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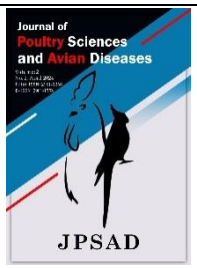

Evaluation of prebiotic supplementation on lipid peroxidation and hematologic parameters of broilers with ascites

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A B S T R A C T

Ascites or pulmonary hypertension syndrome is one of the common complications in the poultry industry, which is associated with symptoms such as increased pulmonary artery pressure, dilatation and hypertrophy of the right ventricle. Previous experiments have proven that the measurement of lipid peroxidation is a key indicator for detecting oxidative stress. It has also been determined that the ratio of heterophile/lymphocyte increases in stress conditions and can be helpful in the diagnosis of oxidative stress. Considering the antioxidant role of prebiotics, there is a possibility that these compounds can reduce the oxidative stress in pulmonary hypertension. In the current study, in order to measure this hypothesis, 135 broilers were purchased and divided into three groups of 45 pieces in three repetitions: sham group: basal diet, control group: basal diet + triiodothyronine (T3) (1.5 mg/kg) to induce ascites, and treatment group: basal diet + T3 (1.5 mg/kg) + Safmannan (1000 grams per ton) from one day old. At the age of 21, 35, and 49 days, 9 chickens from each group were randomly selected and after blood sampling, blood parameters including lymphocytes, heterophil, heterophil/lymphocyte and and the ratio of right ventricular to the total ventricles (RV/TV) weight as well as lipid peroxidation were assayed. According to the findings, the RV/TV increased significantly at the age of 49 days in the positive control group and reached above 0.29, which indicates the presence of ascites in this group ($P<0.05$). The ratio of heterophils to lymphocytes at the age of 49 days in the prebiotic treatment group demonstrated a meaningful reduction compared to the positive control group, and also the amount of lipid peroxidation at the ages of 35 and 49 days in the prebiotic treatment group decreased compared to the positive control group ($P<0.05$). According to the results of the current research, Safmannan prebiotic was able to remarkably decrease the amount of oxidative stress caused by ascites.

Keywords: *Antioxidant, Ascites; Broiler, Lipid peroxidation, Triiodothyronine.*

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1 Introduction

The need to produce as much meat as possible in a shorter period of time, along with a better food conversion ratio in the poultry industry, caused genetics and breeding experts to choose chickens with a faster growth rate in order to achieve better breeds (1). Today, under normal breeding conditions, a one-day-old broiler will gain more than 50 times its weight in less than 50 days, which is almost unique among breeding animals. However, the lung and heart capacity of the new broiler chickens is very similar to the old strains of broiler chickens, which results in high pressure on the cardiovascular system, followed by the occurrence of ascites syndrome (2). In meat-type chickens, this syndrome is a cascade of events that lead to changes including heart enlargement and relaxation, hypertrophy of right ventricle, and fluid accumulation in the ventricular cavity. Adding triiodothyronine (T3) to the diet of chickens increases the occurrence of right ventricular hypertrophy and ascites-related death, which probably occurs secondary to increased oxygen demand (3). In cell membranes, free radicals cause lipid peroxidation and it is known that this process plays a significant role in the formation of pulmonary hypertension. Ascites syndrome occurs in fast-growing chickens, mainly in winter, and is one of the main causes of death in meat-type chickens (4). Maxwell and Robertson in 1997, estimated the average losses due to ascites syndrome from the number of 12.9 billion broiler chickens in 18 countries from the four continents of the world at the rate of 4.7% (equivalent to 560 million chickens) and the annual loss due to the syndrome Ascites in the poultry industry was estimated at more than one billion dollars (5).

Oxidative stress occurs in cells as a result of an imbalance between oxidizing and antioxidant systems. Oxidative stress mainly affects the membrane system of the cell, especially the bilayer phospholipids of the membrane (6, 7). Measuring the peroxidation of lipids in the cell is an important indicator for measuring the level of oxidative stress. In addition to this, the synthesis of free radicals affects the metabolic system, including the heart and gastrointestinal systems in birds with ascites. The intestine needs high amounts of oxygen and hypoxia inhibits the development process of the intestine in broiler chickens (8, 9). Research has shown that the amount of heterophil to lymphocyte increases in stress conditions and can be used as an indicator in stress diagnosis (10). Recently, a new generation of these additives under the title of probiotics, prebiotics and synbiotics is produced and supplied with different brand names as an alternative to antibiotic additives in the nutrition of humans, domestic animals, ruminants, poultry and aquatic animals (11). These products have widely entered a global market and investigating their effects on different body systems in different animals has taken a large share of modern biological science research. By banning the use of antibiotics as a preventive and growth promoting agent by the European Union in 2006, the use of growth promoting additives, especially prebiotics, is expanding rapidly (12). The microflora of the digestive system of animals play a crucial role in health, prevention of diseases, performance and production of livestock and poultry. By affecting the microbial population in the animal's digestive system and preventing the anti-growth effects of detrimental microorganisms, prebiotics improve animal health and growth (13). Prebiotics are much more stable than probiotics against the heat and pressure produced during food processing operations such as pelleting, and they are also economically more affordable because some of them are among the wastes and cheap by-products of food processing (14).

It seems that prebiotics have the mentioned beneficial effects in reducing the ratio of heterophile to lymphocyte and the intensity of lipid peroxidation in ascites chickens. Considering that there has been no previous study in this regard, this research was conducted with the purpose of investigating the effect of probiotic consumption on lipid peroxidation and blood parameters in broiler chickens with ascites.

2 Materials and Methods

2.1 Experimental groups

A total of 135 meat-type chickens (Ross 308) were purchased and divided into three groups of 45 based on a completely random statistical design. The experimental groups were as follows:

Sham group: recipient of the basic ration during the entire experimental period

Control group: recipient of basic ration + triiodothyronine (T3) hormone (Sigma-Aldrich, USA) at a dose of 1.5 mg/kg added to food ration from seven days old to induce ascites (15)

Treatment group: recipient of basic ration + triiodothyronine (T3) hormone at a dose of 1.5 mg/kg to the food ration from seven days of age + Safmannan prebiotic

(Lesaffre, France) at the rate of 1000 grams per ton from one day of age

Each of the groups included 3 pens of 15 pieces. One day after weighing, 15 chicks were selected for each pen so that the average weight of all pens was the same. Chickens were raised from 1 to 49 days old under standard conditions on litter. During the entire rearing period, water and manure were freely provided to the chickens. Before the chickens entered, the hall and pens were disinfected and gassed. Water and feed were freely available to the chickens at all hours of the day and night, and the condition of the animals was checked at least three times a day. Basal diet was formulated based on corn-soybean, which was the same for all groups. In this breeding system, the hall has light for 23 hours and no light for one hour. The temperature of the environment was also adjusted in such a way that at the beginning of the arrival, all the chickens were kept at a temperature of 32ºC. Then, in the first three days, the ambient temperature was reduced by 1ºC daily. After that, the room temperature was reduced by 1ºC every three days until the temperature reached 22ºC on the 21st day of the breeding period. Then, the ambient temperature was kept around 22-21ºC until the end of the study. Also, in order to maintain the health of the chickens and immunize them against some common viral diseases such as Newcastle disease, Infectious bronchitis and Gumboro, vaccination was done. All procedures involving animals were approved by the Islamic Azad University Institutional Animal Care and Use Committee.

2.2 *Assessment of the ratio of right ventricular to the total ventricles (RV/TV) weight*

Based on the results of previous studies, 15 chicks from each experimental group were euthanized on the 21st, 35th, and 49th days of rearing. The hearts of the broilers were isolated and all ventricles were weighed. Then the RV/TV was calculated and recorded. Ascites was induced if the RV/TV was greater than 0.29 (16, 17).

2.3 *Assessment of lipid peroxidation and blood parameters*

On days 21, 35 and 49 of rearing, nine chicks from each group (three chicks from each replication) were randomly selected. One millilitre of blood was collected by sterile cervical syringes, and the volume of blood prepared was taken into account as the empty space for the syringe. After blood collection, the piston of the syringe was pulled to the end and placed at an angle of 30 degrees at room temperature to coagulate the blood. After one hour, the sera were separated and sent to the laboratory to determine the amount of lipid peroxidation. The amount of lipid peroxidation was measured by TBARS test in different samples (18). Also, immediately after blood sampling, multiple cytology smears were prepared on slide and fixed with air dry and using 70% alcohol and stained with Giemsa staining to determine the ratio of heterophile to lymphocyte (19).

2.4 *Statistical analysis*

The findings were analyzed using the SPSS software. The One-way ANOVA was used to determine the statistical differences among the sham, control, and treatment group and followed by Tukey's test. The data were expressed as mean ± SEM and differences between the means at a level of $p < 0.05$ were considered as statistically significant.

3 Results

The ratio of RV/TV in different groups in three rearing periods including 21, 35 and 49 days is presented in Table 1.

Table 1. The RV/TV ratio in the different groups at the ages of 21, 35 and 49 days

Age	Group	RV/TV
21 day-old	Sham	0.25±0.00
	Control	0.27±0.03
	Prebiotic treatment	0.25±0.00
35 day-old	Sham	0.23±0.01
	Control	0.27±0.01
	Prebiotic treatment	0.24±0.02
49 day-old	Sham	0.23±0.01[a]
	Control	0.33±0.02[b]
	Prebiotic treatment	0.25±0.00[a]

Different letters (a and b) in each line shows significant difference at $p<0.05$ level.

No significant difference was observed between all three groups in terms of RV/TV ratio at 21-, and 35-day-old broilers. Finally, the control group showed a significant increase in RV/TV, which indicated right ventricular hypertrophy compared to all treatment groups as well as the sham group on the 49th day of breeding (p<0.05) (Table 1).

Lipid peroxidation was measured as the results of free radical activity in different groups (sham, control and treatment) in three age intervals (Table 2). A significant increase in lipid peroxidation was observed in the control group compared to all treatment and sham groups in 35- and 49-day-old broiler chickens (p<0.05). Less lipid peroxidation was observed in the sham group in all three time periods compared to other groups (p<0.05).

Table 2. Lipid peroxidation in the different groups at the ages of 21, 35 and 49 days

Age	Group	The level of lipid peroxidation (mol per litre)
21 day-old	Sham	0.91±0.03
	Control	1.02±0.05
	Prebiotic treatment	0.97±0.05
35 day-old	Sham	0.68±0.01^{a}
	Control	1.43±0.04^{b}
	Prebiotic treatment	0.72±0.05^{a}
49 day-old	Sham	0.71±0.03^{a}
	Control	1.83±0.04^{b}
	Prebiotic treatment	0.75±0.04^{a}

Different letters (a and b) in each line shows significant difference at p<0.05 level.

The average percentage of heterophil, lymphocyte and heterophil/lymphocyte ratio were counted in two periods of rearing including 35 and 49 days (Table 3). A significant increase in the ratio of heterophils to lymphocytes was observed in 39-day-old chickens in the control and treatment groups compared to the sham group (p<0.05), while on the 49th day of rearing, the ratio of heterophils to lymphocytes was observed in chickens. The treatment group showed a significant decrease compared to the control group (p<0.05).

Table 3. The heterophil, lymphocyte and heterophil/lymphocyte ratio in the different groups at the ages of 35 and 49 days

Age	Group	Heterophil	Lymphocyte	Heterophil/ Lymphocyte
35 day-old	Sham	31.24±1.07^{a}	55.61±0.82^{a}	0.56±0.02^{a}
	Control	51.13±1.04^{b}	43/07±1/04^{b}	1.18±0.03^{b}
	Prebiotic treatment	48.95±1.23^{b}	44.15±0.90^{ab}	1.10±0.02^{b}
49 day-old	Sham	31.21±1.66^{a}	51.18±1.76^{a}	0.60±0.02^{a}
	Control	44.07±0.93^{b}	45.12±1.03^{b}	0.97±0.04^{b}
	Prebiotic treatment	34.51±0.51^{a}	49.05±0.57^{b}	0.70±0.01^{c}

Different letters (a, b, and c) in each line shows significant difference at p<0.05 level.

4 Discussion

An important group of food additives are considered as growth stimulants, by using complex and different mechanisms, increase the usability of the ration, improve the feed efficiency, increase the growth rate, and improve the production characteristics and the general health of the animal (20). Recently, a new generation of these additives under the title of probiotics, prebiotics and synbiotics as a substitute for antibiotic additives in the nutrition of humans, domestic animals, ruminants, poultry and animals with different brand names are being produced and supplied to the world market (11). Their effects on different body systems in different animals have taken a large share of modern biological science research. De Los Santos and colleagues (2005) reported in a research that the addition of prebiotics notably decreased the incidence of ascites syndrome in broilers. Probably, prebiotics reduce the consumption of oxygen by intestinal tissue and more oxygen is available for other tissues (21). Therefore, the current experiments were conducted with the purpose of investigating the effect of safmannan as a prebiotic compound on RV/TV ratio, some blood parameters such as heterophil to lymphocyte ratio and

lipid peroxidation level in broiler chickens with ascites induced by T3. Although peribiotic administration did not have a significant effect on the RV/TV ratio at the age of 21 and 35 days, but at 49 days, it caused an increase in the ratio of RV/TV to more than 0.29 in the control group ($P<0.05$), which confirmed the induction of ascites in the chickens of this group (17). The reduction of RV/TV ratio in all groups receiving Safmannan compared to the control group shows that ascites caused by T3 administration is improved by adding prebiotic compound. Ascites is a metabolic disorder in meat-type chicks in which hypoxia occurs due to increased metabolic demand as well as decreased oxygen availability (22). The imbalance of tissue oxygen demand with oxygen supply caused by T3 administration is associated with increased blood pressure in the pulmonary arteries followed by the gradual development of ascites (23). Administration of T3 causes ascites by increasing cardiac output, basal metabolic rate and hypoxia (24, 25). In previous researches, increased ROS and increased expression of nitric oxide synthase (NOS) and nitric oxide (NO) production have been reported in hypertension (26, 27). Also, the researchers proved that the disruption of NO synthesis in different areas of the heart plays a role in the pathophysiology of heart failure in chickens with ascites. NO plays a key role in regulating heart function due to its role in controlling hypertrophic regeneration, myocardial regeneration and subsequently improving ventricular diastolic expansion (28). The reduction of RV/TV ratio, which indicates the improvement of right ventricular hypertrophy in all chickens with PHS treated with antioxidant agents, can lead to an increase in the antioxidant capacity of birds. An increase in antioxidant capacity leads to a reduction in the ratio of RV/TV due to the elimination of ROS and NO production, which plays an important role in regulating myocardial hypertrophic regeneration and ventricular diastolic expansion (29, 30).

Oxidative stress occurs in cells as a result of an imbalance between oxidizing and antioxidant systems. Oxidative stress mainly affects the membrane system of the cell, especially the bilayer phospholipids of the membrane (29). Measuring the peroxidation of lipids in the cell is an important indicator for measuring the oxidative stress level. In the study of Catala et al. (2009), it was emphasized that the peroxidation of lipids, which leads to the production of hydroperoxide, often occurs following oxidative stress (31). Arab et al. in 2007 showed that the concentration of hydroxyl free radicals is higher in chickens with ascites compared to healthy broilers (32). Bottje and Wideman in 1995 showed that systemic hypoxia can cause cellular hypoxia and increase free radicals production in mitochondria and other organelles (33). The production of free radicals affects the metabolic system including cardiac and gastrointestinal systems in birds with ascites. In another test conducted by Reed et al. (2011), it is stated that lipid peroxidation is a complex process that produces many electrophilic aldehydes in the reactions of radicals derived from oxygen and fatty acids (34). In the current research, the amount of lipid peroxidation at the ages of 35 and 49 days in the prebiotic treatment group reduced compared to the control group, which was statistically meaningful ($P<0.05$). According to the results of the current research, prebiotics could remarkably decrease the amount of oxidative stress caused by ascites, which is in line with the previous findings.

On the other hand, several experiments have proven that the ratio of heterophils to lymphocytes increases in stress conditions and can be considered as a practical index in the diagnosis of oxidative stress (35). This ratio in the present experiment also increased significantly at the age of 49 days in the control group ($P<0.05$), due to the involvement of the chickens in this group in pulmonary hypertension syndrome, and prebiotic administration was able to reduce this ratio so that it has a statistically meaningful difference with the control group ($P<0.05$). In this regard, the experiments conducted by Hester in 1996 and Ghaderi in 2009 indicated that the amount of heterophils to lymphocytes increases in stress conditions (36, 37). This ratio can be used to detect stress and its significance. Also, in another experiment, the effect of ambient temperature on the ratio of heterophil to lymphocyte was evaluated and it was found that this ratio increases with increasing temperature range (38). In a study, Fitko et al. (1993) stated that after exposure to stress in chickens, the metabolic activity of blood heterophils increases (39). Also, Gross and Siegel (1983) suggested that the ratio of heterophil to lymphocyte is a very good indicator to show stress in chickens (40).

Finally, according to the results of this study, prescribed prebiotic (safmannan) was able to decrease complications caused by oxidative stress in broilers with ascites. Therefore, it is recommended to use this compound in feeding broilers.

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Conflict of Interest

The authors declared no conflicts of interest.

Author Contributions

Hamed Zarei: Conceptualization, Formal analysis, Writing - Original Draft.

Sirous Nemati: Investigation.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

Ethical Considerations

This study was approved by the local ethics committee for animal experiments of Islamic Azad University, Garmsar.

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