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Original Article

Azolla as a New Dietary Source in Broiler Feed: a Physiological and Production Study

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Abstract

The high requirement of the broiler to protein in the diet needs more knowledge about protein quality, availability of amino acids, prices or cost, the minimum concentration of anti-nutritional factors (ANFs) and gastrointestinal tract (GIT) health. Azolla is a floating fern with high protein content, vitamins (Vit B12, Vit A, and growth promoters), essential amino acids, and minerals (phosphor, calcium, ferrous and potassium). Azolla can fix nitrogen from the atmosphere through the presence of blue-green algae (Anabaena azollae) in their leaves cavities. This study investigated the physiological and production effect of including different levels of Azolla in broiler feed. Four hundred broiler Ross chicks were randomly divided into four equal groups (n=100). The Control group received a regular diet without any supplementation. Animals in the treatment 1 group (T1) received 15% Azolla in their regular diet formulation, the treatment 2 group (T2) received 30% Azolla in their diet formulation, and the treatment 3 group (T3) received 45% Azolla. The animals had *ad libitum* access to food and water. Body weights and food conversion ratio (FCR) were monitored weekly, and blood samples were collected at the end of the experiment for assessment of lipid profile (cholesterol, triglyceride, HDL, LDL and VLDL), blood proteins (total protein and albumin) and activity of liver enzymes (GOT, GPT and ALP). The results showed an increasing body weight significantly ($P \leq 0.5$) in the T3 group compared with the control along with a significant decrease in FCR at an exact quantity of feed for each group (3 Kg). The results showed no change in physiological parameters (lipid profile, blood protein and liver enzymes activity) except for the levels of GOT, which decreased significantly ($P \leq 0.5$). In conclusion, the inclusion of Azolla at 15, 30 and 45% in broiler feed has a good effect on body weight and FCR, with no adverse effect on the normal physiology of broilers. Also, supplementation of Azolla can decrease production costs in the broiler industry by more than 30%.

Keywords: Azolla, Broiler feed, Lipid profile, Dietary source

1. Introduction

Around the world, the animal feed industry depends on the supply of dietary protein sources from a limited number of the country that is dominant in highly protein-content seed production (soybean, rapeseed, sunflower, peanuts, cottonseed and palm kernels). Therefore, the transport, demand, location, and fluctuation in currency exchange play an essential role in the prices of this seed Iji, Toghyani (1). Researchers give dietary protein the largest attention because it constitutes a major biologically active component in broilers' physiology. Proteins participate in living bodies as enzymes and hormones, which control the performance and production of every living organism. The high requirement of the broiler to protein in the diet needs more knowledge about protein quality, availability of amino acids, prices or cost, the minimum concentration of anti-nutritional factors (ANFs) and gastrointestinal tract (GIT) health (2).

In broiler farms, the farmers spend about 65-75% of the total production cost to produce a complete diet formulation. Protein sources in the diet are considered the significant expansive portion of the broilers' diet formulation; with increased demand for protein sources, the cost also rises, and the necessity to find a new, cost-effective source of dietary protein is necessary very important (3).

Azolla is a floating fern with high protein content, vitamins (Vit B12, Vit A, and growth promoters), essential amino acids, and minerals (phosphor, calcium, ferrous and potassium). Azolla can fix nitrogen from the atmosphere through the presence of blue-green algae (Anabaena azollae) in their leaves cavities (4). Furthermore, it can bio-fertilizer in rice farms and produce biomass rapidly due to its relatively high growth rate with low cost. Therefore, it integrates animal production and agriculture (5, 6).

The increased level of Azolla in the ratio does not affect the protein digestibility, crude fibre and crude fat; broilers can readily digest crud fibre in Azolla, so when it is used in the diet, digestibility is not a limiting factor. The availability of essential amino acids in the Azolla protein makes it a very important source in animal feed and can be considered an excellent unconventional source of protein in broiler diets over 20% (7).

Therefore, this study aimed to investigate the physiological and production impact of Azolla inclusion in broiler diet formulation at different levels.

2. Materials and Methods

2.1. Diet Preparation

Azolla was collected from local rivers surrounding Al-Refaa city and dried, weighed, and mixed with broiler diet ingredients according to the proposed percentages in the experiment; the whole diet was prepared as a pellet and kept until used in the experiment. The nutritional facts of Azolla are tabulated in table 1. The chemical composition of the diet was 22-23% crude protein and 3010 Kcal for the starter, 21-22 crude protein and 3100 Kcal for the grower and finisher. The diet comprises soybean meal, wheat, corn, vegetable oil and premix (amino acids, minerals, multivitamins, choline chloride, calcium di-phosphate).

Table 1. Chemical composition of Azolla meal (8)

Constituents	%
Crude fibre	15.7
Dry matter	10
E. extract	3.47
Total ash	15.05
N. free extract	30
Crude protein	25.78

2.2. Animals

400 (as-hatched) Ross 308 broiler chicks were randomly divided into four groups (100 per each), every 25 chicks were allocated a special cage (120*100*40) feed, and water was *ad-libitum*. The temperature was controlled according to age using a thermostat and electrical heater. All birds were vaccinated against many viral diseases according to the epidemiological condition of the region (infectious bursal d., Newcastle d., infectious bronchitis d. and influenza).

2.3. Experimental Design

The first group served as control and fed the commercial diet throughout the experimental period; the treatment 1 group (T1) was fed 15% Azolla and 85% commercial feed, treatment 2 group (T2) was fed 30% Azolla and 70% commercial feed, and treatment 3 group (T3) fed 45% Azolla and 55% commercial feed.

The experimental period was extended for 39 days. The control group consumed 3 kg at 35 days, while other groups were extended to the 39th day; chicks were weighed at the beginning of the experiment; the weekly weigh recordings were performed until the end of the experiment. The feed conversion and vitality ratio were calculated. Blood samples were collected from the jugular vein, and serum was separated for assessment of lipid profile (cholesterol, triglyceride, high-density lipoprotein HDL, low-density lipoprotein LDL and very low-density lipoprotein VLDL (9, 10) activity of liver enzymes (serum glutamic oxaloacetic

transaminase GOT, serum glutamic pyruvic transaminase GPT and alkaline phosphatase ALK) (11), total blood protein and serum albumen, concentration and activity were assessed by spectrophotometric method.

2.4. Statistical Analysis

Statistical analysis was carried out using a one-way variation analysis, and the Friedman test was used to compare experimental groups. The results were expressed as mean ±SD, and the difference at level $P<0.05$ was considered significant. These results were obtained using GraphPad prism-version 5 (GraphPad, software., Inc. California, USA).

3. Results

3.1. Body Weight

The findings of initial, weekly and final body weight are illustrated in table 2. The recorded data showed insignificant ($P\geq0.5$) differences among the experimental groups during the first week, while, after the second and third weeks, the control group recorded a significant ($P\leq0.5$) increase compared with that of 2nd group. The final weight of the experiment clarifies a significant ($P\leq0.5$) increase in the body weight of T3 compared with the control group. Table 3 clarifies the result of the feed conversion ratio (FCR) and vitality ratio. The results showed a significant ($P\leq0.5$) decrease in the FCR of T3 compared with that of the control and other groups of the present study. On the other hand, the results clarified insignificant ($P\geq0.5$) differences among the experiment groups in vitality ratio throughout the experiment.

Table 2. Impact of Azolla inclusion in broiler feed on body weight

Age per week	control	T1	T2	T3
Initial weight	43±2	41±3	42±5	43±4
1	175±5^{a}	172±4^{a}	176±5^{a}	175±6^{a}
2	483±10^{a}	409±15^{ab}	380±15^{bc}	435±20^{ab}
3	819±30^{a}	730±25^{ab}	700±20^{bc}	750±18^{ab}
4	1680±45^{a}	1410±50^{a}	1450±35^{a}	1470±29^{a}
5	1980±85^{a}	1790±60^{a}	1750±65^{a}	1760±50^{a}
39 days	1980±85^{ab}	1850±80^{b}	1980±71^{ab}	2100±40^{a}

Table 3. Impact of Azolla inclusion in broiler feed on FCR and vitality ratio

Groups	FCR	Vitality r.
control	1.515^{a}	98%^{a}
T1	1.62^{a}	100%^{a}
T2	1.515^{a}	99%^{a}
T3	1.42^{b}	100%^{a}

Numbers refer to mean±SD (n=100 broiler chicks), and letters refer to significant differences ($P\leq0.5$)

3.2. Lipid Profile

Results of blood analysis showed insignificant ($P\geq0.5$) differences among experimental groups in the case of blood lipid profile (Table 4). The results recorded a decrease in cholesterol, triglyceride, HDL and VLDL concentration, while a slight increase was recorded in LDL levels in the T1 and T2 groups but still not significant ($P\geq0.5$). The results showed that cholesterol and HDL concentration increased in the T3 group ($P\geq0.5$) compared with other groups.

Table 4. Effect of Azolla feeding on lipid profile

Groups	Cholesterol	Triglyceride	HDL	LDL	VLDL
control	131.0±8^{a}	75.0±10^{a}	86.3±19^{a}	29.0±3^{a}	15.0±2^{a}
T1	116.5±3^{a}	52.5±3^{a}	71.01±6^{a}	35.0±4^{a}	11.0±1^{a}
T2	118.5±5^{a}	60.0±2^{a}	73.5±1^{a}	33.0±5^{a}	12.0±1^{a}
T3	133.5±7^{a}	75.0±3^{a}	97.0±3^{a}	30.0±4^{a}	15.0±1^{a}

Numbers refer to mean±SD (n=100 broiler chicks), and letters refer to significant differences ($P\leq0.5$)

3.3. Activity of Liver Enzymes and Blood Protein

The recorded data in the case of liver enzymes activity measurement registered a significant ($P\leq0.5$) decrease in GOT activity in T2 and T3 groups compared with the control group (Table 5). While GOT and ALK were insignificant, ($P\geq0.5$) decreased compared to the control group. Table 6 shows the results of total blood protein and albumin concentrations in different groups of the experiment, which recorded insignificant ($P\geq0.5$) differences among the experiment groups.

Table 5. Effect of Azolla feeding on the activity of liver enzymes

Groups	GOT	GPT	ALK
Control	70.3±1[a]	3.5±0.7[a]	1325±133[a]
T1	62±4.2[ab]	1.5±0.7[a]	710±120[a]
T2	37±4[ab]	4.5±2.1[a]	769±130[a]
T3	32±3[b]	4.5±0.7[a]	840±115[a]

Numbers refer to mean±SD (n=100 broiler chicks), and letters refer to significant differences ($P \leq 0.5$)

Table 6. Effect of Azolla feeding on blood protein concentration

Groups	T. protein	Albumen
control	3.45±0.07[a]	1.9±0.1[a]
T1	3.3±0.14[a]	1.7±0.28[a]
T2	3.5±0.14[a]	2±0.14[a]
T3	3.2±0.28[a]	1.85±0.21[a]

Numbers refer to mean ±SD (n=100 broiler chicks), and letters refer to significant differences ($P \leq 0.5$)

4. Discussion

The present study investigated Azolla supplementation in broiler diet formulation at different concentrations. In the previously published works, researchers concluded that Azolla could be used in poultry feed over 5% without any side effects on growth performance and productivity (12, 13). Azolla, with up to 15% inclusion in poultry feed, can enhance performance and growth (14); Ara, Adil (15) found that feeding Azolla over 20% does not have an adverse effect on the carcass. The use of Azolla as a fresh fern or sun-dried and ground makes it a big size; for this reason, the percentage of Azolla inclusion in broiler feed is low; in our study, the compress of whole feed after sun-dried Azolla as pellet makes the size of feed is normal and give us the permitting to increase the percentage. Moreover, production feed costs considerably reduce with increased Azolla inclusion in broiler feed (16).

The finding of our study was registered fluctuation in body weight among experimental groups, which started with significant ($P \leq 0.05$) elevation in body weight of the control group, and gradually this increase subsided, and the difference became insignificant ($P \geq 0.05$), on day 39 of the study T3 was elevated significantly ($P \leq 0.05$). These results were due to feed consumption in treated groups which needed 39 days to consume three Kg compared with the control group (35 days). The increasing percentage of Azolla in broiler feed will negatively affect the birds' appetite, and the growth will consequently reduce; this result was due to high fibre content in a high concentration of Azolla in feed (17).

Feed conversion ratio (FCR) was significantly ($P \leq 0.05$) decreased in T3 (45% Azolla) compared to that of other groups, this finding was agreed with other researchers (17-20), which concluded that the FCR in broiler improved with increase Azolla in diet, while Sharma, Pathak (21) reported insignificant ($P \geq 0.05$) difference in FCR among experimental groups, this finding may be due to the negative effect of Azolla on appetite and the groups were not consumed the same quantity of feed.

Lipid profile measurement recorded insignificant ($P \geq 0.05$) differences among groups; this finding agreed with that of Mishra, Roy (22) and Shukla, Bhattacharyya (23), who found the lipid profile of broiler chick unchanged after feeding Azolla included diet. Also, liver enzymes activity measurements were insignificantly ($P \geq 0.05$) different, except that of T3, which reported a significant ($P \leq 0.05$) decrease in the activity of GOT compared with the control group, this result was asymmetric with that of Mishra, Roy (22) who find GOD increase in the treated group compared with that of control, this may be due to liver enzyme in birds originated from different organs muscles, skeletal and heart, and as a second order from the liver, therefore, its limitation is variable (24). Blood protein concentrations were similar in all groups of the experiment, and this result agreed with that of Shukla, Bhattacharyya (23).

From the present finding, it may be inferred that increasing Azolla inclusion in broiler diet to 45% improves the feed conversion ratio without any adverse effect on the normal physiology of the birds.

Authors' Contribution

Study concept and design: H. A. J. A.
Acquisition of data: H. A. J. A.
Analysis and interpretation of data: H. A. J. A.
Drafting of the manuscript: H. A. J. A.
Critical revision of the manuscript for important intellectual content: H. A. J. A.
Statistical analysis: H. A. J. A.
Administrative, technical, and material support: H. A. J. A.

Ethics

The study design was approved by the ethics committee University of Sumer, Rifai Dhi Qar, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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