher fatty acids. In our study, there was a decrease in triglycerides, however, all changes were within normal limits.

Cholesterol is found in all animal cells and is already necessary in the very early stages of development. Moreover, its total amount in the body remains approximately at the same level under any exogenous influences through the body’s homeostasis mechanism. In our studies, the average cholesterol level in the experiment did not change significantly and was within the limits of physiological norm.

Considering fatty acid composition of muscle tissue of broiler chickens, it should be noted that the additional inclusion of a probiotic preparation in the diet of birds contributed to a significant increase in stearic acid by 28.2% (P≤0.05) and arachinic acid by 4.3 times (P≤0.01), relative to the control group (Table 4). The additional introduction of oak bark extract led to a significant increase in arachinic acid 2.7 times (P≤0.05), and the combined administration of probiotic and oak bark extract increased stearic acid by 26.2% (P≤0.05), in relation to the control.

Today, it is known about the positive effect of some medicinal plants on productive and economic indicators in growing agricultural poultry, in particular, researchers have studied the effect of medicinal herbal mixtures on liver lipid...
metabolism and antioxidant status of body (Schiavone et al. 2008; Shokraneh et al. 2016).

Perhaps herbal supplements can lower LDL by inhibiting intestinal cholesterol absorption through a mechanism similar to ezetimibe (Sweeney and Johnson, 2007; Hajhashemi et al. 2014). In addition, the addition of Heracleum persicum in the above studies with a concentration of 0.5% and 0.75% significantly reduces plasma glucose, which indicates optimal pancreatic function in combination with food intake. In addition, the fact is known that bacteria in probiotics can lower cholesterol, this ability comes from anticholesterol substances produced by bacteria that inhibit cholesterol-producing enzymes, and cholesterol levels also decrease because bacteria absorb a certain amount of cholesterol during growth into cells (Simurat et al. 2009).

In addition, the results of our study are consistent with data from Hasibuan et al. (2019), according to which the introduction of probiotics, Javanese turmeric and plant extract of breadfruit leaves into broiler diet increased productivity and lowered cholesterol, but did not affect the levels of raw protein in broilers. There is also an opinion (Tugiyanti and Susanti, 2017) that the more active antioxidants are in the feed, the more they can maintain protein levels, which can explain the increase in protein in the group treated with oak bark extract.

Thus, it is worth considering that it is likely that some of biologically active substances contained in plant extracts and performing protective functions of plant tissues can have an ambiguous effect on animal organism (Ayaşan, 2013), in particular, on such important processes as an exchange of protein and fat in the body.

In addition, the following fact is important: as part of diets, microelements interact with each other in the composition of broiler diets (Mondal et al. 2010). The property of tannins to bind to enzymes is known, and differences in the chemical structure of these polyphenols can affect such interactions (Hofmann et al. 2006) and, therefore, metabolic processes that change during different periods of poultry rearing. At a certain dose, tannins have a positive effect on productivity (Schiavone et al. 2008). The study showed that during the experiment, fluctuations in the digestibility of substances over the growing periods were observed (Bagirov et al. 2018).

Also, the positive effects of combining probiotic with plant extract noted in our study can be potentiated by the fact that plant extracts have antimicrobial, anticoccidial and fungicidal or antioxidant properties. The antimicrobial effect of essential oils is explained by their lipophilic nature (Camele et al. 2019), due to which essential oils can suppress pathogenic bacteria by penetrating the cell or destroying the bacterial cell membrane. At the same time, the addition of a probiotic promotes colonization of the intestine with normal microflora, which contributes to an increase in

It is worth noting that poultry meat is a valuable product and has many beneficial nutritional properties, among which low lipid content and relatively high polyunsaturated fatty acids are worth noting (İnci and Ayaşan, 2019).

Thus, in the studies of Starcevic et al. (2015), it was noted that experimental groups received supplements containing polyphenols; the researchers also observed an increase of protein in the pectoral muscle of poultry.

Metabolites of phenolic compounds (tannins and other substances), including those contained in Quercus cortex extract, have antioxidant properties (Elansary et al. 2019; Skrypnik et al. 2019), which could affect the fatty acid profile of muscle tissue.

In our study, we noted a decrease in cholesterol levels in all experimental groups on day 42. These data are consistent with the results obtained by Hajhashemi et al. (2014) that reported that essential oil of cow parsnip increases high-density lipoprotein (HDL) levels and lowers low-density lipoprotein (LDL) levels in animals. However, the mechanism by which LDL levels are reduced after phytoextracts are added remains unclear.
the immune status and digestibility of nutrients in the intestine of the bird, which ultimately leads to an increase in feed conversion and an improvement in carcass slaughter quality.

**CONCLUSION**

Thus, we have shown that the use of oak bark extract in the diet of broiler chickens, both separately and in conjunction with probiotic (Lactobifadol), had a positive effect on the lipid content in blood serum of broilers, and a decrease of fat content in the carcass of broiler chickens. It was the most pronounced with the combined use of the extract and probiotic. Thus, our hypothesis presented above was confirmed by research on broiler chickens, where the combined administration of oak bark extract and probiotic drug Lactobifadol contributes to a significant decrease in cholesterol by 20.3% (P≤0.05) and lipids by 27.9% (P≤0.05), compared with the control group. This study has an applied significance. So, it is known that selecting certain additives, it is possible to change the lipid concentration of broiler carcasses and lipid peroxidation indices during meat storage, since the compounds that make up the extract also have antioxidant properties. All this allows us to ensure a better taste of meat and a long shelf life.

**ACKNOWLEDGEMENT**

The research was conducted with financial support from the Russian Science Foundation (Grant № 16-16-10048).

**REFERENCES**


Javandel F., Nosrati M., van den Hoven R., Seidavi A.R., Lavadano F. and Tufarelli V. (2019). Effects of hogweed (*Herculeum persicum*) powder, flavophospholipol, and probiotics as feed supplements on the performance, carcass and blood characteristics, intestinal microflora, and immune response in br-


