

## Effects of *Plantago major* extract on serum protein fractions in broiler diet

Nuriye Tugba Bingol<sup>1</sup>, Semiha Dede\*, Mehmet Akif Karsli<sup>2</sup>, Orhan Yilmaz<sup>3</sup>, Idris Turel<sup>4</sup> and Veysel Yuksek<sup>5</sup>

Biochemistry Department Veterinary Medicine Faculty,  
Yuzuncu Yil University Van, Turkey.

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### ABSTRACT

The aim of this study was to determine the effects of *Plantago major*'s (*P.major*) water extract added into broiler diets at different levels on serum protein fractions. A total of 112 Ross 308 broiler chicks were used in the study. Experiment consisted of control and 3 treatment groups with a 28 chicks within each group. Each experimental group was divided into four subgroups consisting of 4 chicks. A basal (control) diet was prepared and three experimental diets were established by addition of *P.major* into basal diet; *P.major* 1 (5 g/kg feed), *P.major* 2 (10 g/kg feed), *P.major* 3 (15 g/kg feed). Broiler chicks were fed with these diets for 42 days ad libitum. Total protein levels of *P.major* 3 group were lower than other groups and controls. It was determined that the albumin percentages and levels became decreased in a significant ratio in the *P.major* 1 and *P.major* 2, ( $P<0,05$ ), Alpha 1 globulin percentage and concentration were found significantly high in *P.major* 2 group than the other groups ( $P<0,05$ ), Alpha 2 level and percentage in *P.major* 1 group was seen significantly higher than the control group, the A/G ratio in *P.major* 1 and *P.major* 2 was seen significantly lower than the control group. There was no significant difference between groups for beta and gamma globulins as percentage.

**Key words:** Broiler, Diet, *Plantago major*, Serum protein fractions.

### INTRODUCTION

It has been determined that the antibiotics added to the feed of broiler chicks for long years in order to decrease the risk of getting caught an illness and in order to increase the performance had some leftovers in animal products and such substances develop cross resistance with the antibiotics used human illness treatments (Buchanan *et al.* 2008). For this reason, in EU countries, since January 2006, the use of antibiotics as a growth factor in feeds has been banned (Pirgozliev *et al.*, 2008). Instead of the antibiotics in the rations, as alternative natural growth stimulus; many alternative products such as organic acids, probiotics, prebiotics, plant extracts, essential oils have started to be used (Ball, 2000).

*Plantago major* is a perennial plant, the leaves of which are used in wound healing for many years. The plant consists alkaloids, caffeic acid derivatives (anti-inflammatory effected plantamajoside and antioxidant acteoside), flavonoids (antioxidant effect), iridoid glycosides (antiinflammatory and antimicrobial effected Aucubin, Aucubigenin) and such active ingredients (Samuelsen, 2000). It has been indicated that in the pharmacological studies done with the juicy and methanol extract of the plant; it was notified that the plant has many effects such as antiinflammatory, antibacterial, antioxidant, immune modulator, anti-ulcerogenic effect and

wound healing effect (Ravn and Brimer, 1988; Campos and Lissi, 1995; Guillen *et al.*, 1997; Basaran *et al.*, 1997; Samuelsen, 2000; Hosein Farzaei *et al.*, 2014). An extract of *P. Major* Seeds has potential as a therapeutic agent to combat diabetic complications (Matsuura *et al.*, 2014).

The normal serum protein has two main proteins: albumin and globulins. Serum proteins can be separated electrophoretically and can detect 5 main protein fractions (albumin,  $\alpha 1$ ,  $\alpha 2$ ,  $\beta$  and  $\gamma$ -globulins) respectively. The serum protein electrophoretic profile help to determine various conditions, such as feeding, different infectious diseases, liver disorders, acute inflammatory and proliferative cases, immunity, tissue damage like trauma, and many other physiological disorders. Especially, the immunoglobulins which take place in gama globulin fraction are important for immune metabolism (Manojlovic *et al.*, 1993; Erstad, 1996; Karagul *et al.*, 2000; Mehmetoglu, 2002; Murray, 2003).

When the related studies about *Plantago major* are considered, it has been thought that it has a significant value, especially in increasing the immunity in broiler chicken feeding and it is thought that it has a role in increasing the resistance to the illnesses with the anti-bacterial effect it has. This study was planned to investigate the effects of *Plantago major* extract with water on serum protein fractions in broiler chicken.

\*Corresponding author's e-mail: sdede@yyu.edu.tr

<sup>1</sup>Animal Nutrition and Animal Dieases Dept. Veterinary Medicine Faculty, Yuzuncu Yil University Van, Turkey.

<sup>2</sup>Animal Nutrition and Nutritional Dieases, Dept. Veterinary Medicine Faculty Kirikkale University, Kirikkale, Turkey.

<sup>3</sup>Pharmacy Faculty, Yuzuncu Yil University, Van, Turkey.

<sup>4</sup>Pharmacy Faculty Adiyaman University, Adiyaman, Turkey.

<sup>5</sup>Ozalp Vocational High School, Yuzuncu Yil University, Van, Turkey.

## MATERIALS AND METHODS

In the study, 112 Ross 308 type broiler chicks have been used. The experiment was organized in a way that 3 experiment group and a control group were set up, each made up with 28 chicks. Each experiment group was divided into 4 sub-groups made up with 7 chicks.

The mixed feed used in the study was prepared in a feed-factory in accordance with the mixed feed ingredients as they should be. The content of the feed was prepared according to NRC (1994) and it has been given in Table 1.

The plant of *Plantago major* L. which constitutes the core material of this study was collected in May-June month's period of 2008, in Van, and the type of the plant was identified in Yuzuncu Yil University, Science and Letters Faculty, Biology Department. The collected plants were ground with a mill run by electricity, after the plants were dried in shade, and the plants went through sieves with 0.4mm diameter. The plants which went through the sieve were kept in colored glass jars. For the juice extract of *Plantago major* plant; the plant in the amounts of 5 gr, 10 gr, 15 gr was measured and they were put it the 100cc beakers. 100cc of the boiled distilled water which was cooled down to 60°C was taken and the water was poured onto thee beakers; within which 5 gr, 10gr, 15 gr *Plantago major* plant was and in the double boiler which has been adjusted to 60 °C, the mixtures was kept for 1 hour by mixing with a glass rod. The plant-water mixture which was taken out of the double boiler was left to cool. After it was totally cold, the mixture was filtered

into sterile container from the Whatman filter paper and it was made ready for use. Daily prepared *P.major* juicy extract was added to the feed through squirting; a homogeny mixture of 5 (*P.major* 1), 10 (*P.Major* 2) and 15 (*P.major* 3) g/kg feed was gained. These prepared feed was given to the animals ad libitum after they had dried. Animals were put subjected to group feeding in groups of 7 each, they were fed ad libitum. There was always clean water in manual kerbs. As base material, wood flour was used; illumination was provided 24 hours; in day time daylight was availed; and at nights, bulbs were used. All chicks were fed with starter-feed during 0-3rd weeks of the study and they were fed with growth feed in 4th-6th weeks of the study.

After completion of the study, the blood samples of the chicks were taken from the below of the wing vein, according to the proper way, into the glass tubes. The blood samples were collected from the all groups and serum samples was separated by centrifugation at 500 g for 10 min. Total protein concentrations were analyzed using the biuret method. The serum protein fractions were separated using the Helena Lab-Titan III® Serum Protein Electrophoresis device (Cat No. 3023), Helena Lab -Titan III Cellulose acetate cards and Electra HR Buffer (Cat No. 5805) tampon solutions (Helena, Bioscience Europe, UK), then stained with Ponceau S Stain solution.

The bands attained after the investigation of the serum protein fractions electrophoretically were assessed in

**Table 1:** Botanical and chemical composition of the experimental diet.

Content (kg/100kg)	Start	Finishing
Corn	49.00	57.50
Soybean Meal	42.00	33.70
Dicalciumphosphate	0.90	0.13
Limestone	1.50	2.00
Vegatable oil	5.80	6.00
Vitamin-Mineral Premixes*	0.30	0.30
Methionine-Cystine	0.15	0.07
Salt	0.35	0.30
Chemical compositions of mixed feeds that calculated		
Crude protein	22.95	19.75
Metabolizable energy (Kcal/kg)	3105	3207
Calcium	1.00	0.90
Available phosphorus		0.40
Chemical compositions that determined by analysis of mixed feeds (%)		
Dry Matter	92.72	97.71
Crude Protein	24.23	21.49
Crude Oil	6.19	6.41
Crude Cellulose	5.026	5.04
Ash	7.91	5.38
Nitrogen-free extract substances	49.13	54.39
Metabolic Energy (Kcal/kg)**	3230	3356

\*Vitamin-Mineral premixes (1U or mg/kg diet): vitamin A, 12000 IU ; vitamin D3> 1500 IU; vitamin E, 30 mg ; vitamin K3> 5 mg; vitamin B1> 3 mg ; vitamin B2, 6 mg; vitamin B6> 5 mg ; vitamin B12> 0.03 mg ; nicotinamide, 40 mg ; calcium-D-pantotenat, 10 mg ; folic acid, 0.075 mg ; choline chloride, 375 mg; antioxidant, 10 mg manganese, 80 mg ; ferrous, 80 mg ; zinc, 60 mg ; copper, 8 mg ; iodine 0.5 mg ; cobalt, 0.2 mg ; selenium, 0.15 mg. \*\* Calculated according to Titus ve Fritz (1971)

Platinum 3.0 program and the concentrations and percentages of the protein bands were ascertained.

The data were analyzed with a One Way Variance analysis. Duncan test was applied for multiple comparisons. Differences were considered as significant, when the p value was less than 0.05. using the SPSS 22.0 statistical software.

## RESULTS AND DISCUSSION

The data obtained after the evaluation of the percentage of serum protein fractions in total protein were summarized in Table 2. It has been determined that the percentages of albumin in all experiment groups decreased compared to the control group and the decrease in *P.Major* 1 and *P.Major* 2 was important ( $p < 0.05$ ). Alpha 1 globulin percentage were found significantly high in *P.Major* 2 group than the other groups ( $p < 0.05$ ). It has been found that the Alpha 2 fraction was highest in *P.Major* 1 group, it was lowest in *P.Major* 3 group. It has been found that only *P.Major* 1 group was significantly higher than the control group. There was no significant difference between groups for beta and gamma globulins as percentage.

It has been found important that the levels of albumin in all experiment groups were decreased compared to the control group, the decrease in *P.Major* 2 and *P.Major* 3 was significant compared to the control group ( $p < 0.05$ ) (Table 3). Alpha 1 globulin levels were found significantly high in *P.Major* 2 group than the other groups ( $p < 0.05$ ). It has been found that Alpha 2 level was high in *P.Major*, it was found lowest in *P.Major* 3. Just, it has been found that *P.Major* 1 group was high significantly compared to the control group. There was no significant difference between groups for beta and gamma globulins levels. Total protein levels of *P.Major* 3 group were lower than other groups and controls. It was determined that A/G ratio was significantly low in *P.Major* 1 and 2 than the control group.

This study was undertaken for the purpose of researching on the effects of *Plantago major* plant which

was added to serum protein fractions as the mixed feed of the broiler chicks.

Albumin helps in contributing osmotic pressure balance alongside the help for the lipid and steroid hormones' being carried. The rest of the serum proteins are made up total serum globulin fraction (2-3,5 gr/dl), various carrier and acute phase proteins and immunoglobulins (Adkins *et al.*, 2002; Jacobs *et al.*, 2005).

Serum proteins might vary both quantitatively and qualitatively in such physiological situations as age, heat, pregnancy; and in situations as nutrition, gender, environmental factors and genetic polymorphism (Karagul *et al.*, 2000; Onat *et al.*, 2002). In this study, total protein levels of *P.Major* 3 group were lower than *P.Major* 1 group and controls. In this situation, application of higher doses of *P.Major* significantly decreased the total protein levels; it has been drawn attention that this situation is valid for all protein fractions.

In this study, in the conclusion of the statistical evaluation of the percentages and concentrations of the protein fraction, it was determined that the data attained was compatible. Albumin is one of the most abundant proteins in blood. Albumin has several functions in the blood including protecting the feature of fluid balance between the blood and other tissues. Albumin decreases in malnutrition, severe protein loss, severe hunger and progressive chronic diseases (Adkins *et al.*, 2002; Metzler and Metzler, 2003; Prinsen *et al.*, 2004; Jacobs *et al.*, 2005). In the present study, it has been determined that the albumin percentages and levels were low in all experiment group compared to the control group ( $p < 0.05$ ). When the tasks of the albumin in the organism are considered, it has been thought that especially the use of albumin is important in the metabolism of the substances which enter the body as the source of *P.Major*. Mainly, albumin concentration decreases with the reasons such as hunger, decrease in hepatic synthesis, increase in albumin destruction, great loss via the intestines and peeing (Prinsen *et al.*, 2004).

**Table 2:** The Frequency of serum protein fractions (%)

	Albumin	Alpha1	Alpha2	Beta	Gamma
Control	47.43±1.91 <sup>a</sup>	8.92±1.05 <sup>a</sup>	11.58±1.46 <sup>ab</sup>	11.18±1.17	20.89±1.54
P.Major 1	34.19±6.48 <sup>b</sup>	8.84±1.03 <sup>a</sup>	15.17±1.89 <sup>b</sup>	10.82±1.23	21.82±1.72
P.Major 2	32.89±2.49 <sup>b</sup>	17.14±1.78 <sup>b</sup>	12.81±1.01 <sup>ab</sup>	14.28±0.72	22.88±1.46
P.Major 3	43.99±1.51 <sup>ab</sup>	8.96±0.97 <sup>a</sup>	10.10±1.19 <sup>a</sup>	13.53±1.47	23.41±1.48

a, b: Means with different superscripts within a column are significantly different, ( $P < 0.05$ )

**Table 3:** The concentrations of serum protein fractions (g/L)

	Albumin	Alpha1	Alpha2	Beta	Gamma	Total Protein	A/G Ratio
Control	1.640±0.120 <sup>a</sup>	0.305±0.038 <sup>a</sup>	0.393±0.044 <sup>ab</sup>	0.400±0.067	0.747±0.108	3.480±0.279 <sup>a</sup>	0.92±0.07 <sup>c</sup>
P.Major 1	1.518±0.210 <sup>a</sup>	0.317±0.022 <sup>a</sup>	0.588±0.132 <sup>b</sup>	0.562±0.154	0.805±0.072	3.790±0.452 <sup>a</sup>	0.68±0.065 <sup>ab</sup>
P.Major 2	1.043±0.084 <sup>b</sup>	0.562±0.087 <sup>b</sup>	0.415±0.051 <sup>ab</sup>	0.457±0.040	0.727±0.051	3.198±0.221 <sup>ab</sup>	0.50±0.06 <sup>a</sup>
P.Major 3	1.056±0.099 <sup>b</sup>	0.222±0.043 <sup>a</sup>	0.256±0.056 <sup>a</sup>	0.326±0.042	0.572±0.079	2.434±0.295 <sup>b</sup>	0.79±0.05 <sup>bc</sup>

a, b: Means with different superscripts within a column are significantly different, ( $P < 0.05$ )

Serum globulin levels can provide information along with other tests which are important for the determination of liver diseases, and, increases in acute and chronic liver diseases, chronic infections, acute diffuse glomerulonephritis, sarcoidosis, carcinoma and autoimmune diseases (Karagul *et al.*, 2000; Onat *et al.*, 2002).

$\alpha_1$ -Globulin fraction consists ( $\alpha_1$ - antitrypsin,  $\alpha_1$ - acid glycoprotein,  $\alpha_1$ - lipoprotein (Apolipoprotein A),  $\alpha_1$ - fetoprotein (AFP), transkörtine, protein connecting thyroxine, glycoprotein,  $\alpha_1\alpha_1$ -antichymotrypsin) (Turgut, 2000; Mehmetoglu, 2002). These proteins can be effect in the cases of severe hunger or progressive chronic diseases, enteropathy, nephropathies (Karagul *et al.*, 2000; Onat *et al.*, 2002). In this study, Alpha 1 globulin percentage and concentration were found significantly high in *P.Major* 2 group than the other groups ( $p < 0.05$ ).

$\alpha_2$ -macroglobulin, haptoglobin, ceruloplasmin which are among alpha 2-globulins are significantly affected from many metabolic situations (Mehmetoglu, 2002; Gungor *et al.*, 2004). In this study, it has been found that alpha 2 level and percentage were highest in *P.Major* 1 group, and in it was lowest in *P.Major* 3 group. It has been determined that only the *P.Major* 1 group was significantly higher than the control group. It is seen that the proteins which are in alpha 1 and 2 globulin fractions are affected from the *P.Major* application which is implemented in various doses. These data can be used as a starting point for further studies which will investigate the proteins in *P. Major* metabolism and their tasks in the pterygota-metabola.

There was no significant difference between groups for beta and gamma globulins as percentage. Although there exists knowledge that *Plantago* applications has beneficial effects on the lipoprotein metabolism within  $\beta$ -globulin fraction (Chung *et al.*, 2008), in this study beta globulin is not affected from *P.Major* in pterygota-metabola. This situation is thought to have a connection with the used dose, readiness method and the duration.

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Immunoglobulins and the C-reactive protein (CRP) migrate in the  $\gamma$ -globulin area. In the evaluation of serum protein fractions, the decrease in albumin is balanced with the increase in immunoglobulins in the gamma fraction (Erstad 1996; Karagul *et al.*, 2000; Mehmetoglu 2002; Murray 2003). Despite this, it has been seen that although albumin levels are affected after *P.Major* application, changes in gamma globulins are not significant statistically. It was interpreted that this situation depends on the dose of the extract and the usage duration. In addition to this, although there exist publications (Gomez-Flores *et al.*, 2000; Chiang *et al.*, 2003; Dorhoi *et al.*, 2006, Akram *et al.*, 2014) notifying *P.Major*'s immunoregulator effect; attention has been drawn to the fact that gamma globulin fractions do not affect significantly in the poultry in these doses. It was determined that also A/G ratio in *P.Major* 1 and *P.Major* 2 groups were low significantly due to increase in total globulin level. It was seen that *P.Major* 3 group was not different from the control group.

Although the studies researching on the effects of *P. Major* extract are scant, it is notified that its being added to the feed of broiler does not affect alive weight and alive weight increase, feed consumption and the ratio of benefiting the feed, carcass, empty carcass, viscera, abdominal fat and the weight of the intestines (Bingol *et al.*, 2010). According to this, it is of importance that the effect of *P.Major* must be found in the protein metabolism added to the performance and efficiency of the broiler chicks. In this study; in which the effects of *P.Major*'s juice extract's being added to the feeds in different doses on protein fractions are studied, as a conclusion; it was determined that Alpha 1 and 2 globulin in control group total protein increased; while albumin percentages and levels and A/G ratio were decreased; there was no significant difference between groups for beta and gamma globulins as percentage. However according to the conclusions attained, especially the protein fractions and the proteins said to contain and affect - might be worth researching by exploring and detailing in further studies.

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