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ORIGINAL ARTICLE

Effect of feed additives from marine hydrobionts on the protein metabolism condition in broiler chickens

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There was studied the effect of two feed additives made from finely ground wastes of the primary processing of marine aquatic organisms on the biochemical parameters of the protein metabolism of the blood serum of broiler chickens of *Ross 308* cross. The composition of the mineral feed additive (MFA) included mussel leaves and seawater, the protein-mineral feed additive (PMFA) – the leaves of large and the body of small mussels, seaweed *Phyllophora nervosa* and seawater. The determination of blood serum indices was performed using an automatic biochemical analyzer *GBG ChemWell 2910* (USA) and the use of test systems *Global Scientific* (USA). According to the results of biochemical studies of the broiler chickens' blood serum, the diet of which was supplemented with feed additives, there was established an increase in the total protein content, incl. albumin and globulin. Compared with MFA, the use of PMFA to a greater extent increased the content of total protein, albumin, and globulin. The total protein content changes and its fractions occurred due to an increase in the activity of transamination enzymes: alanine and aspartate aminotransferase and the absence of changes in glucose content. Due to the relatively smaller increase in the amount of albumin compared to globulin, the albumin-globulin ratio in the chickens of the experimental groups was lower. Among the globulin fractions, the greatest changes were observed in the relative content of ά2-globulins. Based on the results of the research, a conclusion was drawn on the stimulating effect of feed additives of marine aquatic organisms on the protein metabolism condition of broiler chickens.

Key words: broiler chickens, feed additive, marine hydrobionts, total protein, albumin, globulin, alanine and aspartate aminotransferase.

Introduction

The deficiency of high-quality sources of protein, minerals, biologically active substances threatens both the health and productivity of the poultry bird. Fish meal flour is rich of sulfur-containing amino acids and is often used in poultry feeding. However, the increasing expenses of catching fish, the general perception that it should be used only for human nutrition, limits its use in animal husbandry. Other hydrobionts, such as mussels, as well as waste from their primary processing, may be an alternative to fish. Mussels' meat has a high protein content, similar to fish in chemical composition and their leaves are a source of important inorganic substances, primarily Calcium and Phosphorus (Berge et al., 1989; Jonsson et al., 2009) and can be a valuable feed ingredient in accordance with organic standards (Morris et al., 2019). On the other hand, the primary processing waste of sea mussels may cause ecological and economic problem and its recycling into animal feed is a solution (McLaughlan et al., 2014).

Other hydrobionts the red seaweed are a rich source of lipids, polysaccharides, proteins, bioactive compounds, and secondary metabolites such as polyphenols and minerals that are beneficial to health (Pujol et al., 2002; Bansemir et al., 2004; Lins et al., 2009; Gómez-Ordóñez et al., 2012; Souza et al., 2012). It has been shown that the major seaweed carrageenan polysaccharides have antiviral features as well as antitumor, anticoagulant and immunomodulatory effects (Campo et al., 2009; de Jesus Raposo et al., 2015). Other compounds of red seaweed, such as derivatives of brominated furanones, have antimicrobial features (Janssens et al., 2008; Kulshreshtha et al., 2015; Kulshreshtha et al., 2014). Components of cultured red seaweed enhance the immune response (Liu et al., 2013).

The great attention is paid to protein in poultry feeding due to its importance as a basic structural material and component of biologically active substances (Beski et al., 2015). The growth process and development of broiler chickens is a very intense period of weight gain in a very short time, accompanied by significant metabolic changes in the structure of serum proteins (Szabó et al., 2005). Adequate protein content in the diet of broiler chickens, especially at an early age, promotes early bowel development and digestive physiology, and improves broiler growth and immunity (Allison, 2012).

The total protein concentration in the serum of birds is about half that in mammals (approximately 40 g/l), due to the extremely high blood glucose concentration, which reduces the protein concentration to maintain colloid osmotic pressure (Scanes et al., 2015). Poultry serum proteins are an important dynamic marker of nonspecific immunity, productivity, as well as an marker that responds to changes in diet and changes with age and are the basis of a general biochemical condition that allows identification of metabolic changes (Piotrowska et al., 2011; Tóthová et al., 2019).

Thus, the total protein, albumin, serum content of broiler chickens is lower than the high mycotoxin content of deoxynivalenol and zearalenone in the compound feed (Faixová et al., 2010). The total protein content increase by 15.0-31.0%, including, albumin by 10.3-20.7%, globulin by 30.0-60.0% in serum of chickens during feeding feed additives of a mixture of black pepper, coriander seeds, turmeric powder reports (Abou-Elkhair et al., 2014). High albumin content helps to maintain metabolic balance during the period of quick growth of new animals (Piotrowska et al., 2011; Szabó et al., 2005).

The purpose of the work is to determine the effect of mineral and protein-mineral feed additives made from the products of primary processing of marine hydrobionts on the protein metabolism condition of broiler chickens.

Methods

The experiments were performed on Ross 308 cross meat chickens. Chickens were kept within the same room in different cages under the same microclimate parameters according to hygienic requirements. During the experiment, the bird was clinically healthy. The chickens were kept and manipulated in accordance with the European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes (Strasbourg, 1986). The bird were fed standard compound feed according to national standard DSTU 4120-2002, irrigation was performed from nipple like bowls. Broilers of the control group were fed only the basic diet (BD), the experimental groups from 20 to 42 days old in addition to BD were fed feed additives made from marine hydrobionts. Chickens of the first and second experimental groups (D1 and D2) received mineral feed addictive (MFA), third and fourth group (D3 and D4) were fed protein-mineral feed additive (PMFA). Moreover, broilers D1 and D3 group by weight of feed received 93% of BD and 7% respectively of MFA and PMFA, D2 and D4 groups in addition to the BD (100%) 7% respectively of MFA and PMFA. Broilers' blood collection was performed at the end of the rearing period during the slaughter through the jugular vein of five heads from each group.

MAF (Patent of Ukraine for utility model number 34634), is a finely ground leaves of the Black Sea mussel (*Mytilus galloprovincialis*) and seawater. PMFA (patent of Ukraine for utility model No. 42687) contains milled leaves of large and small mussels, waste of primary processing of *Phyllophora nervosa* seaweed and seawater.

The feed additive effect of on the biochemical condition of the broilers' body was evaluated by protein metabolism ratio (total protein content and its fractions), as well as the activity of the reamination enzymes – alanine aminotransferase (ALT) and aspartate aminotransferase (AST) serum.

The study on the biochemical parameters was performed in the laboratory of biochemistry of the research department of LLC "*Center for Veterinary Diagnostics*" (Kiev) with the help of the automatic biochemical analyzer *GBG ChemWell* 2910 (USA) and the use of test systems produced by *Global Scientific (USA).*

Results and discussion

The values of biochemical parameters of protein metabolism of chickens in the experimental groups were generally in accordance with species and age norms (Table 1). In chickens in the control group, the total protein content was at the extreme upper limit of normal, albumin was lower, and globulin was higher. With the use of feed additives there was a significant increase in the content of total protein in the serum: in chickens D1 group by 35.8% (p≤0.01), D2 group by 23.3% (p≤0.01), D3 groups by 38.4% (p≤0.001), D4 groups by 41.8% (p≤0.05).

Table 1. Serum biochemical parameters of 42-days-chickens with feed additives (M ± m, n = 5)

Index	Group Name				
	Control	D1	D2	D3	D4
		93 % BD + 7 % MFA	100 % BD + 7 % MFA	93 % BD + 7 % MFA	100 % PMFA + 7 %
Total protein cont., g/L	35.2±0.55	47.8±3.30**	43.4±2.33**	48.7±1.64***	49.9±4.32*
Albumin, g/L	12.0±0.55	14.2±1.02	12.8±0.49	14.8±0.80*	15.2±1.20*
Globulin, g/L	23.2±1.76	33.6±2.55*	30.6±1.32*	33.9±1.05***	34.7±2.07**
ά1-globulins, %	7.2±0.86	7.4±0.51	7.6±0.68	8.6±0.68	6.6±0.51
ά2- globulins, %	12.0±0.84	16.4±0.51**	14.2±0.86	14.6±0.75*	13.8±0.58
β- globulins, %	13.4±1.08	15.4±0.87	14.2±1.02	13.2±0.58	15.2±1.02
γ- globulins, %	33.2±1.88	31.0±1.92	34.4±1.03	33.4±1.47	33.8±1.16
Coef, A/G	0.52±0.06	0.43±0.02	0.42±0.02	0.43±0.01	0.44±0.03
ALT, U/L	2.2±0.20	2.8±0.37	2.6±0.40	2.6±0.40	3.0±0.32
AST, U/L	320.0±7.88	342.6±60.55	372.6±17.47*	288.8±14.5	421.6±40.99*

* - P≤0.05; ** - P≤0.01; *** - P≤0,001 compared to the control group.

At the same time, within MFA feeding contained only the minerals of the mussel leaves, its content was higher by 23.3-35.8%, and the PMFA containing, in addition to the minerals, the mussel leaves, as well as their meat and biologically active substances of algae by 38.4-41.8%. Our data is coordinated with the information of Nogovytsina (2018) on the increase of total protein content in the serum of caterpillars within feeding vermiculite and Buryakov et al. (2014), on the increase of the total protein content in the serum of chickens using the mineral complex "*Proteal Tri Plus*" in the form of protein and carbonate, zinc and manganese salts in sulphate and chelate form, as well as plant extracts.

It should be noted that the total protein content increase in the serum of chickens of the experimental groups was less due to albumins, and to a greater extent due to the globulin fraction. Thus, the chickens of D1 group have an increased albumin content by 18.3%, D2 groups by 6.7%, D3 groups by 23.3% ($p \leq 0.05$), D4 groups by 26.7% ($p \leq 0.05$). Globulin content increased in broilers of D1 group by 44.8% ($p \leq 0.05$), D2 groups by 31.9% ($p \leq 0.05$), D3 groups by 46.1 % ($p \leq 0.001$), D4 groups by 49.6% ($p \leq 0.01$). Among the globulin fractions, chickens of D1 group it was higher have the largest changes in the relative content of ά2-globulins: in by 4.4% ($p \leq 0.01$), D2 groups by 2.2%, D3 groups by 2.6% ($p \leq 0.05$), D4 groups by 1.8%. The change in the relative content of other fractions: ά1-, β- and γ-globulins was not significant ($p > 0.05$).

In addition, both albumin and globulin content increased to a greater extent in the serum of chickens fed PMFA and, to a fewer extent, by the use of MFA. Thus, the albumin content of serum of chickens D1 and D2 in the group fed MFA was higher by 18.3 and 6.7% ($p > 0.05$), and D3 and D4 groups, which was fed PMFA by 23.3 and 26.7% respectively. The content of globulins in chickens D1 and D2 group increased significantly by 44.8 and 31.9%, D3 and D4 groups by 46.1 and 49.6%.

Alpha globulin fractions consist of various lipoproteins, including α1-, α2-lipoproteins, as well as pre-β-lipoproteins, which are mainly intended to transport lipid molecules and other substances though biological fluids (Allison et al., 2012). Thus, the higher concentration of α1- and α2-globulins in chicken's body using feed additives may be caused by an increased intensity of substances transporting for more intense metabolic reactions during this period and for their use in tissue structures (Filipović et al., 2007).

It is established that many diagnostically important serum proteins, such as acute phase proteins (α1-acid glycoprotein, ceruloplasmin, and amyloid A), are included in the α-globulin fraction (Tóthová et al., 2016). However, increased concentrations of active phase proteins are not always a consequence of an activated reaction, as their index can be increased by influencing the body stressors, changes in environmental conditions (Pradeep, 2014). Therefore, the higher concentrations of α-globulins we have found in chickens body under using feed additives may be related to the need for substance transport and more intense metabolic processes due to changes in nutrition (Takahashi et al. 2009; O'Reilly et al., 2014; O'Reilly et al., 2018). It should be noted that a significant increase in the relative content of ά2-globulins by 4.4% ($p \leq 0.01$) and 2.6% ($p \leq 0.05$), which was established in broilers of D1 and D3 groups where MFA and PMFA replaced 7% of the basic diet. The content increase of ά2-globulins was unreliable due to the enrichment of feed addictive. According to the study's results, we found a general tendency to increase the content of β-globulins in the broilers' serum of the experimental groups (except D3 group). A concentration increase of β-globulins in serum during feeding is reported by (Filipović, al. 2007). In the β-globulin fraction, proteins such as transferrin, ovotransferrin, fibronectin and hemopexin have been identified (Xie et al., 2002). Transferrin belongs to the group of Ferum connecting glycoproteins involved in the metabolism of the Ferum (Lambert, et al., 2005; Rath et al., 2009). In addition, the β-fraction contains complement components C3 and C4 that are involved in immune responses (Roman et al., 2009; Gueguinou et al., 2014).

As is known, the γ-fraction of globulins contains mainly immunoglobulins. Some classes of immunoglobulins (IgM and IgG) can merge with the β-globulin fraction (Capitelli et al., 2013). According to our research, the content of γ-globulins in the broilers' blood for the feed additives use made from hydrobionts did not showed any significant changes. It should be noted according to the technology of manufacturing MFA and PMFA there was used concentrated hydrochloric acid having bactericidal properties for the inorganic substances dissolution of the mussel leaves and their disinfection. Thus, feed additives were cleaned from microorganisms and while feeding did not induce the synthesis of γ-globulins in broiler chickens' organisms.

Due to a more significant relative content increase of globulins compared to albumins, the albumin-globulin coefficient within the experimental groups tended to decrease and in the D1, D2, D3 and D4 groups respectively 0.43 ± 0.02; 0.42 ± 0.02; 0.43 ± 0.01 and 0.44 ± 0.03 versus 0.52 ± 0.06 in the control group. The lower albumin-globulin ratio in the studied groups of broiler chickens reflects a greater degree of globulin content increase than albumin, which is probably due to the maintenance of the metabolic balance of the chickens' body while using feed additives.

It is known that the liver, as the "central laboratory" of the body is actively involved in the regulation of almost all metabolic processes. Such a sensitive test of the protein metabolism condition, as the activity of hepatospecific enzymes – ALT and AST is widely used to evaluate its possible disorders (Min, Tang). Thus, the increase of ALT and AST activity in broilers' serum occurred due to the high content of mycotoxins in the combined feed (Faixová et al., 2010), and against the background of the increase of total protein content and globulin it occurred due to the high content of amino acids threonine (Min et al., 2017) in the diet. The replacement of soybean flour to fish in the diet of broiler chickens caused a dose-dependent ALT and AST activity increase in serum with a very high correlation coefficient (Aletor et al., 1990). Due to growth retardation syndrome, the serum of chickens was characterized by a higher activity of AST and ALT, a reduced number of total protein content (Rani et al., 2011). The use of probiotics, prebiotics and its combinations to laying hens caused a decrease in the activity of ALT and AST (Tang et al., 2017). The heat stress caused a decrease in body weight of the chickens, increased activity of serum ALT and AST (Bueno et al., 2017). The use of dried tomato pulp these ratio corresponded to control (Hosseini-Vashan et al., 2016).

According to the results of the studies, the feed additives use made of hydrobionts tended to increase the activity of ALT, which content in the chickens body of the experimental groups was higher by 18.2-36.4% (in all cases, $p > 0.05$). The AST content was significantly higher than the enriched diet with both MFA and PMFA used at a dose of 7% in addition to the basic diet, respectively by 16.4% ($p \leq 0.05$) and 31.8% ($p \leq 0.05$). Such changes are likely to indicate a metabolism increase by the greater

income of minerals and protein into the bird body. Under condition that 7% of the diet was supplemented with feed additives, AST activity did not change significantly.

Conclusion

The use of broiler chickens feed additives made from the processing waste of marine hydrobionts such as sea mussels and red algae causes protein metabolism changes. Feeding the mineral and protein-mineral additives there was observed an increase of the total protein, albumin and globulin content in the serum. The use of protein-mineral additive compared to mineral increased the content of total protein, albumin and globulin to a greater extent. Due to the relatively smaller increase of albumin content compared to globulin, the albumin-globulin ratio in within test group chickens was smaller. In the globulin composition, the largest changes occurred in the α- and β-globulin fractions. The use of feed additives contributed to the higher activity of the reamination of alanine and aminotransferase enzymes.

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