



EFFECT OF PARTIAL AND TOTAL SUBSTITUTION FOR AZOLLA PLANT (*AZOLLA PINNATA*) POWDER INSTEAD OF SOYBEAN MEAL IN BROILER CHICKENS DIETS ON BLOOD BIOCHEMICAL TRAITS

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Abstract

This experiment was conducted in the poultry field, Poultry Department belonging to the Center for Livestock and Fisheries, Directorate of Agricultural Research, Ministry of Science and Technology for the period from 8/7/2018 to 19/8/2018 in order to study the effect of partial and total substitution for Azolla plant (*Azolla pinnata*) powder instead of soybean meal in broiler chickens diets on blood biochemical traits. A 105 chick of unsexed broiler chickens (Ross strain), with one day's age were used, where they were randomly divided into five treatments, with a rate of 21 birds per treatment and each treatment consisted of three replicates (7 birds per replicate). The experimental treatments were as follows: The first treatment: the feed provided to the chicks of this treatment for the duration of the experiment and without any addition or substitution, it was used as a control group, the second treatment: the feed provided to the chicks by substituting Azolla plant 5% instead of soybean meal, the third treatment: the feed provided to the chicks by substituting Azolla plant 10% instead of soybean meal, the fourth treatment: the feed provided to the chicks by substituting Azolla plant 15% instead of soybean meal and the fifth treatment: the feed provided to the chicks by substituting Azolla plant 20% instead of soybean meal. The study included the following traits: concentration of uric acid, total protein, concentration of albumin, concentration of globulin, G / A ratio, concentration of total cholesterol, concentration of triglyceride, concentration of High-density lipoprotein, concentration of low-density lipoprotein, thyroid hormones T3 and T4, Alanine transaminase enzyme (ALT), Aspartate Aminotransferase (AST) enzyme and concentration of glutathione peroxidase enzyme. The results indicated that the substitution of Azolla plant with the percentage of (5, 10, 15, 20%) led to raising the concentration of total protein, globulin, high-density lipoproteins, thyroid hormones T3 and Glutathione peroxidase as well as a significant decrease in total cholesterol, triglycerides and low-density lipoproteins were recorded. It is concluded from the current experiment that the substitution of the Azolla plant instead of soybean meal can lead to improving some of the biochemical traits for broiler chickens blood.

Key words: Azolla plant, blood biochemical traits, broiler chickens.

Introduction

Livestock production in developing countries suffers from a shortage of feed materials and many of the traditional materials used in poultry diets, such as soybean meal, are becoming increasingly expensive. Research has shown that unconventional sources can replace imported and expensive feeds, therefore there is an urgent need to look for alternative protein sources. Aquatic plants can be provided as a cheap animal feed and as a partial substitute to high-priced traditional proteins in the diets of broiler chickens, where aquatic plants are considered important to any ecosystem and source for supplying the

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water with oxygen as well as rich sources with protein, carbohydrates, food for humans and fish as well as aquatic birds (Amitav *et al.*, 2016). We have recently seen introducing many medicinal plants in the feeding of broilers chickens and laying hens. Therefore, the use of herbs and plant extracts in poultry feeding by considering it as safe and natural materials and that one of these plants is Azolla, it is a small fern plant living afloat on the surface of the water and in the rice fields submerged with water, which is a plant with Rhizoids and live in temperate or hot climates. Sun-dried Azolla is considered a good protein and carbohydrate source, although its ash content is high. Azolla works on fixing nitrogen in the soil and it has a high protein content reach to (25-44%). Where Azolla

content of protein is close to the soybean content of (Alalade and Iyayi, 2006) which is a rich mineral source (10-15%) from dry weight as well as essential amino acids (7-10%) and vitamins and carotenoids (20-30%) (Naghshi *et al.*, 2014). Azolla is used as feed for laying hens, broilers chickens, fish and sheep (Parthasarathy *et al.*, 2002). Azolla can be used in poultry diets, with a percentage of (20-50%) from the concentrated feed and some studies have shown their use with percentage amounted to 45% with a natural growth rate compared to the control treatment and ducks can be fed n it either green or after being dried and slightly moistened with water, Azolla has relatively low fibers (11-13% based on dry matter) and ash content amounted to 20% (Singh and Subudhi, 1978). In view of the above, this study aims to determine the effect of partial and total substitution for Azolla plant (*Azolla pinnata*) powder instead of soybean meal in broiler chickens diets on blood biochemical traits and knowing the best proportions that can be substituted in the diet.

Materials and Methods

This experiment was conducted in the poultry field, Poultry Department belonging to the Center for Livestock and Fisheries, Directorate of Agricultural Research, Ministry of Science and Technology for the period from 8/7/2018 to 19/8/2018, where the field and laboratory study

was applied in order to study the effect of partial and total substitution for Azolla plant (*Azolla pinnata*) powder instead of soybean meal in broiler chickens diets on blood biochemical traits. A 105 chick of unsexed broiler chickens (Ross strain), with an average weight (41 g) and one day's age were randomly distributed in a closed hall containing vertical batteries and each battery consist of 4 stories. Four treatments were used. Each treatment consisted of three replicates (7 birds per replicate), where the chicks were randomly distributed. A continuous lighting program (24 hours/day) has been used since the experiment began, at the end of the sixth week of the birds' age, the halls were supplied with light bulbs. The chicks were fed freely on a crushed diet as shown in table 1, where 21.14 protein and 3032 kcal metabolic energy were supplied per kg of feed. The diet was divided into five sections according to the following nutritional treatments: The first treatment: the feed provided to the chicks of this treatment for the duration of the experiment and without any addition or substitution, it was used as a control group, the second treatment: the feed provided to the chicks by substituting Azolla plant 5% instead of soybean meal, the third treatment: the feed provided to the chicks by substituting Azolla plant 10% instead of soybean meal, the fourth treatment: the feed provided to the chicks by substituting Azolla plant 15% instead of soybean meal and the fifth treatment: the feed provided

Table 1: Percentages of feed materials involved in the composition of diets used in the experiment with the calculated chemical composition.

Percentage of feed materials (%)	First treatment	Second treatment	Third treatment	Fourth treatment	Fifth treatment
Yellow corn	43	43	43	43	43
Wheat	12	12	12	12	12
Barley	9	9	9	9	9
Soybean meal	20	20	20	20	20
Azolla	0	0	0	0	0
The concentrated animal protein	12	12	12	12	12
Oil	3	3	3	3	3
Food salt	0.3	0.3	0.3	0.3	0.3
Lime	0.7	0.7	0.7	0.7	0.7
Total	100%	100%	100%	100%	100%
Crude protein	21.24	23.96	23.97	23.97	23.93
Metabolic Energy (kcal/kg feed)	3032	3032	3017	3020	3020
Lysine (%)	1.43 g	1.43 g	1.43 g	1.43 g	1.43 g
Methionine (%)	0.82 g	0.82 g	0.82 g	0.82 g	0.82 g
Methionine + cysteine (%)	1.07 g	1.07 g	1.07 g	1.07 g	1.07 g
Raw fibers	2.16	4.02	4.37	4.72	5.05

* The concentrated protein from Belgian, each kilogram contains: 2200 kcal metabolic energy, 40% crude protein, 8% fat, 3.5% fiber, 25% ash, 8% calcium, 3.1 available phosphorus, 1.2% lysine, 1.2% Methionine, 1.8% Methionine + 70 mg, 30 mg Vitamin B1, 300 mg Vitamin E, 2500 IU Vitamin D3, Cysteine A, 2% Chlorine, 10,000 IU 12 mg Folic Acid, 250 B12, 120 mg Pantothenic acid, 400 mg niacin, 50 mg vitamin B6, 5000 mg Choline chloride, 450 mg iron, 70 mg copper, 600 mg C, 600 mcg biotin, 1000 mg two special vitamins, 750 manganese, 5 mg iodine, 1 g cobalt And antioxidants; ** By chemical composition, according to feed materials analysis (NRC, 1994).

to the chicks by substituting Azolla plant 20% instead of soybean meal. The study included the following traits: concentration of uric acid, total protein, concentration of albumin, concentration of globulin, G/A ratio, concentration of total cholesterol, concentration of triglyceride, concentration of High-density lipoprotein, concentration of low-density lipoprotein, thyroid hormones T3 and T4, Alanine transaminase enzyme (ALT), Aspartate Aminotransferase (AST) enzyme and concentration of glutathione peroxidase enzyme. Blood samples were collected after slaughtering birds in tubes that did not contain anticoagulants. Blood plasma was separated by centrifuges at a speed of (3000 rpm) for 15 minutes. The serum was kept in clean tubes at a temperature of -20°C . A diagnostic kit from Jordanian origin was used to measure cholesterol (mg / 100 ml) according to (Franey and Elias, 1968). Triglycerides, low-density, high-density lipoproteins were estimated according to (AOAC, 1980), the total protein (g / 100 ml) and uric acid (mg / 100 ml) based on the method described in (Henry *et al.*, 1982). As for the concentration of globulin was calculated according to the following equation: (concentration of total protein - concentration of albumin). Liver enzymes were estimated according to the method described in (Reitman and Frankle, 1957). Table 1, shows the used feed materials and their calculated chemical composition during the experiment period. The Completely Randomized Design (CRD) was used to study the effect of different treatments on the studied traits, Significant differences between the averages were compared using Duncan's Multiple Range Test (Duncan, 1955) and the SAS program (SAS, 2010) was used to analyze the data.

Results and Discussion

Table 2, shows the effect of partial and total substitution of Azolla plant in the diet of broiler chickens on blood plasma traits at the age of (6) weeks, where the table shows that there were no significant differences between all treatments in the concentration of uric acid and albumin in the blood of birds, while the results indicated

Table 2: Effect of partial and total substitution of Azolla plant in the diet of broiler chickens on blood plasma traits at age (6) weeks.

Treatments	Concentration of uric acid (mg/dl)	Total protein (g/100 ml)	Concentration of albumin (g/100 ml)	Concentration of globulin (g/100 ml)	G/A ratio
First treatment (control)	26.67 ± 5.63	5.793 ± 0.02 b	3.253 ± 0.35	2.540 ± 0.05 b	1.286 ± 0.005 a
Second treatment (5% Azolla)	26.78 ± 6.05	6.367 ± 0.08 a	3.405 ± 0.08	2.962 ± 0.03 a	1.166 ± 0.002 ab
Third treatment (10% Azolla)	27.08 ± 4.30	6.208 ± 0.05 a	3.150 ± 0.11	3.058 ± 0.06 a	1.030 ± 0.003 b
Fourth treatment (15% Azolla)	26.17 ± 4.82	6.250 ± 0.09 a	3.250 ± 0.25	3.000 ± 0.04 a	1.087 ± 0.004 b
Fifth treatment (20% Azolla)	27.11 ± 5.61	6.319 ± 0.07 a	3.142 ± 0.23	3.177 ± 0.07 a	0.988 ± 0.008 bc
Significant level	N.S	*	N.S	*	*

NS: no significant difference between treatments. * There were significant differences at the level of ($P < 0.05$).

that there were significant differences between the treatments in the concentration of total protein and the concentration of globulin, where it is observed from the results of the table that the treatments of Azolla plant were significantly ($P \leq 0.05$) excelled on the control treatment (first). As for the G/A ratio, the control treatment recorded the highest percentage of G/A, with a significant difference ($P \leq 0.05$) from the treatments (third, fourth and fifth). The reasons for increasing the averages of total protein and globulin in the treatments of Azolla plant may be due to the fact that it is considered a medicinal plant, which helps to improve digestion in birds, this contributes to an increase in the percentage of a nutrient, including glutathione-related protein in the liver and consequently to increase the percentage of Y-Globulin protein in the blood (Basak *et al.*, 2002), or it may be that Azolla works to reduce the exposure of birds to any type of stress through increasing thyroxine secretion and thus increase the rates of metabolic and increase the vital reactions in the body and then build muscle tissue in the body, which leads to maintaining a high average of total protein and globulin in the birds' blood of the study treatments compared to the control treatment (Balaji *et al.*, 2009).

Table 3, shows the effect of partial and total substitution of Azolla plant in the diet of broiler chickens on the concentration of cholesterol, blood fat and thyroid hormones at the age of (6) weeks, where the results show that there were significant differences between the treatments in the concentration of cholesterol, low-density lipoproteins and thyroid hormone T3. It indicated significant improvement in these traits except for the results of triglycerides, high-density lipoproteins and thyroxine T4 which did not show significant differences between treatments and the control treatment in the averages of this trait. From the table observation, the results showed that the substitution treatments of Azolla plant (second, third, fourth and fifth) recorded a significant decrease in the concentration of total cholesterol and low-

Table 3: Effect of partial and total substitution of Azolla plant in the diet of broiler chickens on the averages concentration of cholesterol, blood fat and thyroid hormones at age (6) weeks.

Treatments	Concentration of Cholesterol (mg / 100 ml)	Concentration of Triglyceride (mg / 100 ml)	Concentration of high-density lipoproteins (mg / 100 ml)	Concentration of low-density lipoproteins (mg / 100 ml)	Thyroxine hormone T3 (ng / ml)	Thyroxine hormone T4 (ng / ml)
First treatment (control)	6.03±181.5 a	161.30±0.06	47.59±1.45	5.03±100.673 a	2.56±1.54 b	1.35±7.46
Second treatment (5% Azolla)	7.10±169.2 b	158.03±1.11	47.08±1.32	6.21±90.77 b	0.46±1.66 a	2.12±7.81
Third treatment (10% Azolla)	5.41±172.42 b	161.21±2.54	46.67±1.12	4.42±93.13 b	0.75±1.87 a	1.46±7.79
Fourth treatment (15% Azolla)	6.53±167.04b	162.43±3.42	49.92±1.72	6.53±84.48 b	0.22±1.84 a	1.74±8.07
Fifth treatment (20% Azolla)	4.31±161.12b	160.31±1.53	48.71±2.83	6.44±81.37 b	0.12±1.83 a	1.83±7.39
Significant level	*	N.S	N.S	*	*	N.S

NS: no significant difference between treatments. * There were significant differences at the level of ($P<0.05$).

density lipoprotein compared to the first treatment (control) which recorded the highest concentrations in these traits. As for the thyroid hormone T3, the substitution treatments of Azolla plant (second, third, fourth and fifth) significantly ($P\leq 0.05$) recorded the highest level compared to the control treatment which recorded the lowest level for this traits. The decrease in the concentration of total cholesterol, low-density lipoprotein for Azolla plant treatments may be due to the role of Azolla, which reduces enzymes that are excreted from the liver and builds fatty acids or the decrease in the concentration of triglyceride may be due to inhibition of the Acetyl CoA Synthetase, which is considered a necessary enzyme in the fatty acid

Table 4: Effect of partial and total substitution of Azolla plant in the diet of broiler chickens on the concentration of ALT and AST and the concentration of glutathione peroxidase for the broiler chicken blood serum at the age of 6 weeks.

Treatments	Concentration of ALT (IU/L)	Concentration of AST (IU/L)	Concentration of Clotathione peroxidase (mg/100 ml)
First treatment (control)	25.87±0.14	63.00±0.18	0.397±0.08 c
Second treatment (5% Azolla)	23.92±0.25	59.42±0.31	0.474±0.13 a
Third treatment (10% Azolla)	25.42±0.21	59.83±0.33	0.438±0.26 b
Fourth treatment (15% Azolla)	25.75±0.63	60.25±0.19	0.451±0.28 ab
Fifth treatment (20% Azolla)	22.43±0.81	60.25±0.11	0.463±0.18 ab
Significant level	N.S	N.S	*

NS: no significant difference between treatments;

* There were significant differences at the level of ($P<0.05$).

synthesis process (Bhattacharyya *et al.*, 2015).

Table 4, shows the effect of partial and total substitution of Azolla in the diets of broiler chickens on the concentration of ALT and AST and the concentration of glutathione peroxidase for the broiler chicken blood serum at the age of 6 weeks, where it was not observed a significant difference between all treatments in the concentration of Alanine transaminase, where the rise and decrease in the levels of these enzymes reflect the health status for the bird and liver function and health status (Ganong, 2005). As for the concentration of the glutathione peroxidase, we note that the substitution treatments of Azolla plant were significantly ($P\leq 0.05$) excelled compared to the first treatment (control), which recorded the lowest concentration of the enzyme. The high concentration of enzyme in Azolla treatments may be due to the role of active substances in Azolla plant, which has the ability to inhibit the activity of free radicals because it has an effective antioxidant role within the body as considering one of the most important natural antioxidants (Mishra *et al.*, 2016).

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