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Article:

The Impact of Feeding Sun-Dried Azolla Meal on Growth Performance and Some Serum Biochemical Parameters of Broiler Chickens.

Mahmoud Abd El-Kareem[1]*, Mohamed A. Mousa[2], Gamal M. Mosaad[1].

[1]Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Minia University, [2]Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Sohag University.

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Abstract

The current study was conducted to assess the impact of feeding sun-dried azolla meal on growth performance and some serum biochemical parameters of broiler chickens. A total number of 80 one-day-old broiler chicks (Ross 308 strain) were individually weighed and allocated randomly into four treatment groups (20 birds/group), each group consists of two replicates each of 10 birds. The control group (T1) received a basal diet, while the experimental groups T2, T3, and T4 were fed diets containing 3%, 6% and 9% sun-dried azolla, respectively. Over a 35-day period, results showed a clear trend of significantly increased ($P<0.05$) weight gain and increased feed intake with higher levels of Azolla inclusion, with T4 exhibited the highest final body weight and feed intake. Feed conversion ratios (FCR) improved insignificantly ($P>0.05$) in the Azolla-fed groups compared to the control. Serum total protein, albumin, and globulin levels were unaffected by treatment ($P>0.05$). No significant differences ($P=0.093$) were observed among different groups in LDL levels. T3 and T4 had significantly higher HDL values ($P<0.05$). Additionally, Azolla supplementation significantly decreased cholesterol and triglyceride levels ($P<0.05$). Antioxidant enzymes activity (superoxide dismutase, catalase and glutathione peroxidase) was enhanced with groups fed with dried azolla compared with the control group. It can be concluded that sun dried Azolla meal is a promising and beneficial non-conventional feed ingredient that can be used up to 9% in broiler diets for improving growth performance and enhancing the serum biochemical constituents in broilers.

Keywords: Antioxidant, Azolla, Biochemical parameters, Broiler, Performance.

Introduction

Broiler production accounts for 33% of global meat production and is a crucial source of protein for human nutrition **[1]**. The cost of poultry feeding accounts for about 70 to 80% of the overall production expenses, rendering it the costliest aspect and the key factor within the poultry production sector. Soybean meal and maize are conventional but expensive feedstuffs used in broiler production systems. The utilization of locally accessible non-conventional feed resources is a feasible and potentially the most effective approach to reduce the feed cost **[2, 3]**.

Azolla is a highly prevalent aquatic fern. It is one of the most extensively spread plants. Swamps, wetlands, ditches, and rivers and lakes with non-turbulent water are all suitable aquatic places for Azolla growth all over the world **[4]**. It is characterized by its tiny, scale-like leaves floats horizontally on the water's surface **[5]**.

Azolla is a photosynthetic aquatic macrophyte that is multiplying rapidly. It can double its biomass within 7 days. Per hectare of pond, it may yield nine tons of protein

IJCVR ***Corresponding author:*** Mahmoud Abd El-Kareem, ***Email:*** Mahmoud-karem@mu.edu.eg, ***Address***:*Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Minia University, Minia, Egypt*

annually. Because of its capacity to fix nitrogen from the atmosphere through a symbiotic relationship with *Anabaena azollae* (a blue-green algae), azolla can flourish in environments where nitrogen is scarce. Its rapid biomass growth also allows for superior carbon fixation rates compared to other plants **[4, 6]**.

Azolla is viewed as a very promising non-conventional feed due to its simple production process, low water requirements for growth, great yield, and excellent nutritional value. Azolla has high protein content and contains all the indispensable amino acids. Vitamins (vit B_{12}, A, and beta-carotene), growth-promoting intermediaries and biopolymers are found in azolla along with Ca, P, Mg, K, Cu, Fe and other minerals **[7]**. Azolla possesses a diverse array of pharmacological characteristics and may serve as both a nutritional source and an antioxidant. It acts as an immunological booster, improving cell-mediated immune responses. It also functions as a phytoremediator, bioremediator and hepatoprotector (protecting liver from hepatotoxic substances). Its concentrated decoction possesses antioxidant, anti-inflammatory, and anti-apoptotic characteristics **[8, 9]**.

The abundance of flavonoids and phenolic chemicals in azolla is primarily responsible for its potent antioxidant activity. These bioactive substances are widely recognized for their capacity to reduce oxidative stress and scavenge free radicals **[10]**. Therefore, the current research was carried out to investigate the impact of feeding broilers on diets containing different levels of dried azolla meal on growth performance parameters, some biochemical parameters, and serum antioxidant parameters.

Materials and Methods

The performance of the bird is a reflection of its physiological activity; therefore, the purpose of this study was to assess the impact of adding sun dried Azolla meal to broiler diets as a protein source on the growth performance and some serum biochemical parameters.

Ethical approval

The housing, handling and sample collection procedures for the broilers were conducted in full compliance with established ethical guidelines. Measures were implemented to minimize any potential pain or discomfort, following the animal welfare protocols approved by the Faculty of Veterinary Medicine, Minia University, under approval number IRB-FVM-MU-2024-110 with a date 7/1/2024.

Experimental Design

A total number of 80 one-day-old unsexed broiler chicks (Ross 308 strain) were individually weighted and allocated randomly into four treatment groups (20 birds/ group), each group consists of two replicates each of 10 birds. The initial average weight of chicks was 41±0.17. Chicks were weighed weekly, and feed consumption was recorded.

Management and feeding

Experimental groups

The control group (T1) received a basal diet, while the experimental groups T2, T3, and T4 treatment groups were fed diets containing 3%, 6% and 9% sun-dried Azolla, respectively. Chicks of each replicate were placed in a separate cage in tiered battery system consisted of vertical three cages. All birds were subjected to identical managerial, vaccination, hygienic, and housing circumstances including water, food, spacing and lighting.

Diets and feeding

Diets were formulated to meet the nutrient requirements of Ross 308 broiler chicks catalog as modified from **NRC [11]**. It included a two-phase feeding regime lasted for 35 days. It consisted of a starter diet (1-14 days) containing 23% crude protein (CP) and about 3000 Kcal/kg metabolizable energy (ME) and a grower-finisher diet (15-35 days) containing 21% CP and about 3100 Kcal/kg ME. The composition of sun-dried Azolla meal was chemically analyzed according to **AOAC [12]**: 2291 kcal/kg diet ME, 25.07% CP, 14.64% CF, 3.24% EE, 30.18% NFE, 17.39% Ash, and 90.52% DM. The ME content of sun-dried Azolla meal was calculated using the formula derived by **Lodhi et al. [13]**. However, the chemical composition of other feedstuffs aligns to **NRC [11]** guidelines, **Table (1)**. Diets was provided ad-libitum in the form of mash. Fresh water was provided ad libitum.

Table 1 The chemical composition of feedstuffs other than Azolla

Ingredients	Composition %						ME (kcal/kg)
	DM	CP	EE	CF	NFE	Ash	
Corn	88	8.5	3.8	2.2	72.4	1.1	3350
SBM	89	46	0.8	7	30.2	5	2230
Corn gluten	90	60	2.5	1.3	24.2	2	3720
Soybean oil	99	--	99	--	--	--	8375

Table 2 The physical and chemical composition % of the experimental diets.

Ingredients	Starter				Grower finisher			
	T1	T2	T3	T4	T1	T2	T3	T4
Yellow corn	55.16	53.66	52.16	50.65	61.08	59.58	58.08	56.58
Soybean meal	33.06	31.66	30.26	28.86	27.66	26.26	24.86	23.46
Corn gluten	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Sunflower oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Dried Azolla	0.00	3.00	6.00	9.00	0.00	3.00	6.00	9.00
Limestone	1.34	1.24	1.14	1.04	1.41	1.31	1.21	1.11
Dicalcium phosphate	1.48	1.46	1.44	1.43	1.05	1.03	1.01	0.99
L-Lysine HCl	0.03	0.05	0.07	0.09	0.07	0.09	0.11	0.13
DL-Methionine	0.13	0.13	0.13	0.13	0.03	0.03	0.03	0.03
Common salt	0.50	0.50	0.50	0.50	0.40	0.40	0.40	0.40
***Min. & Vit. Premix**	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Calculated nutrients								
DM	89.20	89.25	89.30	89.35	89.09	89.14	89.19	89.24
Cp	23.00	23.00	23.00	23.00	21.00	21.00	21.00	21.00
ME (kcal/kg)	3029	3017	3005	2993	3104	3092	3080	3068
CF	3.59	3.90	4.21	4.52	3.34	3.65	3.96	4.27
EE	5.49	5.51	5.54	5.57	5.67	5.70	5.73	5.75
NFE	51.13	50.53	49.92	49.31	53.78	53.18	52.58	51.97
Ash	5.83	6.15	6.47	6.79	5.18	5.50	5.81	6.13
Ca	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
P	0.45	0.45	0.45	0.45	0.35	0.35	0.35	0.35
Lysine	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00
Methionine	0.50	0.50	0.50	0.50	0.38	0.38	0.38	0.38

**Each kg of premix contained; Vit A, 12000 IU; Vit E, 10 IU; Vit k3, 3mg; Vit D3, 2200 ICU; Vit B1, 2.2 mg; Vit B2, 10 mg; Ca pantothenate,10 mg; niacin, 20 mg; Vit B6, 1.5 mg; Choline, 500 mg; Vit.B12, 10µg; Folic acid, 1 mg; Biotin, 50µg; Mn, 55mg; Zn*

Measurements

Performance

Live body weight, feed intake, and feed conversion ratio were monitored on a weekly basis for each of the treatment group.

Serum biochemical parameters

Serum samples were collected to measure the total protein and its fractions using spectrophotometer with the commercial test kits (BioMed diagnostics; Egy-Chem for lab technology, Heliopolis, Cairo, Egypt). Serum LDL, HDL, cholesterol and triglycerides concentrations were estimated using spectrophotometer with the commercial test kits (BioSystems, Costa Brava, Barcelona, Spain). Antioxidant enzymes (SOD, CAT and GPx) activity was estimated by using Sinothinker SK3002B (semi-auto chemistry analyzer, made in China) and commercial kits (Biodiagnostic, Dokki, Giza, Egypt).

Statistical analysis

The obtained results have been expressed as the mean ±SE. All data have been analyzed using one way analysis of variances (ANOVA) followed by Duncan test using IBM SPSS 20.0 (statistical package for social science) software.

Results

Body weight development

The results presented in **Table (3)** illustrated the impact of dietary inclusion of dried Azolla meal at different levels on broiler body weight development over the five weeks experimental period. There were no significant differences (P=0.882) in the initial body weights between the control group and the Azolla-fed groups. By week 1, broilers receiving 3% and 6% Azolla showed significantly (P=0.05) higher body weights compared to the control group. But the 9% Azolla group exhibited insignificant improvement (P>0.05) in the body weight compared to the control group. The 9% Azolla group achieved a better, though not significantly higher weight than the control group. From the second week onward, the group receiving 9% Azolla consistently exhibited the highest (P=0.000) body weights, followed by the groups receiving 6% and 3% Azolla, respectively, showing a clear trend of increased weight gain with higher levels of Azolla inclusion.

Feed intake and feed conversion ratio

Our data showed that broilers fed with 9% Azolla recorded the highest feed intake, reaching 4193.0 g. The 6% Azolla group followed closely with 3961.0 g, surpassing the control group's intake of 3860.6 g. Interestingly, the 3% Azolla group consumed the least feed at 3719.5 g. This trend of increased feed intake with higher levels of Azolla inclusion highlights the potential of increasing Azolla levels to stimulate higher feed consumption in broilers.

The results presented in **Table 4** illustrated the influence of dietary inclusion of dried Azolla meal on the feed conversion ratio over the five weeks experimental period. The FCR of the control group was the least efficient across all weeks with the average (1.70), whereas the Azolla fed groups exhibited improved overall feed efficiency, with values of (1.58) for the 3% azolla group, (1.62) for the 6% azolla group, and (1.66) for the 9% azolla group. However, the P-value (P=0.847) indicated that these differences were not statistically significant across different groups.

Serum biochemical parameters

The results presented in **Table 5** revealed insignificant improvement (P>0.05) in total protein levels or its fractions, including albumin and globulin, nor in the albumin to globulin ratio among the different groups. The data were also showed, no significant differences (P=0.093) among different groups in low-density lipoprotein (LDL) levels. However, the groups fed 6% and 9% dried Azolla recorded numerically lower LDL values, suggesting a more favorable effect associated with the inclusion of dried Azolla in the diet. Group fed with 9% dried azolla recorded significant (P<0.05) higher HDL values, followed by the group fed with 6% dried azolla compared with the control group. Significant differences (P<0.05) were observed in the cholesterol levels in the dried azolla fed groups compared to the control. Triglyceride levels were highest in the 6% dried Azolla group, followed by the control, 9% and 3% dried Azolla groups, respectively.

The inclusion of dried Azolla meal significantly (P<0.05) improved the activity of key antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase, with the most notable increases observed at higher Azolla inclusion rates. SOD levels were significantly higher (P=0.018) in the 6% and 9% Azolla groups. Catalase activity was significantly higher (P=0.023) in all dried Azolla fed groups compared to the control, with the highest value observed in the 9% group. Glutathione peroxidase activity significantly increased with the inclusion of Azolla, with the 9% dried azolla fed group showing the highest activity. These results suggest that dried Azolla supplementation can enhance the antioxidant defense system in broilers.

Table 3 The influence of dietary inclusion of dried Azolla meal on the body weight development (g/week/bird).

Week	Control	Dried azolla meal			P value
		3%	6%	9%	
0	40.85±0.52	40.10±0.77	40.60±0.67	40.70±0.77	0.882
1	177.2±3.64^{b}	189.5±3.24^{a}	189.5±4.35^{a}	186.2±2.66^{ab}	0.050
2	470.4±4.09^{c}	506.4±3.00^{b}	559.7±5.36^{a}	569.4±8.83^{a}	0.000
3	990.3±8.70^{c}	1060.1±11.09^{b}	1152.6±7.26^{a}	1175.8±9.78^{a}	0.000
4	1593.8±5.71^{d}	1684.9±7.46^{c}	1782.8±8.41^{b}	1825.9±9.46^{a}	0.000
5	2189.7±6.01^{d}	2294.5±10.99^{c}	2387.4±6.73^{b}	2463.1±8.19^{a}	0.000

**Means with significant differences at (p<0.05) are indicated by different superscripts within the same row.*

Table 4 The influence of dietary inclusion of dried Azolla meal on the feed conversion ratio.

Week	Control	Dried azolla meal			P value
		3%	6%	9%	
1	1.37	1.35	1.37	1.39	
2	1.52	1.42	1.44	1.46	
3	1.77	1.63	1.68	1.70	
4	1.84	1.71	1.77	1.81	
5	2.01	1.80	1.84	1.92	
Average	1.70	1.58	1.62	1.66	0.847

**Means with significant differences at ($p<0.05$) are indicated by different superscripts within the same row.*

Table 5 The influence of dietary inclusion of dried Azolla meal on serum biochemical parameters.

Parameters	Unit	Control	Dried azolla meal			P value
			3%	6%	9%	
T.P	g/dL	4.23±0.44	5.09±0.26	4.77±0.24	5.04±0.20	0.251
ALB	g/dL	2.48±0.31	2.81±0.12	2.47±0.28	3.11±0.15	0.222
GLO	g/dL	1.76±0.14	2.27±0.38	2.29±0.04	1.92±0.05	0.242
A/G		1.41±0.07	1.24±0.28	1.08±0.14	1.62±0.03	0.212
LDL	mg/dL	92.79±4.97	94.38±3.60	80.10±2.73	86.89±3.21	0.093
HDL	mg/dL	52.70±3.59^{b}	43.23±0.25^{c}	54.68±1.39^{ab}	57.69±1.04^{a}	0.000
CHOL.	mg/dL	189.26±2.55^{a}	182.59±8.85^{ab}	157.34±1.61^{c}	167.41±4.97^{bc}	0.010
T.G	mg/dL	109.65±1.76^{b}	92.25±0.84^{c}	118.66±3.21^{a}	104.71±3.19^{b}	0.000
SOD	U/ml	3.15±0.21^{b}	3.26±0.25^{ab}	3.48±0.31^{a}	3.77±0.12^{a}	0.018
CAT	U/L	3.33±0.16^{b}	3.54±0.44^{a}	3.79±0.27^{a}	4.12±0.02^{a}	0.023
GPx	mU/mL	1.69±0.13^{b}	1.84±0.09^{a}	1.94±0.08^{a}	2.11±0.06^{a}	0.002

**Means with significant differences at ($p<0.05$) are indicated by different superscripts within the same row. **T.P, total protein; ALB, albumin; GLO, globulin; A/G ratio, albumin/globulin ratio; LDL, low density lipoprotein; HDL, high density lipoprotein; CHOL., total cholesterol; T.G, triglycerides; SOD, superoxide dismutase enzyme; CAT, catalase enzyme; GPx, glutathione peroxidase enzyme. ***U/ml, Units per milliliter; U/L, Units per liter; mU/mL, milliunits per milliliter.*

Discussion

This discussion focuses on evaluating the effects of incorporating varying levels of dried Azolla meal into broiler chicken diets on the growth performance and biochemical serum parameters which reflect the overall health of birds. Offering insights into the efficacy of dried azolla as alternative or complementary feed ingredients in broiler production systems.

The findings of this study **(Table 3)** are consistent with those of **Ibrahim et al. [14]**, who observed an increase in final body weight as the level of dried Azolla was increased from 4% to 12%. In their study, the 12% dried Azolla group achieved a final body weight of 2231.3 g, while the control group reached 2064.9 g. Similarly, **Samad et al. [15]** reported that broilers fed 15% Azolla exhibited significantly higher body weights compared to other groups. Aligning with our results, **Rathod et al. [16]** found that feeding 6% dried Azolla to Satpuda breed broiler chickens resulted in the highest final body weight compared to other treatment groups (2%, 4%, and the control). Similarly, **AL-Shwilly [17]** fed broiler chickens with various Azolla inclusion levels (0%, 15%, 30%, and 45%) and reporting that the highest inclusion level yielding the most substantial improvement in body weight. The findings were in line with those of **Kamel and Hamed [18]**, who reported that dietary supplementation with dried Azolla at levels of 4%, 8%, and 12% had a significant linear improvement in the final body weights of broilers. Additionally, our findings are in agreement with those of **Rawat et al. [19]**, **Tawasoli and Nabi [20]**, **Kumar et al. [21]**, **Kumar et al. [22]** and **Arram et al. [23]** who demonstrated a significant improvement in body weight of broilers fed different levels of Azolla. The improvement in live body weights may be attributed to the high protein content of Azolla, along with the presence of essential amino acids and minerals. In addition, Azolla spp. contains carotene and biopolymers, both of which play a role in promoting animal productivity and overall health **[24]**. On the contrary, our findings disagreed with the results of **Islam and Nishibori [25]** who reported lower body weights in broilers fed 5% Azolla (1262g) and 7% Azolla (1358g) compared to the control group (1440g). Similarly, **Islam [26]** in his study recorded that Azolla supplementation reduced the body weight of broilers compared with the control. The variations in body weight development observed at different inclusion levels of Azolla may be attributed to differences in the species or the maturity stages of the used azolla. According to **Flyman and Afolayan [27]**, the nutritional composition of Azolla can be significantly affected by its stage of maturity and the harvesting period, which in turn affects the overall growth performance of broiler chickens. In a more neutral position, studies by **Balaji et al. [28]**, **Dhumal et al. [29]** and **Al-Hamed and Al-Husseiny [30]** reported no significant differences in live body weight between the different treatment groups. This reinforces the idea that replacing a portion of the standard diet's protein with azolla protein can result in comparable performance in broiler chicks. This suggests that Azolla can serve as an effective alternative protein source without compromising growth performance.

The current findings (**Table 4**) concur with those of **Tawasoli and Nabi [20]** who found that more feed intake was recorded in the 6% azolla fed group compared to the control. Similarly, **Kumar et al. [21]** observed a highly effect of azolla feeding on feed consumption, with the highest feed intake recorded in the 7.5% Azolla group (3900g) while the control group recorded (3734g). Also, **Rathod et al. [16]** reporting that feed intake was increased in chicks fed ration with 6% dried azolla than 2% and 4% dried azolla and control groups. Our results were in line with those of **Islam and Nishibori [25]**, **Islam [26]**, **Kumar et al. [22]** and **Arram et al. [23]** who reported linear increase in feed intake in azolla fed groups compared to the control group. In contrast to our findings, **Ibrahim et al. [14]** reported a reduction in feed intake with increasing levels of Azolla (4%, 8%, 12%) in the ration. Similarly, **Kamel and Hamed [18]** observed that dried Azolla at different inclusion levels significantly decreased total feed intake, with the lowest feed intake recorded in broilers fed 8% Azolla compared to the control group. Similarly, **Yadav and Chhipa [31]** observed that while the average weekly feed intake was unaffected at 5% Azolla inclusion, it decreased when Azolla was incorporated at 10% and 15% levels. Unlike our findings, **Ara et al. [32]** indicated no significant differences in the amount of food consumed among Azolla fed groups.

In agreement with our results, **Balaji et al. [28]** reported no significant variations in feed conversion ratio among the dietary groups. Similarly, **Dhumal et al. [29]** reported no significant variations in the FCR at the sixth week of age, although the treated groups (2.5% and 5% Azolla) showed improved FCR compared to the control group. Also, **Saikia et al. [33]** found that broilers receiving 5% Azolla meal exhibited feed efficiency comparable to the control group. **Chichichi et al. [34]** also observed no significant differences in FCR among groups fed with 5% and 10% Azolla. **Rawekar et al. [35]** and **Kashyap et al. [36]** further support these results, as they reported no significant variations in FCR in groups fed different Azolla levels. Our findings align with those of **Samad et al. [15]** who reported insignificant improvement in the feed conversion ratio among Azolla fed groups. Moreover, **Sharma et al. [37]** noted that weekly FCR did not significantly differ among broiler groups receiving different levels of Azolla (1.5%, 2.5%, 3.5%, 4.5%, and 5.5%).

Sharma [38] reiterated these findings, showing no significant weekly or cumulative differences in FCR between the above treatment groups. **Abdelatty et al. [39]** also confirmed that Azolla supplementation had no significant impact on the FCR. Similarly, **Al-Hamed and Al-Husseiny [30]** observed no significant differences in feed conversion coefficients among groups receiving different levels of Azolla (4.5%, 9%, and 13.5%).

Contrary to our findings, **Rathod et al. [16]** reported that chicks fed 6% dried Azolla achieved a superior FCR compared to the control group, followed by those fed 4% and 2% dried Azolla. **Arram et al. [23]** also found that the FCR for chicks fed 10% and 5% Azolla (1.45) was significantly better than the control group (1.71). Similarly, **Ibrahim et al. [14]** noted that Azolla-fed birds had a better FCR than the control, with improvements observed as the Azolla level increased from 4% to 12%, with the control group recording an FCR of 1.71 and the 12% Azolla group achieving 1.46. Similarly, **Yadav and Chhipa [31]**, **Tawasoli and Nabi [20]**, **AL-Shwilly [17]**, **Kamel and Hamed [18]** and **Kumar et al. [21]** found that the FCR significantly improved with Azolla meal supplementation. In contrast, **Islam and Nishibori [25]** reported that the FCR was worst in the group fed 7.5% Azolla (1.79) compared to the control group (1.48) and the 5% Azolla group (1.47). Similarly, **Islam [26]** noted that the Azolla-fed groups (5% and10%) exhibited worse FCR values (1.81 and 1.83, respectively) compared to the control group (1.73).

In agreement with our findings **(Table 5)**, several studies have reported no significant effects of Azolla supplementation on total protein, albumin, and globulin levels in broilers. **Chichichi et al. [34]** observed no significant variation in total protein and its fractions across treatments with 5% and 10% Azolla inclusion. Similarly, **Al-Rekabi et al. [40]** found no significant differences between all Azolla fed groups in albumin concentration. **Shukla et al. [41]**, **Najim et al. [42]**, **AL-Shwilly [17]** and **Adil et al. [3]** similarly found no significant differences in the total protein or albumin and globulin concentrations between different Azolla fed groups. **Arram et al. [23]** also observed no significant differences in total protein between control, 5%, 10%, and 15% Azolla groups, although a significant increase in total protein was recorded in the 20% Azolla group compared to the control. Contrary to our results, **Al-Rekabi et al. [40]** found significant increase in the concentration of total protein and globulin among broilers fed 5%, 10%, 15%, and 20% Azolla. Also, **Kamel and Hamed [18]** observed that feeding broiler chicks with varying levels of dried Azolla (4%, 8%, and 12%) significantly increased albumin and total protein concentrations compared to the control group. **Ajit Singh et al. [43]** found that the amount of serum total protein was significantly different between the control group and the group fed 15% Azolla.

In concurrence with our results, **Balaji et al. [44]** reported that supplementing broiler diets with 4.5% Azolla can significantly lower serum cholesterol levels, supporting the lipid-lowering potential of Azolla supplementation at moderate levels (1.5%, 3%, and 4.5%). In agreement with our result, **Islam and Nishibori [25]** observed no significant differences in LDL levels across dietary groups. However, LDL values tended to decrease with 5% and 7.5% Azolla inclusion compared to the control. Also, HDL concentrations were higher in the 5% and 7.5% Azolla-fed groups, alongside reductions in total cholesterol and triglycerides particularly in the 5% Azolla group. **Najim et al. [42]** further corroborated these findings by demonstrating a significant reduction in plasma cholesterol levels with the 5% Azolla treatment. **Al-Rekabi et al. [40]** reported a significant increase in HDL concentrations and a decrease in total cholesterol and triglycerides, which aligns with our results, especially with higher Azolla inclusions (5%, 10%, 15%, and 20%). **Kamel and Hamed [18]** similarly noted that feeding broilers with 4%, 8%, and 12% Azolla significantly increased HDL concentrations compared to the control, with the 12% Azolla group showing the highest HDL value. Cholesterol concentrations were also significantly lower in the Azolla-fed groups, while triglyceride levels did not show significant differences. Moreover, **Arram et al. [23]** found that broilers fed 5%, 10%, and 15% Azolla recorded lower triglyceride values compared to the control group, further supporting the cholesterol-lowering and lipid-modulating effects of Azolla in broiler diets. These findings collectively reinforce the beneficial impact of Azolla on lipid metabolism and serum lipid profiles in broilers. Contrary to our findings, **Islam [26]** reported no significant differences in the lipid profile parameters among Azolla fed groups. But the 5% Azolla-fed group exhibited an insignificantly better lipid profile than both the control and 10% Azolla groups, with a slight reduction in LDL and TG, and an increase in HDL. Similarly, **AL-Shwilly [17]** found no significant differences in the blood lipid profile. While cholesterol and HDL concentrations showed a slight increase in the 45% dried Azolla-fed group and LDL levels were slightly elevated in the 15% and 30% dried Azolla-fed groups. **Adil et al. [3]** also found no significant differences in cholesterol levels among the different treatment groups. **Arram et al. [23]** reported no significant changes in HDL levels across all Azolla-treated groups (5%, 10%, 15%, and 20%). Additionally, there were no significant differences in cholesterol levels between the control and groups fed with 5%, 10%, and 15% Azolla.

Information in the literature regarding the effect of Azolla on serum antioxidant enzymes activity in broiler

chicken is scarce. Antioxidant enzymes act as the first line defense antioxidants by suppressing or preventing the formation of free radicals or reactive species in cells. The SOD enzyme catalytically dismutate the superoxide radical, generated in tissues through metabolic processes or cellular reactions, into hydrogen peroxide (H_2O_2) and oxygen (O_2). Catalase breaks down H_2O_2 into water and molecular oxygen, thereby reducing potential damage from free radicals. Since catalase is absent in the mitochondria, the reduction of H_2O_2 to water and lipid peroxides to their corresponding alcohols is carried out by glutathione peroxidase enzyme **[45]**. On the contrary to our results, **Shukla et al. [41]** reported no significant difference in serum superoxide dismutase levels among treatment groups supplemented with 5% Azolla or given azolla free choice alongside other feeds. Similarly, **Punyatong et al. [46]** found that the glutathione (GSH) levels remained similar across all groups, regardless of the percentage of fresh Azolla supplementation (5%, 10%, 15%, 20%, and 25%). Thus, more studies are needed to confirm the current findings. In alignment with our findings, **Seth et al. [47]** reported that catalase activity was significantly higher in Azolla-supplemented groups compared to the control. Similarly, **Chichilichi et al. [48]** observed that catalase activity was significantly higher in the 10% Azolla group compared to the 5% and control groups, reinforcing the association between Azolla supplementation and enhanced antioxidant enzyme levels. This enhancement in catalase levels in birds fed increasing levels of Azolla might be attributed to its high iron and copper content. Moreover, **Al-Rekabi et al. [40]** reported that serum glutathione peroxidase levels in the Azolla substitution treatments (5, 7.5, 10% azolla) significantly surpassed those of the control treatment, which exhibited the lowest enzyme concentration. The elevated enzyme concentration in Azolla treatments may result from the active substances in the Azolla plant, which possess the capacity to inhibit free radical activity due to their potent antioxidant properties, thereby serving as significant natural antioxidants **[49]**. **Kamel and Hamed [18]** also demonstrated that dried Azolla supplementation at different levels (4%, 8%, and 12%) significantly increased catalase and glutathione peroxidase values compared to the control group, with the 8% Azolla group exhibiting the highest enzyme levels. This further supports the beneficial effects of Azolla on oxidative stress markers in broilers.

Conclusion

It can be concluded that sun dried Azolla meal is a promising and beneficial non-conventional feed ingredient that can be used up to 9% in broiler diets for improving growth performance and enhancing the serum biochemical constituents in broilers.

Conflict of interest

There is no conflict of interest.

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