



# Effect of Dried Azolla on Growth Performance, Hematological, Biochemical, Antioxidant Parameters, and Economic Efficiency of Broiler Chickens

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**Abstract** | A total of 208 healthy one-day-old broiler chicks (Cobb-500) were allocated randomly in to 4 groups (4 replicates / each). Broiler chicks fed different diets containing 0%, 4%, 8%, and 12% dried Azolla (DA) for a period of 42 days. The results clarified that dietary DA at different levels significantly ( $P=0.012$ ) decreased total feed intake. The lowest total feed intake value was for broilers fed 8% DA (3151.3g). Broilers group fed with 12% DA had the highest significant ( $P<0.001$ ) average final body weight and body weight gain. The feed conversion ratio was significantly ( $P=0.002$ ) better among all broiler groups fed with DA. Dietary treatments with different levels of DA showed significant improvement in immune cells count such as lymphocytes ( $P<0.001$ ), and heterophils ( $P= 0.003$ ). Dietary inclusion of DA significantly increased ( $P < 0.05$ ) albumin, total protein, and high-density lipoprotein concentrations. Urea, creatinine, aspartate aminotransferase, alanine aminotransferase, uric acid, and cholesterol concentrations were significantly ( $P < 0.05$ ) decreased in all broilers fed DA. Dietary inclusion of DA improved antioxidant potency. Economically broiler group fed with 12% DA diet significantly ( $P<0.001$ ) recorded the highest total return, and net return values (3.25, 0.94 \$/chicken, respectively). The highest net return per Kg gain, and economic efficiency values were found for group fed 12% Azolla (0.42, and 0.82, respectively). In conclusion, the dietary inclusion of DA up to 12%, can improve the growth performance, blood parameters, antioxidant properties without adverse effect on the health condition of broiler chicks, and increase the profitability for broiler chickens.

**Keywords** | Broiler chickens, Dried Azolla, Performance, Economic, Efficiency.

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## INTRODUCTION

Poultry industry becomes one of the most expanding sectors in the world as it helps to fulfill the gap between the requirement and the availability of high-quality protein for human consumption, leading to increase demand for higher and safer protein source (Alkhalif et al., 2010). Feed is considered the largest single production cost

in broiler production and can constitute up to 80% of total livestock production costs and 86- 87% of the total variable production cost (Davis et al., 2013). The shortage of feed is the most important problem that hinders the development of the poultry industry all over the world (Naghshi et al., 2014). The incorporation of conventional feed ingredients, like maize, soya bean, fish etc., in poultry feed has caused an enormous increase in the cost of feed, thus

any attempt to reduce the cost of feed may lead to a significant reduction in the total production cost (Swain et al., 2014). Poultry raisers and farmers are in search of sustainable and economical feed ingredients for poultry feeding. Many scientists are working on different unconventional feed sources to increase the productive and economic efficiency (Abeke et al., 2008). Feeds of plant origin, like green plants, are considered excellent sources of protein and fat. Recently, aquatic plants are gaining much interest in food and biomedical research due to its broad range of uses such as human food, animal feed and bio-fertilizers. Among these aquatic plants, floating fern *Azolla pinnata* has been widely used as an alternative feed source for poultry (Samad et al., 2020). *Azolla* is a free-floating fresh water fern belonging to the family *Azollaceae* and order *Pteridophyta* (Kumar et al., 2018). It contains almost all essential amino acids, vitamins (Vit. A, Beta carotene and Vit. B12), growth promoter intermediaries and minerals such as iron, calcium, potassium, magnesium, phosphorus, manganese etc. (Dhumal et al., 2009). *Azolla* contains probiotics, carotenoids and biopolymers (Parashuramulu and Nagalakshmi, 2012). Additionally, it can be used as an anti-bacterial and antioxidant agent in medicine, due to its high phenolic and flavonoid content (Noor Nawaz et al., 2014). So, the objective of this study is to evaluate the effect of diet containing different levels of dried *Azolla* on growth performance parameters, hematological, biochemical parameters, antioxidant properties, and economic efficiency of broiler chickens.

## MATERIAL AND METHODS

The current experiment was approved by the Committee of Animal Care and Welfare, Benha University, Faculty of Veterinary Medicine, Egypt (BUFVTM:01-12-20). The experimental period was extended for 6 weeks from September 24<sup>th</sup> to November 5<sup>th</sup> in the year 2020 at the Experimental Animal Research Center, Faculty of Veterinary Medicine, Benha University, Egypt.

### BIRDS, HOUSING AND MANAGEMENT

A total of 208 one-day-old broiler chicks (Cobb-500), obtained from El-NILE Company for poultry and feeding – Egypt, were used in this study. The average initial weight of chicks was about  $43.61 \pm 0.15$  g/chick. The chicks were individually weighed, wing banded for their identification and randomly allocated into four groups of different treatments, each group composed of four replicates (13 birds / replicate). The chicks were housed in a clean well-ventilated deep litter pens and the floor was covered with wood shaving up to 5 cm height. The house was provided with heaters to adjust the environmental temperature according to the age of chicks. All birds have same managerial, hygienic and housing conditions including water, food,

spacing and lighting. In the first 3 days, the brooding temperature was 33°C, then it was gradually lowered to 28°C by the end of the 2<sup>nd</sup> week of age, and thereafter kept constant till the end of experiment, and the relative humidity was between 60-70%, with 23h/d light throughout the experimental period. Fresh water and feed were provided ad libitum. The chicks were vaccinated against Newcastle disease, infectious bronchitis disease, influenza virus (H5) and infectious bursal disease.

### BIRD, DIET AND EXPERIMENTAL DESIGN

*Azolla* was collected as green plant from Ahmed Azzam Company for Agricultural Projects Management, Giza governorate, Egypt. It was sun dried immediately after harvesting, after complete sun drying *Azolla* was then ground. Dried *Azolla* (DA) sample was chemically analyzed before being used in the broiler diets. Following the recommendation by National Research Council (NRC, 1994), four iso-energetic and iso-nitrogenous diets were formulated for Cobb chicks. The chicks were fed starter diet from day zero till the 10<sup>th</sup> day of age. After that, chicks were fed on grower diet that was given till the 22<sup>nd</sup> day of age. Finally, chicks were fed on finisher diet till the end of the experiment (42<sup>nd</sup> day of age). The experimental diets contained yellow corn, soybean meal, corn gluten meal, in addition to mineral and vitamin supplements. The diets were formulated to contain 0%, 4%, 8%, and 12% DA for C (control), T1, T2, and T3, respectively as presented in Table 1.

### DATA COLLECTION

Body weights (BW), and feed intake of the birds were recorded on day 1, 7, 14, 21, 28, 35 and 42. The feed intake (FI/chick) was calculated by dividing the amount of feed consumed in grams during the week by the number of chicks of this group during the same week. Total feed intake (TFI) was calculated for all the experimental period for each chick per grams. Body weight gain (BWG) was calculated weekly for each chick by subtracting the body weight between two successive weights. Feed conversion ratio (FCR) was calculated by dividing feed intake with weight gain.

At day 42, five birds from each group were randomly selected for blood sampling. Blood samples (3 ml) were harvested from the bird's wing vein, with one part being used in Eppendorf tubes for serum separation (3000 rpm; 15 min; 4 °C). The sera were stored at - 20 °C for the determination of serum chemistry, and antioxidant enzymes. The other part was stored in a vacutainer tube containing EDTA as an anticoagulant for hematological analysis. Total leukocytic count (TLC) and counts of red blood cells (RBC) were performed using Neubauer hemocytometer in a dilution of 1:200 with Natt and Herrick solution. Differential leukocyte count, hemoglobin (Hb) concentration and

packed cell volume (PCV) were determined as described by (Campbell, 1995). Serum biochemical analysis of triglycerides (TG), total protein (TP), albumin (A), globulin (G), A/G ratio, High density lipoprotein (HDL), cholesterol, urea, uric acid, creatinine, Serum aspartate aminotransferase (AST), and Alanine aminotransferase (ALT) were performed using commercial kits (Centronic, Germany) on Chem 7, USA. To measure reduced glutathione (GSH) and catalase (CAT) enzymes, a liver tissue sample (250 mg) was mixed in one ml of cold phosphate buffer saline (PBS) solution (pH 7.4) and centrifuged at 4000 rpm for 15 min. The supernatant was removed for assaying. CAT level was determined according to Aebi (1984), and GSH activity was measured as described by (Beutler et al., 1963) using a commercial kit (bio-diagnostic, Egypt).

Economic efficiency parameters were determined via calculation of total costs (TC) and total returns (TR). TC comprised total variable cost (TVC) and total fixed cost (TFC). TVC included the total feed intake cost (TFI cost) for each experimental group. It was estimated for each chick in each group per dollar during the experimental period. TFC involving the costs of labor, purchased chick cost, veterinary management cost, equipment depreciation, water, electricity, fuel, building rent values and other miscellaneous costs. The building and equipment depreciation was calculated by straight line method equation according to (Sankhyan, 1983; Sarkar et al., 2008).

Value of equipment depreciation for each broiler chick:

$$\text{Equipment depreciation} = \frac{(\text{Value of equipments (L.E.)} / \text{No of years}) / \text{No of project cycles per year}}{\text{Total number of broiler}}$$

Value of building depreciation for each broiler chick:

$$\text{Building depreciation} = \frac{(\text{Value of building (L.E.)} / \text{No of years}) / \text{No of project cycles per year}}{\text{Total number of broiler}}$$

TR was calculated as the summation of litter selling return per number of birds at the end of the experiment and the chicken selling return per gram. This chicken selling return was calculated by multiplying the final body weight per gram  $\times$  market price per gram. Net return (NR) was estimated by using the following equation:  $\text{NR} = \text{TR} - \text{TC}$ . Feed cost per kg weight gain (\$/kg) = feed cost (\$/kg)  $\times$  feed intake per chicken (kg/chicken)/weight gain per chicken (kg/ chicken). NR per kg gain (\$/kg) = NR/kg body weight gain. Economic efficiency = NR per kg gain/feed cost per kg gain (El-Aziz et al., 2019).

## STATISTICAL ANALYSIS

Differences between studied groups were analyzed by using the statistical software package for Social Sciences (SPSS v. 23.0, Inc., Chicago, IL, USA), for one way analysis of variance. Duncan's Multiple Range-Test (Duncan,

1955) was used to test differences among means. Statistical significance between mean values was set at ( $P < 0.05$ ). Results were reported as means and standard error of the mean (SEM).

## RESULTS

### PERFORMANCE TRAITS

The effects of dietary treatment with different levels of DA on growth performance parameters are illustrated in (Table 2). Growth performance parameters including FI, BW, BWG, and FCR were differed significantly among different treated groups. The obtained results revealed that the dietary DA at different levels significantly ( $P = 0.012$ ) decreased TFI. The lowest TFI value was for broilers that fed 8% Azolla (3151.3g). DA 8% decreased TFI (- 393.8 g/chick) compared with the control group.

The initial BW of the chicks did not differ significantly ( $P = 0.926$ ). The obtained results revealed that dietary supplementation of DA at each level significantly ( $P < 0.001$ ) improved the average final BW, BWG. Broilers group fed with 12% DA had the highest significant ( $P < 0.001$ ) average final BW and BWG. Final BW increased by (166.4 g/broiler) in group fed with 12% DA as compared with the control group. The FCR was significantly ( $P = 0.002$ ) better among all the broiler groups fed with DA than the control group which showed the poorest FCR value.

### HEMATOLOGICAL, BIOCHEMICAL INDICES, AND ANTIOXIDANT PARAMETERS

As shown in Table 3, It was found that the hemoglobin and total leukocytic count values were differed significantly ( $P < 0.05$ ) among different dietary groups. While, values of RBC and PCV revealed non-significant ( $P > 0.05$ ) differences. Concerning Hb value, broiler groups fed with DA had significant ( $P = 0.038$ ) higher values compared with the control group. The highest value of Hb was for group fed 12% DA (9.89). Regarding TLC values, also all groups fed with DA recorded higher significant ( $P < 0.001$ ) values compared with the control group, the highest value was for group fed with 4% DA (35.6) and the lowest value was for the control group (24.2). Dietary treatments with different levels of DA showed significant improvement in immune cells count such as lymphocytes ( $P < 0.001$ ), and heterophils ( $P = 0.003$ ). Broiler group fed 4% DA had the highest Lymphocytes percentage value (73.3%). Concerning the value of heterophils, the highest value was recorded for broiler group fed with 12% DA (36.1).

As shown in Table 3, feeding broiler chicks on different levels of DA significantly increased ( $P < 0.05$ ) albumin, total protein, and HDL concentrations compared to the control group. The highest value for albumin, and total protein

**Table 1:** Ingredients Composition, calculated chemical analysis, and cost of the experimental diets (g/kg).

Feed ingredients	Starter				Grower				Finisher			
	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
DA <sup>1</sup>	0.0	40.0	80.0	120.0	0.0	40.0	80.0	120.0	0.0	40.0	80.0	120.0
Yellow corn	588.0	578.0	548.0	546.0	625.0	603.0	580.0	554.0	665.0	647.0	646.0	618.0
SBM <sup>2</sup>	185.0	197.0	187.0	132.0	156.0	145.0	122.0	110.0	97.0	78.0	79.0	64.0
CGM <sup>3</sup>	140.0	126.0	123.0	149.0	122.0	120.0	125.0	124.0	142.0	145.0	138.0	137.0
Wheat bran	29.0	0.0	0.0	0.0	32.0	25.0	26.0	22.0	34.0	29.0	0.0	0.0
Soybean oil	14.0	15.0	19.0	11.0	24.0	26.0	27.0	30.0	23.0	23.0	20.0	24.0
L-lysine	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
DL-Methionine	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vit.&Min. mix. <sup>4</sup>	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Salt	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Limestone	15.0	15.0	14.0	13.0	14.0	14.0	13.0	13.0	14.0	13.0	12.0	12.0
DCP <sup>5</sup>	15.0	15.0	15.0	15.0	13.0	13.0	13.0	13.0	11.0	11.0	11.0	11.0
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Calculated chemical composition (%) <sup>6</sup>												
CP	21.99	22.0	22.1	22.1	20.0	20.0	20.02	20.03	19.0	19.0	19.04	19.0
CF	3.41	3.72	4.16	4.42	03.29	3.67	4.06	4.46	3.05	3.41	3.69	4.1
Ca	0.96	0.98	0.97	0.95	0.87	0.89	0.88	0.90	0.81	0.80	0.79	0.80
Available P	0.47	0.47	0.47	0.47	0.43	0.43	0.44	0.44	0.38	0.39	0.38	0.38
Lysine	1.35	1.4	1.4	1.32	1.27	1.27	1.25	1.25	1.14	1.12	1.14	1.14
Methionine	0.56	0.55	0.55	0.57	0.52	0.52	0.52	0.52	0.52	0.53	0.52	0.52
Sodium	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
ME (kcal/kg)	3035.7	3037.5	3035.3	3037.9	3110.4	3109.1	3108.5	3108.3	3181.0	3180.9	3182.1	3182.8
Cost per kilogram (\$)	0.389	0.379	0.371	0.362	0.380	0.372	0.364	0.356	0.378	0.370	0.357	0.350

<sup>1</sup>DA Dried Azolla: (Proximate chemical composition as % of dry matter basis, was determined according to the procedures of the AOAC. (2000): Crude protein= 22.48%, Crude fiber% = 14.7%, Dry matter (DM) = 4.70%, Ether extract (EE) = 4.50 %, Ash = 17.34%, Nitrogen-free extract (NFE) = 40.9%, Metabolizable energy = 2458.4 kcal/kg. Metabolizable energy (ME kcal/g) was calculated according to the formula derived by Lodhi *et al.* (1976). ME (Kcal/g) = 1.549 + 0.0102 CP + 0.0275 EE + 0.0148 NFE - 0.0034 fiber. <sup>2</sup> Soybean meal (44% crude protein). <sup>3</sup>Corn gluten meal. <sup>4</sup>Hy-Mix commercial broiler premix purchased by Misr feed additives company, Egypt. Composition (per 3kg): Vitamin A = 12,000,000 IU, D<sub>3</sub> = 4,000,000 IU, E = 60,000 mg, K<sub>3</sub> = 3,000 mg, B<sub>1</sub> = 2,000 mg, B<sub>2</sub> = 6,500 mg, B<sub>6</sub> = 5,000 mg, B<sub>12</sub> = 20 mg, Niacin = 45,000 mg, Biotin = 75 mg, Folic acid = 2,000 mg, Pantothenic acid = 12,000 mg, Choline chloride = 1000,000 mg, Zinc = 80,000 mg, Manganese = 100,000 mg, Iron = 45,000 mg, Copper = 10,000 mg, Iodine = 1,000 mg, Selenium = 200 mg, Cobalt = 100 mg, Calcium carbonate to 3kg. <sup>5</sup>Dicalcium phosphate (21% calcium and 20% phosphorus). <sup>6</sup>According to Feed Composition Tables for broiler chickens (NRC for poultry 1994). Control: 0% dried Azolla, T1: 4% dried Azolla, T2: 8% dried Azolla, T3: 12% dried Azolla

**Table 2:** Effect of dietary treatments on growth performance of broiler chickens.

Parameter	Experimental groups				SEM	P-value
	Control	T1	T2	T3		
Initial BW (g/chick)	43.60	43.90	43.63	43.67	0.15	0.926
Final BW (g/chick)	2114.9 <sup>d</sup>	2237.5 <sup>b</sup>	2182.8 <sup>c</sup>	2281.3 <sup>a</sup>	19.2	<0.001
TFI (g/chick)	3545.1 <sup>a</sup>	3316.4 <sup>b</sup>	3151.3 <sup>b</sup>	3267.2 <sup>b</sup>	50.5	0.012
BWG. (g/chick)	2071.2 <sup>d</sup>	2193.6 <sup>b</sup>	2139.1 <sup>c</sup>	2237.7 <sup>a</sup>	19.2	<0.001
FCR.	1.71 <sup>a</sup>	1.51 <sup>b</sup>	1.47 <sup>b</sup>	1.46 <sup>b</sup>	0.03	0.002

Control: 0% dried Azolla, T1: 4% dried Azolla, T2: 8% dried Azolla, T3: 12% dried Azolla  
a,b,c means with different superscripts in each row are significantly different at  $P < 0.05$ .

**Table 3:** Effect of dietary treatments on hematological, biochemical indices, and hepatic antioxidant parameters of broiler chicken

Parameter	Experimental groups				SEM	P-value
	Control	T1	T2	T3		
Hematological indices						
RBC (x10 <sup>6</sup> /μl)	2.43	2.48	2.30	2.53	0.05	0.349
Hb (g/dl)	8.71 <sup>c</sup>	9.43 <sup>ab</sup>	8.75 <sup>c</sup>	9.89 <sup>a</sup>	0.18	0.038
PCV (%)	27.2	29.4	26.6	29.6	0.55	0.115
TLC (x10 <sup>3</sup> /μl)	24.2 <sup>b</sup>	35.6 <sup>a</sup>	31.8 <sup>a</sup>	33.0 <sup>a</sup>	1.14	<0.001
Hetero. (x10 <sup>3</sup> /μl)	30.2 <sup>b</sup>	28.1 <sup>b</sup>	35.7 <sup>a</sup>	36.1 <sup>a</sup>	1.05	0.003
Lym. (x10 <sup>3</sup> /μl)	59.9 <sup>d</sup>	73.3 <sup>a</sup>	62.9 <sup>c</sup>	66.0 <sup>b</sup>	1.20	<0.001
Biochemical indices						
Total protein (g/dl)	12.7 <sup>b</sup>	11.7 <sup>b</sup>	16.3 <sup>a</sup>	14.6 <sup>ab</sup>	0.60	0.016
Albumin (g/dl)	2.42 <sup>ab</sup>	2.58 <sup>ab</sup>	3.35 <sup>a</sup>	1.23 <sup>b</sup>	0.37	0.026
Globulin (g/dl)	10.27	9.19	13.06	13.45	0.76	0.122
HDL (mg/dL)	189.7 <sup>ab</sup>	240.2 <sup>a</sup>	149.4 <sup>b</sup>	276.1 <sup>a</sup>	16.8	0.025
Urea (mg/dl)	3.39 <sup>a</sup>	2.19 <sup>ab</sup>	1.36 <sup>b</sup>	1.91 <sup>b</sup>	0.27	0.045
Creatinine (mg/dl)	0.77 <sup>b</sup>	0.53 <sup>c</sup>	0.92 <sup>a</sup>	0.79 <sup>b</sup>	0.04	<0.001
Uric acid (mg/dl)	4.05 <sup>a</sup>	2.16 <sup>b</sup>	1.37 <sup>b</sup>	2.25 <sup>b</sup>	0.29	0.002
Cholesterol (mg/dl)	410.3 <sup>a</sup>	283.2 <sup>ab</sup>	187.1 <sup>b</sup>	242.8 <sup>b</sup>	30.2	0.044
Triglyceride (mg/dl)	45.8	58.5	37.2	39.6	3.59	0.148
AST (u/l)	208.1 <sup>a</sup>	151.9 <sup>ab</sup>	111.7 <sup>b</sup>	103.3 <sup>b</sup>	15.6	0.041
ALT(u/l)	7.33 <sup>a</sup>	5.63 <sup>b</sup>	3.43 <sup>c</sup>	3.76 <sup>c</sup>	0.42	<0.001
Antioxidant parameters						
CAT (U/gm)	2.61 <sup>b</sup>	3.92 <sup>ab</sup>	4.85 <sup>a</sup>	3.39 <sup>ab</sup>	0.29	0.032
GSH (nmol/gm)	10.3 <sup>b</sup>	13.7 <sup>ab</sup>	16.3 <sup>a</sup>	14.6 <sup>ab</sup>	0.80	0.044

RBC: Red blood cells; Hb: Hemoglobin; PCV: Packed cell volume; TLC: Total leukocytic count; Hetero. (Heterophils); Lym. (Lymphocytes); HDL: High density lipoprotein; ALT: Alanine transaminase; AST: Aspartate aminotransferase; CAT (Catalase); GSH (Glutathione). Control: 0% dried Azolla, T1: 4% dried Azolla, T2: 8% dried Azolla, T3: 12% dried Azolla. a,b,c means with different superscripts in each row are significantly different at *P* < 0.05.

**Table 4:** Effect of dietary treatments on economic efficiency parameters of broiler chickens.

Parameter	Experimental groups				SEM	P-value
	Control	T1	T2	T3		
Cost parameters						
TFI cost (\$/chick)	1.35 <sup>a</sup>	1.23 <sup>b</sup>	1.13 <sup>c</sup>	1.15 <sup>c</sup>	0.03	<0.001
TFC(\$/chick)	1.16	1.16	1.16	1.16	-	-
TC(\$/chick)	2.51 <sup>a</sup>	2.39 <sup>b</sup>	2.29 <sup>c</sup>	2.31 <sup>c</sup>	0.027	0.001
Return parameters						
Broiler selling (\$/chick)	2.98 <sup>d</sup>	3.15 <sup>b</sup>	3.07 <sup>c</sup>	3.21 <sup>a</sup>	0.027	<0.001
Litter selling (\$/chick)	0.038	0.038	0.038	0.038	-	-
TR (\$/chick)	3.02 <sup>d</sup>	3.19 <sup>b</sup>	3.11 <sup>c</sup>	3.25 <sup>a</sup>	0.027	<0.001
NR (\$/chick)	0.51 <sup>c</sup>	0.80 <sup>b</sup>	0.82 <sup>b</sup>	0.94 <sup>a</sup>	0.049	<0.001
Feed cost / kg gain (\$/kg gain)	0.65 <sup>a</sup>	0.56 <sup>b</sup>	0.53 <sup>ab</sup>	0.51 <sup>c</sup>	0.01	<0.001
NR / kg gain (\$/kg gain)	0.25 <sup>c</sup>	0.36 <sup>b</sup>	0.38 <sup>ab</sup>	0.42 <sup>a</sup>	0.02	<0.001
Economic efficiency	0.37 <sup>c</sup>	0.65 <sup>b</sup>	0.72 <sup>ab</sup>	0.82 <sup>a</sup>	0.05	<0.001

TFI cost: Total feed intake cost; TFC: Total fixed cost; TC (Total cost); TR: Total return; NR: Net return.

Control: 0% dried Azolla, T1: 4% dried Azolla, T2: 8% dried Azolla, T3: 12% dried Azolla

a,b,c means with different superscripts in each row are significantly different at *P* < 0.05.

was recorded in group fed with 8% DA (3.35, and 16.3, respectively). The highest value of HDL was for group fed 12% DA (276.1), while the lowest value was for group fed with 8% DA (149.4). Nevertheless, the opposite trend was noticed with urea, creatinine, AST, ALT, uric acid, and cholesterol concentrations whereas they were significantly ( $P < 0.05$ ) lower in broilers fed with 4, 8, 12 % DA than those of the control group, while triglyceride values did not show any significant differences among different dietary treatments.

The current results revealed a significant difference of catalase (CAT) and reduced glutathione (GSH) among all the experimental groups. The dietary DA at each level had a higher significant increase in CAT ( $P=0.032$ ), and GSH ( $P=0.044$ ) values than the control group. The highest value for CAT and reduced GSH was found for broiler group fed with 8% Azolla (4.85 and 16.3, respectively), while the lowest value was for the control group (2.61 and 10.3, respectively).

#### ECONOMIC EFFICIENCY PARAMETERS

Results in Table 4, cleared that there was a significant ( $P<0.05$ ) difference in the TFI cost, TC, TR, NR, NR per Kg gain, and economic efficiency among all the experimental groups. The dietary DA at different levels significantly decreased TFI cost ( $P < 0.011$ ), and TC ( $P=0.010$ ) compared with the control group, the lowest values were recorded for broiler group fed with 8% Azolla (1.13, 2.29 \$/chicken, respectively). DA 8% decreased TC (- 0.22 \$/chicken) compared with the control group. TR, NR, NR per Kg gain, and economic efficiency values were significantly ( $P<0.05$ ) higher for all broiler groups fed DA at different levels compared with the control group. Moreover, broiler group fed with 12% DA diet significantly ( $P<0.001$ ) recorded the highest TR, and NR values (3.25, 0.94 \$/chicken, respectively) compared with the control group. The highest NR per Kg gain, and economic efficiency values were found for group fed 12% Azolla (0.42, and 0.82, respectively).

## DISCUSSION

#### PERFORMANCE TRAITS

The result of the current study clarified that the growth performance of broiler chicken fed with DA significantly improved. Lower feed intake and better FCR have been detected in all the broiler groups fed with DA compared with the control group. Lower feed intake might be attributed to the inability of birds to eat much of the bulky Azolla, and the high content of crude fiber in Azolla (Bacerra et al., 1995). The current findings are in consent with those got by Joysawal et al. (2018) who stated that there was a significant ( $P \leq 0.01$ ) decrease in feed consumption of all

the groups fed with Azolla in diet compared to control. Also, Chatterjee et al. (2020) recorded a decrease of feed consumption with an increase in the level of Azolla up to 15% in the diet of poultry birds. Similar to our findings of better FCR of dietary treated groups with DA, Acharya et al. (2015) stated that the inclusion of Azolla spp. in the diets could help improve the feed efficiency of the chickens. Wuthijaree et al. (2012) also recorded that supplementation of 10% and 15% of Azolla in the ration of the broilers improved the FCR. On the contrary, Rawat et al. (2015) reported that the broiler groups fed with diet supplemented with 5% Azolla showed a higher FI. Also, Samad et al. (2020) mentioned that the birds fed with Azolla did not show significant differences in FCR. This variation might be due to the used broiler strain with capacity variation of fiber degradation and different environmental factors (Rout et al., 2017).

Moreover, the dietary supplementation of DA improved the final weights, and BWG. The significant improvement of the final BW and BWG of the broiler group fed with 12% DA might be due to the fact that Azolla is rich in protein contents, particularly the essential amino acids. Moreover, vitamins, and a significant content of mineral elements such as iron, calcium, potassium, magnesium, phosphorus, manganese etc. added a beneficial effect on the growth performance (Dhumal et al., 2009). In addition to that, Azolla contains carotenoids and biopolymers which are natural antioxidant and immunomodulation agents that play an important role in the development of the productivity and health condition of the animals (Acharya et al., 2015). The present finding was in accordance with (Fadzlin et al., 2020) who reported that chicken group fed with 15% Azolla spp. significantly recorded the highest BW and BWG. On the other hand, these results disagreed with Paudel et al. (2015) who said that the broiler chickens that are not supplemented with Azolla showed the highest BWG in the 6<sup>th</sup> week, but the lowest BWG was observed for chickens fed 20% Azolla. Islam (2017) recorded that the live weight is the highest ( $P<0.05$ ) in the birds that are not fed Azolla. These variations observed in the result of BW, and BWG of feeding Azolla at different levels could be attributed to the differences in the species of Azolla and the stage of maturity of the used plant species. The stage of maturity of the Azolla and the time of harvesting can influence the nutritional composition of the plant (Michael and Anthony, 2008).

#### HEMATOLOGICAL, BIOCHEMICAL INDICES, AND ANTIOXIDANT PARAMETERS

Dietary DA had significant improvement at different levels in some hematological parameters compared with the control group. Both Hb and TLC values increased in all groups fed with DA compared with the control group. In

the line of this result came [Shinde et al. \(2017\)](#) who stated that there was a significant increase in hemoglobin values in groups fed with diet supplemented with Azolla. Oppositely, this result disagreed with [Kumar et al. \(2018\)](#) who recorded that supplementation of DA at 2.5, 5, 7.5, and 10 % had no effect on the HB and TLC. Dietary DA resulted in non-significant difference of RBC and PCV values. Conversely, [Alagan et al. \(2020\)](#) mentioned that there were significant ( $P \leq 0.01$ ) differences in the PCV and RBC for the birds provided with diet supplemented with 5% Azolla.

Dietary treatments with different levels of DA had a positive effect on immune cell count. Heterophils and Lymphocytes percentage values were increased in broilers fed DA up to 12% compared to the control group. This incremented value could be due to the fact that Azolla has high phenolic and flavonoid content ([Noor Nawaz et al., 2014](#)). In harmony with the obtained result, [Mishra et al. \(2016\)](#) stated that bird groups fed with Azolla at 5% and 7.5% have higher heterophils, and lymphocytes values than control ones. Meanwhile, [Kumar et al. \(2018\)](#) recorded that supplementation of dried Azolla at 2.5, 5, 7.5, and 10 % had no effect on the heterophils level. The current results indicate that the birds fed with Azolla diet were adequately nourished, and thus do not suffer from anemia or show any signs of infectious disease or parasitic problems.

For the antioxidant potency of DA, the catalase, and glutathione values significantly increased in DA treated groups than the control group. This improvement in the antioxidant potency might be due to the presence of active substances in Azolla which prevent the activity of the free radicals ([Mishra et al., 2016](#)). These results agreed with [AL-Rekabi et al. \(2020\)](#) who noted that the concentration of glutathione is increased significantly ( $P < 0.05$ ) for chickens fed with diet supplemented with Azolla compared to the control group. [Nayak et al. \(2015\)](#) recorded that the high phenolic and flavonoid content of Azolla allow it to be used as an antibacterial and antioxidant agent.

The current study demonstrated also a significant increase in albumin, total protein, and HDL concentrations in broiler chickens fed with different levels of DA compared to the control group. [Yaman et al. \(2000\)](#), stated that the total plasma proteins are a common parameter utilized to estimate the avian body condition. Albumin, one of the main serum proteins, serves as the most favorable source of amino acids for synthesis of tissue proteins. Additionally, feeding Azolla plays an important role in reducing the exposure of the birds to any type of stress through increasing the secretion of thyroxine, thus increasing the rates of metabolism and increasing the vital reactions in the body, building muscle tissue, which leads to maintaining a high average of total protein in the birds' blood than

the control ([Balaji et al., 2009](#)). These results agreed with the finding of [AL-Rekabi et al. \(2020\)](#) who revealed that the broiler chickens that are fed with Azolla had a higher concentration of total protein in comparison to the control group. On the other hand, [Alagan et al. \(2020\)](#) reported a non-significant difference among different dietary treatments on albumin, globulin, and albumin to globulin values. DA feeding resulted in a considerable decrease in the concentrations of urea, creatinine, AST, ALT, uric acid, and cholesterol, while triglyceride values did not show any significant differences among different dietary treatments. These results might be due to the better utilization of protein in broiler groups which are fed with Azolla, and the presence of polyunsaturated fatty acids of Azolla which act as cholesterol lowering agents ([AL-Rekabi et al., 2020](#)). In accordance with the current results, [Rieta and Cabaral, \(2018\)](#) mentioned that the broilers fed with a diet supplemented with 100% Azolla showed lower blood cholesterol ( $P < 0.05$ ) than the broilers that take 50% Azolla and the control ones. On the other hand, [Shambhvi et al. \(2021\)](#) said that there is a non-significant difference in the plasma concentration of uric acid or creatinine in birds fed with Azolla.

#### ECONOMIC EFFICIENCY PARAMETERS

From the economic point of view, the dietary DA at different levels significantly decreased TFI cost and TC compared with the control group. This result might be due to the decreased TFI among all groups fed DA. Broiler group fed 8% Azolla recorded the lowest TFI value, resulting in reducing the feed intake cost, consequently reducing the TC. These results agreed with the finding of [Joysowal et al. \(2018\)](#) who mentioned that Azolla is an unconventional feed of low price that reduces the feed cost, so it can be used as poultry feed. In a previous study, [Mahanthesh et al. \(2018\)](#) said that the replacement of commercial feed by 20% Azolla reduced the feed cost. This result also agreed with [Chatterjee et al. \(2020\)](#) who recorded that birds fed with 5% Azolla reduced the production cost compared with the control group.

Higher growth performance of broilers fed with DA, and the significant increase of final BW reflected on the TR, NR, NR per Kg gain, and economic efficiency which were significantly higher for all broiler groups fed with DA at different levels compared with the control group. Interestingly, the current results reported that broilers fed with 12% DA diet showed significantly the highest profitability and economic efficiency compared with the control group. Out of the abovementioned results, the diet containing 12% DA is considered the best economical diet for feeding broiler. This result might be due to more than one reason: a greater feed efficiency by feeding Azolla ([Rout et al., 2017](#)), better FCR ([Naghashi et al., 2014](#)), the improvement in

the BW of broilers fed with DA (Ara et al., 2015), and lower production cost (Rieta and Cabaral, 2018). Consistently with these findings, Chichilichi et al. (2014) recorded that birds fed with Azolla with different levels showed the highest profit margin compared with the control ones. On the other hand, Yadav and Chhipa (2016) indicated that the broilers that did not receive Azolla diet had the highest return compared with the broilers that received diet containing Azolla.

## CONCLUSION

The dietary inclusion of 4%, 8%, and 12% DA, can improve the growth performance, hematological and biochemical parameters, and antioxidant properties without adverse effect on health condition of broiler chicks. Additionally, the inclusion of DA up to 12% can increase the profitability for broiler chickens.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHORS' CONTRIBUTION

Material preparation and data collection were performed by both authors. Data analysis was performed by Eman Ramadan Kamel. Eman Hamed prepared the first draft of the manuscript. The final draft of the manuscript was rewritten, reviewed, and edited by Eman Ramadan Kamel.

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