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The potential of *Azolla* as a nutritious feed alternative for broilers: A study on physiology and production

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Abstract
The effects of various *Azolla* levels in broiler feed on production and physiology were examined in this study. A total of 400 Ross broiler chicks were split into four identical groups for this reason (100 per each). Food and drink were ad libitum to all groups, but the groups that were treated (T_1, T_2, and T_3), as called the control group, obtained varying percentages of *Azolla* (15 percent, 30 percent, and 45 percent), respectively. Weekly measurements of body weight and FCR (feed conversion ratio) were conducted, and samples of blood were taken at the conclusion of the experiment to assess the liver enzyme activity (GOT, GPT, and ALP), blood proteins (albumin and total protein), and lipid profile (triglyceride, cholesterol, VLDL, HDL, and LDL). With the exception of the activity of GOT, which was ultimately ($p \leq 0.5$) decreased, the findings revealed no alteration in the physiological parameters (liver enzyme activity, blood protein, and lipid profile) and an increase in body weight ($p \leq 0.5$) in the T_3 (third group that was treated) in comparison to the control, where the FCR was decreased, at the same amount of feed (3 kg). To conclude, the addition of *Azolla* percentages (15, 30, and 45) to feed of broil improves both FCR and body weight without negatively impacting the broiler body's normal physiology. Moreover, it can save feed production expenses by over 30%.

Keywords: *Azolla*, dietary source, lipid profile, broiler feed

Introduction
The supply of dietary protein sources, which are restricted to a few dominant countries in the production of protein rich content seeds (sunflower, cottonseed, soybean, peanuts, rapeseed, and palm Kernels), is essential to the animal feed industry. As a result, factors such as transportation, demand, location, and fluctuations in currency exchange rate significantly impact the price of this seed (Paul *et al*., 2017) [20].

Because dietary protein makes up a significant portion of the biologically active component of the broiler body, researchers focus much of their emphasis on it. Both hormones and enzymes, which regulate the functions and synthesis of all living things, contain proteins. The rising demand for broiler protein in the diet needs a greater understanding of the following topics: minimum concentrations of anti-nutritional factors (ANFs), pricing or costs, availability of amino acids, and the health of the gastrointestinal tract (GIT) (Salman, 2015) [20].

To create a complete diet, farmers must spend between 65 and 75 percent of their entire production costs, where protein sources are the most expensive portion. Since costs rise in tandem with increased demand, it is critical to discover a modern, widely accessible, reasonably priced, and distinctive source of dietary protein (Ahmed *et al*., 2020) [2].

Azolla is a type of aquatic ferns that is enriched with protein, minerals, and essential amino acids (ferrous, calcium, phosphor, and potassium), vitamins (A, B12, β carotene, growth promoters). When Anabaena *Azolla*e (blue-green algae) are present in the leaf sinus of *Azolla* plants, nitrogen can be fixed in the atmosphere by the plants (Dev *et al*., 2015) [8]. Furthermore, due to its comparatively rapid growth rate and reasonably priced, it can be used as a biofertilizer in rice farms and produce biomass quickly, integrating agriculture and animal production (Lakshmanan *et al*., 2017; Karpa *et al*., 2021) [14, 12].

Crude fiber, crude fat, or the digestibility of proteins are unaffected by the rise in *Azolla* in the ratio. Furthermore, when *Azolla* is utilized in a diet, digestibility is not a limiting problem because the crude fiber is very digestive by broilers.

Azolla's protein is highly valuable in animal feed because it contains essential amino acids. It can also be regarded as a suitable non-traditional source of protein for broiler diets that contain more than 20 percent protein (Mamata *et al*., 2018) [15]. The aim of this research was to examine the production and physiological impacts of *Azolla* inclusion in broiler feed containing more than 20 percent.

Materials and methods

This experiment was accomplished at the College of Agriculture (Animal House), Sumer University, Iraq, throughout the interval from March 21, 2021, to April 29, 2021.

Preparation of feed

Azolla was gathered from the nearby rivers around Al-Refaa city, dried, weighted, combined with broiler feed in a percentage-based manner, and pulverized. The entire diet was then made into pellets at Al-Mustafa factory and stored until the experiment's use. The diet of Al-Waha factory had a chemical makeup of 3100 kilocalories for the grower and finisher and 21-22 percent crude protein, and also the starter with 3010 kilocalories and 22–23 percent crude protein. Vegetable oil, wheat, corn, Soybean meal, and premix, which consists of multivitamins, minerals, amino acids, choline chloride, and calcium diphosphate, make up the diet.

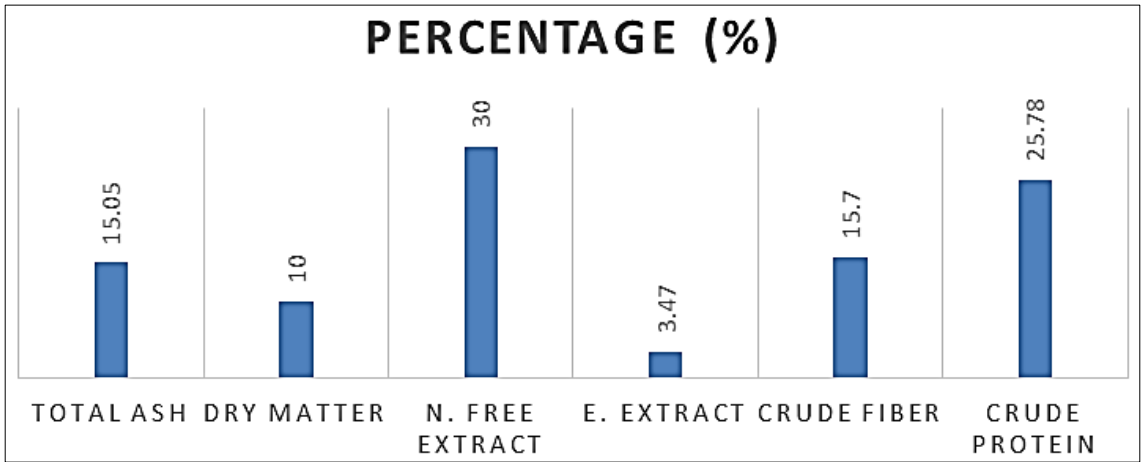


Fig 1: The chemical content of *Azolla* meal

Animals

Four hundred chicks (Ross 308) for broil (As-hatched) were randomly split into four groups, every group consists of 100 chicks, 25 of them were assigned to a cage in dimensions (120 x 100 x 40), where water and food were ad-libitum. A thermostat and an electrical heater were used to regulate the temperature based on age. Vaccinations against several viral infections were given to all birds based on the epidemiological conditions of the area (Infectious bronchitis, Newcastle, infectious bursal, and influenza diseases).

Experimental design

Four hundred chicks for broil (as-hatched) were randomly split into four groups (100 per group). Throughout the experiment, the commercial diet was fed to the first 100 chicks, which served as a control; the second group (T_1) had 85% commercial feed and 15 % *Azolla*; the T_2 (Third group) had 70% commercial feed and 30% *Azolla*; and the T_3 (fourth group) had 55% commercial feed and 45% *Azolla*.

The duration of the experiment was prolonged by 39 days; this duration was established based on the amount of ingested by each group (we specified 3 kilograms for each bird). As a result, the control group at 35 days ingested 3 kg, while the other groups' periods were elongated to 39 days. In order to evaluate the lipid profile (VLDL- very low-density lipoprotein, LDL- low density lipoprotein, HDL-high density lipoprotein, cholesterol, and triglycerides), the chicks were weighted at the beginning, weekly, and at the end of experiment. Vitality ratio factor and feed conversion were also obtained, and serum prepared and samples of blood were drawn from the jugular vein to assess the lipid profile (Verley, 1975; Tietz, 1998) [25, 24]. The spectrophotometric approach was used to evaluate the liver enzymes' activity (ALK- alkaline phosphatase, GOT- serum glutamic oxaloacetic transaminase, and GPT-serum glutamic pyruvic transaminase), total blood protein, and serum albumen activity and levels (Tietz, 1970) [23].

This statistical study was done with one-way analysis of variation, and the test of Friedman was employed for comparing the groups of the experiment. The findings were stated as mean± SD, and the variation was classified as significant at p level less than 0.05. The mentioned findings were acquired by applying Version 5 of GraphPad Prism program.

Results

The weight of body

Table 1 presents the results of the initial, every week, and last weight of body measurements. The experiment groups' differences were found to be inconsequential ($p \geq 0.5$) during the first week of the results. However, following both the second and third weeks, the group, which is called control group, experienced a significant ($p \leq 0.5$) increment in a compare to the second group. In contrast to the control group, the experiment's final weight shows that T_3's body weight increased significantly ($p \leq 0.5$). Figure 2 presents the (FCR) and vitality ratio data. It is evident that there was a substantial ($p \leq 0.5$) drop in T_3's FCR in comparison to other groups and the control one in the current study. However, the findings explained minor ($p \geq 0.5$) variations between the groups of the experiment in vitality ration via the period of the experiment.

Table 1: Effects of adding *Azolla* to broiler feed on body weight at weekly and final intervals.

Age / week	Initial weight	1	2	3	4	5	39 days
Control	43±2	175±5 a	483±10 a	819±30 a	1680±45 a	1980±85 a	1980±85 ab
T_1	41±3	172±4 a	409±15 ab	730±25 ab	1410±50 a	1790±60 a	1850±80 b
T_2	42±5	176±5 a	380±15 bc	700±20 bc	1450±35 a	1750±65 a	1980±71 ab
T_3	43±4	175±6 a	435±20 ab	750±18 ab	1470±29 a	1760±50 a	2100±40 a

Mean is referred to in numbers ±SD (n=100 chicks of broil), Significant changes are indicated by letters (p≤0.5).

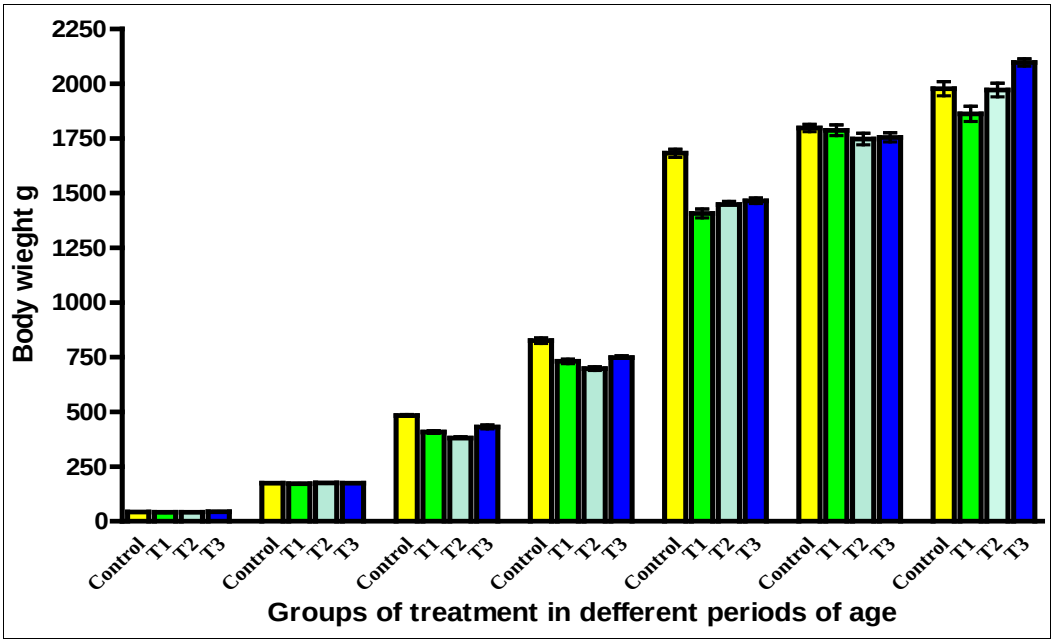


Fig 2: Effects of adding *Azolla* to feed of broil on vitality ratio and FCR.

Mean is referred to in numbers ±SD (n=100 chicks of broil), Significant changes are indicated by letters (p≤0.5).

Lipid profile

Figure 3 illustrates the lipid profile data, which illustrated insignificant (p≥0.5) variations across the groups of the experiment. The research results showed an increase in LDL in both T_1 and T_2, but no considerable change in cholesterol, triglyceride, HDL, or VLDL concentrations (p≥0.5). When group T_3's cholesterol and HDL concentrations were compared to the other groups, there was an insignificant (p≥0.5) rise.

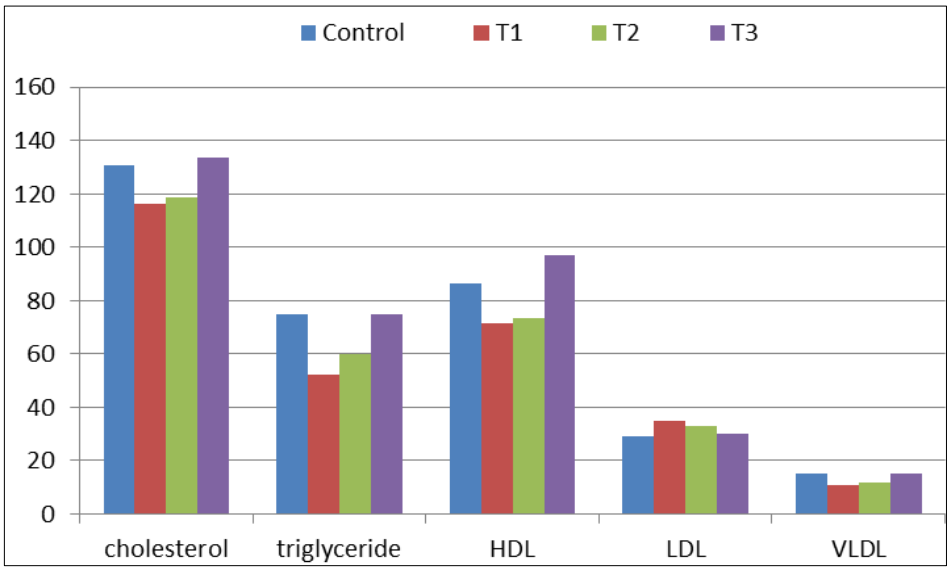


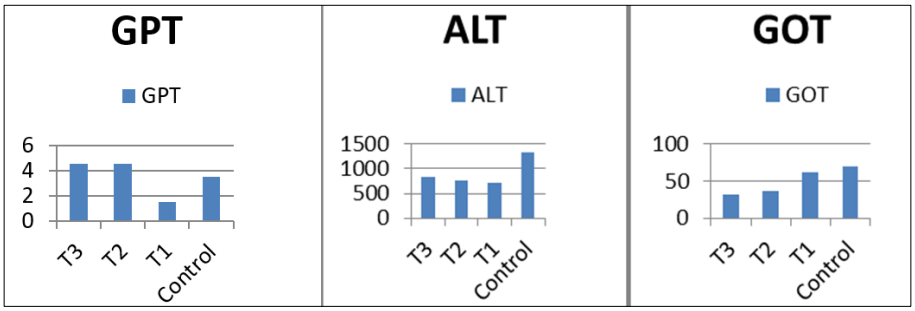
Fig 3: *Azolla* feeding's impact on the profile of lipid.

Mean is referred to in numbers ±SD (n=100 chicks of broil), Significant changes are indicated by letters (p≤0.5).

Hepatic enzyme and blood protein behavior

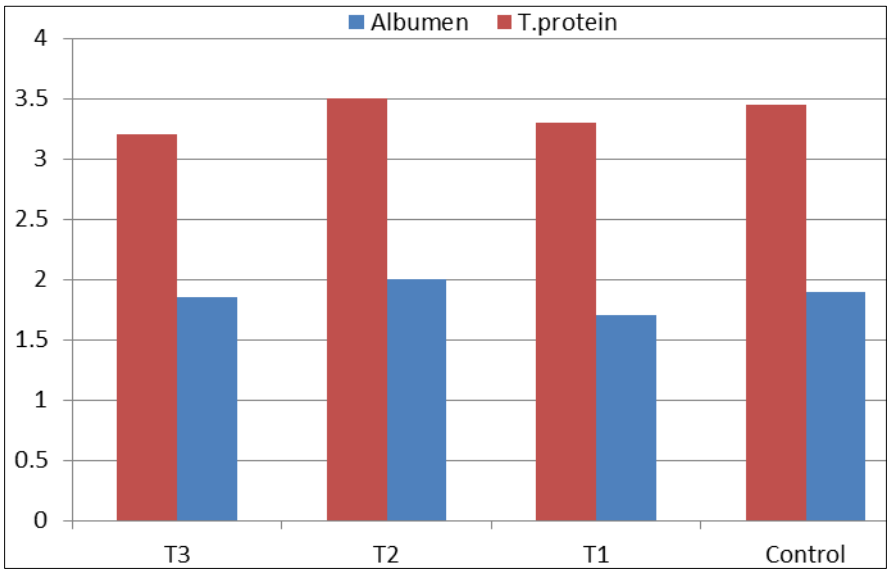
When comparing the GOT activity of groups T_2 and T_3 to the control group Figure 4, liver enzyme activity assessment revealed a substantial (p≤0.5) decline, in contrast to the control group, ALK and GOT showed an insignificant

(p≥0.5) decrease. The obtained findings of total blood protein and albumen levels in the various experiment groups are displayed in Figure 5, wherein the differences between the groups were found to be statistically insignificant (p ≥ 0.5).



Mean is referred to in numbers ±SD (n=100 chicks of broil), Significant changes are indicated by letters ($p \leq 0.5$).

Fig 4: Impact of *Azolla* feeding on hepatic enzyme activity.



Mean is referred to in numbers ±SD (n=100 chicks of broil), Significant changes are indicated by letters ($p \leq 0.5$).

Fig 5: *Azolla* feeding's impact on protein levels in blood.

Discussion

The objective of the current study was to find out if broiler feed with more than 30% *Azolla* could be beneficial. Numerous studies have come to the conclusion that adding *Azolla* to poultry feed in amounts greater than 5% does not negatively impact growth or performance (Seth *et al*., 2013; Abdelatty *et al*., 2020) [22, 1]; however, adding *Azolla* to poultry feed in amounts up to 15% can improve growth and performance (Fadzlin *et al*., 2020) [10]; and feeding *Azolla* in amounts greater than 20% does not negatively impact carcass. Because *Azolla* is used as a fresh fern or when it is pulverized after drying it under the sun, it is large in size; as a result, broiler feed contains a small percentage of *Azolla*. In our study, the feed's typical size was achieved by compressing the entire feed after sun-drying *Azolla* pellets, which allowed us to raise the percentage. Moreover, having more *Azolla* added to the feed of broil considerably decreases the feed production cost (Kumar *et al*., 2018) [13].

In the beginning, the study reveals a significant increase in body weight in the control group, indicating a change in the experimental groups' weight. Nevertheless, the study found that the body weight gain decreased over time and became inconsiderable (p≥0.05), but at day 39, the group T_3 showed an ultimate increase (with a p level ≤ 0.05). The reason behind obtaining this result was the feed consumed by the treated groups, which were 39 days to finish three kg, while it took only 36 days for the control group to consume the same amount of feed. Broiler nutrition's high fiber content and *Azolla* concentration can negatively impact birds' growth and eating desire, as Saikia *et al*. (2014) [21] demonstrated.

Research shows that increased *Azolla* in nutrition enhances feed conversion ratio in broilers (Dhumal *et al*., 2009; Alalade & Lyayi, 2006; Saikia *et al*., 2014) [9, 3, 21]. However, Rohan *et al*. (2020) [19] found in T_3 a significant (With a level of $p \leq 0.05$) reduction in FCR (45% *Azolla*), possibly due to differing feed intake and *Azolla*'s negative appetite effect.

The study found minor variations in lipid profiles across groups, which was (p≥0.05), but broiler chicks' lipid profile remained unchanged after *Azolla*-containing diet feeding (Deepesh *et al*., 2016; Mayank *et al*., 2018) [7, 16]. Additionally, there were no observations of considerable differences (p≥0.05) in the measurements of hepatic enzyme activity, with the exception of T_3, which indicated a high

($p \leq 0.05$) reduction in the behavior of GOT in comparison to the control group. This conclusion did not match the observations of Deepesh *et al*. (2016) [7], who saw a rise in GOD in the treatment group relative to the control group. This could be because the liver enzymes in birds come from several organs, including the muscles, skeleton, heart, and liver, in a second order; as a result, their limitations are not constant (Brugere *et al*., 1987) [6]. All experimental groups had comparable blood protein concentrations, and this finding was not in disagreement with Mayank *et al*.'s (2018) [16].

Based on the current research, it can be concluded that adding 45 percent more *Azolla* to the broiler diet increases the feed conversion ratio without negatively affecting the birds' typical physiology.

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Conflict of Interest statement
The authors declare no conflicts of interest.

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