



## Economic Evaluation of Using Azolla on Growth Performance of Broiler Chickens: Gene Expression Impact



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**T**HE purpose of this study was to examine the effects of feeding Azolla to broiler chickens on growth performance, gene expression, and its financial impact. 120 one-day-old chicks (Cobb 500) in total were divided into four groups at random. For 42 days, broiler chickens were fed several diets that contained 0%, 4%, 8%, and 12% dried Azolla (DA). Broilers group fed with 12% DA had the highest significant average final body weight and body weight gain. Azolla dietary treatments at various doses resulted in a noticeable improvement in the expression profile of growth (*IGF-I*, *GH*, *MYLK4*, *MEF2B*, *TRPV1*, *STAT5A*, *PGAM2*), immune (*IL-4*, *IL-6*, *IL-8*, *IL-1 $\beta$* , and *AVBD9*), antioxidant (*SOD*, *CAT*, *GPX*, *PRDX2*, and *PRDX6*) and intestinal health (*MUC2*, *gastrotropin*, *Cath-B*) genes. The highest net return per Kg gain, and economic efficiency values were found for group fed 12% Azolla. In deduction, the dietary enclosure of DA up to 12%, can advance the growth performance, antioxidant characteristics without negatively affecting on the health condition of broiler chicks, and upsurge the profitability for broiler chickens.

**Keywords:** Economic evaluation, Azolla, growth performance, Broilers, Gene expression

### Introduction

The global increase in poultry production has made the poultry business one of the fastest growing industries in the world, which has led to a shortage and rising prices for conventional feed ingredients [1]. The most significant obstacle to the growth of the poultry industry globally is the lack of food, which has led researchers to focus on alternative food sources and unorthodox food products. Azolla is utilised as a substitute in the grill chickens' diet to help with the high cost of the feed, which accounts for roughly 70% of overall production costs [2].

Azolla may treble its biomass in 3 to 10 days and is typically found in shallow water basins [3]. Azolla is a good food source for species of poultry, livestock, and aquatic life because of

its low input costs and ease of growing in the wild and under regulated farm conditions [4]. Azolla is an excellent source of protein and a rich supply of probiotics, vital amino acids, vitamins, minerals, and trace minerals [5]. Feeding Azolla to chicken lowers feed costs and mortality rates while improving meat quality in terms of colour, flavour, tenderness, and juiciness [6].

Up to 5% of air-dried Azolla can be substituted in commercial diets without negatively affecting performance or economic metrics [7]. Because azolla has a significant amount of crude protein, minerals, and vitamins that satisfy the majority of a bird's nutritional needs, it promotes faster growth in poultry feeding diets. At 21 to 42 days of age, broiler diets containing Azolla at levels ranging from 5 to 25% significantly reduce the average daily growth ( $P > 0.05$ ) [8, 9].

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Supplementing the broiler meal with Azolla at a rate of 5 to 20% has a significant impact on FI and daily weight improvement. The feed consumption of broilers fed 5% Azolla meal was comparable to that of broilers not given Azolla [10]. Adding dried Azolla at a rate of 5% to the diet for broilers increases feed consumption, body weight gain, and feed conversion without having an adverse effect on health or economic gain [11]. At 21 to 42 days of age, the feed conversion ratio and feed efficiency are dramatically reduced when azolla is used in the broiler diet at a rate of 5 to 10% [12]. When compared to chicks not fed Azolla meal, the cost of feed consumed throughout the production phase is significantly reduced ( $P < 0.01$ ) in the Azolla-fed chicks [13]. Broilers fed 15% Azolla had the highest feeding cost per kg of live weight growth, however broilers fed 5% Azolla meal showed lower feeding cost when compared to broilers not fed Azolla [10].

In comparison to birds fed 10% Azolla and birds not given Azolla, birds fed 5% Azolla demonstrated the largest profit margin [14]. The birds that were not given Azolla and those that were fed 10% Azolla had the highest cost of production per bird, highest net income, and highest gross income, whereas the birds that were fed 15% and 20% Azolla had the lowest cost of production per bird [15]. The group that received 5% Azolla had the highest profit per bird, and the birds who received 5%, 15%, 10%, and 0% Azolla, respectively, had the highest net profit per bird [10,16].

The objectives of this study was investigating the effect of feeding altered diets comprising 0%, 4%, 8%, and 12% dried Azolla on growth performance, gene expression and economic parameters of broilers.

## **Material and Methods**

### *Ethics Declaration*

All animal supervision measures, established experimental collecting, and sample disposal were carried out below the direction of the University of Sadat City's Veterinary Medical School in agreement with IACUC strategies (code VUSC-016-1-23)

### *Birds' management*

A total of 120 day-old chicks (Cobb 500) were purchased from the Egyptian poultry company El-Dakahlia. The starting weight of each chick was approximately  $44.53 \pm 0.21$  grams. The chicks were weighed independently before being

arbitrarily divided into four groups: a control group, three treatment groups, and three groups of 10 chicks each.

The chicks were kept in tidy, well-ventilated deep litter pens that had wood shavings that reached a height of 5 cm. Heaters were installed in the home so that the temperature could be adjusted based on the age of the chicks. The same housing conditions apply to all groups, including water, food, density, and illumination. After the period of brooding, the temperature remained around  $28^{\circ}\text{C}$  until the experiment's conclusion, with 23 hours per day of light. Ad libitum access to clean water and rations is granted. The birds received immunizations against infectious bronchitis, infectious bursal disease (Gamboro), and Newcastle disease.

### *Diet formulation*

Azolla was gathered as a green plant from agricultural banks and shallow water areas, dried in the sun right away, and then pulverised once fully dry. Before being used in the grill diets, the dried Azolla sample underwent a chemical analysis. In accordance with the National Research Council's recommendation [17], four sets of iso-energetic and iso-nitrogenous diets were created. The chicks were fed starter food starting on day 0 and continuing through day 10 of age, grower diet starting on day 22 of age, and finisher diet continuing through day 35 of age. As shown in Table 1, the diets were designed to contain 0% dried Azolla D1 for control and 4%, 8%, and 12% dried Azolla for D2, D3, and D4, respectively.

### *Data Collection*

On days 1, 7, 14, 21, 28, and 35 of the experimental period, the chicks' initial BW, weekly BW, and FI were recorded. The ending body weightness growth is the change between the beginning and final body weights. Body weight gain was measured as the alteration between two succeeding body weights per week [18].

$$\text{BWG} = \text{BW1} - \text{BW2}$$

The weekly feed consumption in each group was divided by the number of chicks in that group to determine the FI. The following equation [19] calculates FCR based on the amount of feed necessary to produce a unit weight:

$$\text{FCR} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

*Economic evaluation**Total Variable Costs (TVC)*

Labour, feed, chicks, veterinary managing, production-related costs, and additional costs made up the total variable costs (TVC) [20].

*Total Fixed Costs (TFC)*

Depreciation on land, buildings, and equipment were involved in the total fixed costs (TFC). The depreciation rate for buildings was calculated using a 25-year time setting, while the 5-year time period was used to calculate the equipment depreciation rate [21].

$$\text{Depreciation rate} = \frac{\text{value of asset} / \text{age of asset (year)} / \text{no of production cycles per year}}{\text{Total number of birds}}$$

*Total Costs (TC)*

The total costs were comprised of the entire fixed costs plus the whole variable costs. (TC) [22].

$$TC = TVC + TFC.$$

*Reduction % in the feed costs*

The percentage reduction relies on the price of the ingredients used in each diet as well as the cost of manufacturing each diet per tonne for each group. Additionally, the cost of the feed ingested throughout the trial, which is dependent on how much feed each group consumed, affects the reduction percent. Comparisons between the reduction percentages of the experimental groups and the control group were made [23].

*Total Return*

The equation previously discussed was employed to calculate the overall return [24]. Additionally, all prices were established in accordance with the going rate during the time of the study.

$$\text{Total return (TR)} = \frac{\text{price of broiler meat/ kg} \times \text{total live BW in Kg}}$$

*Net Return*

The following equation [25] was used to determine the net income.

$$\text{Net income (NR)} = \text{total return} - \text{total costs.}$$

$$\text{Gross margin (GM)}$$

GM was considered by the following equation [26].

$$GM = \text{total return (TR)} - \text{total variable cost (TVC)}.$$

*Relative gross margin (RGM)*

The RGM determined using the subsequent

equation [27].

$$RGM = \text{GM of tested group} / \text{GM of control group}.$$

*Economic efficiency*

The economic effectiveness of the various grill groups was determined by dividing the return by the total cost of the feed consumed for each group. The cost of each kg of diets for each group is determined based on the market price of feed ingredients and the amount of azolla utilized in the diet. Economic efficiency was calculated using the following equation [28].

$$\text{Economic efficiency \%} = \frac{\text{(net return)}}{\text{(total feed cost)}} \times 100$$

*Investigational samples*

For liver, muscle, spleen, and intestine sample collection, six birds from every group were haphazardly picked and slaughtered. For the purpose of quantifying gene expression, samples were sterile collected, cleaned in phosphate buffer saline (PBS), instantly frozen in liquid nitrogen, and then kept at 80 °C.

*Total RNA extraction, reverse transcription and quantifiable real time PCR*

Following the manufacturer's directions, whole RNA was extracted from the tested chickens with Trizol reagent (RNeasy Mini Ki, Catalogue no. 74104). By means of a NanoDrop® ND-1000 Spectrophotometer, the isolated RNA's amount was determined and confirmed. Each sample's cDNA was formed according to the manufacturing method (Thermo Fisher, Catalogue no. EP0441). Valuation of the expression configuration of growth (*IGF-I*, *GH*, *MYLK4*, *MEF2B*, *TRPV1*, *STAT5A*, *PGAM2*), immune (*IL-4*, *IL-6*, *IL-8*, *IL-1β*, and *AVBD9*), antioxidant (*SOD*, *CAT*, *GPX*, *PRDX2*, and *PRDX6*) and intestinal health (*MUC2*, *gastrotropin*, *Cath-B*) genes was performed using SYBR Green PCR Master Mix (2x SensiFast™ SYBR, Bioline, CAT No. Bio-98002) and quantitative RT-PCR.

SYBR Green PCR Master Mix was utilized in real-time PCR to conduct comparative quantification of mRNA levels (Quantitect SYBR green PCR kit, Catalogue no. 204141). Primer structures were formed via the *Gallus gallus* sequence that was issued in PubMed, as indicated in Table 2.

For the normalization, the housekeeping gene *GAPDH* was employed as a constitutive control.

The reaction combination was approved in a entire capacity of 25  $\mu$ l comprised of entire RNA 3  $\mu$ l, 4  $\mu$ l 5x Trans Amp buffer, 0.25  $\mu$ l reverse transcriptase, 0.5  $\mu$ l of each primer, 12.5  $\mu$ l 2x Quantitect SYBR green PCR master mix and 8.25  $\mu$ l RNase free water. The concluding reaction mix was sited in a thermal cyler and the subsequent program was carried out: reverse transcription at 50 °C for 30 mins, primary denaturation at 94 °C for 8 mins followed by 40 cycles of 94 °C for 15 s, annealing temperatures as presented in Table 2, and 72 °C for 30 s. Following the amplification phase, a melting curve analysis was performed to confirm the specificity of the PCR product. The comparative expression of each gene per sample in contrast with *GAPDH* gene was carried out and considered conferring to the  $2^{-\Delta\Delta Ct}$  mode [29].

#### Statistical analysis

The means and standard errors of the means (SEM) are used to present data. One-way analysis of variance (ANOVA) and the post hoc Duncan's multiple comparison assessment were employed to analyze the data. There was statistical significance between various groups when the P value was less than 0.05.

### Results

#### Growth performance

As revealed in table 3, the treated groups showed significantly different growth performance indicators, such as FI, FCR, BW, and BWG. The acquired results indicated that, when compared to the control group, dietary treatments with DA at various levels considerably reduced FI and increased FCR. The control group had the highest FI, whereas D3 (the 8% DA treated group) had the lowest FI. The best FCR value was observed for D4 (12% DA treated group), whereas the control

group had the worst FCR, according to the FCR data, which showed that as the DA content in the diet increases, so does the FCR.

When compared to the control group, the BWG and BW considerably improved when different levels of DA were included in the diet, according to the data in table 3. The D4 (12% DA treated group) final BW and BWG measurements were 2231.3 gm and 2187.7 gm, respectively, while the control group's final BW and BWG measurements were 2064.9 gm and 2021.2 gm, respectively.

#### Economic evaluation parameters

Results in Table 1 showed that adding DA to broiler diets at various levels had a significant impact on the percentage of diet costs that each group saved in comparison to the control group. The uppermost percentage of savings came from D4 (12% DA), while the lowest percentage came from D2 (4% DA).

Regarding the TC, TR, and NR results in table 4, the treatment diets of DA at various levels considerably reduced TC in comparison to the control group, with the control group recording the highest value of TC and D3 (8% DA) recording the lowest. When compared to the control group, for all groups fed treatment meals containing DA at varied amounts, TR and NR values were noticeably higher, with D4 (12%) recording the highest returns and D1 the lowest. The GM, RGM, and economic efficiency percentage results are all in line with each other and show that the DA treated groups improve all economic parameters and outperform the control group. The highest GM, RGM, and efficiency percentage were recorded in D4 (12% DA), and the lowest percentage were recorded in D1 (control).

TABLE 1. Reduction % in diet cost in control and treated diets.

Group	Ration type	Cost of diet (LE)/kg	Reduction % in diet cost	Total Reduction % in diet cost
D1 (Control)	Starter	21.40	0	0
	Grower	20.50	0	
	Finisher	19.05	0	
D2 (4% DA)	Starter	20.80	2.80%	2.48%
	Grower	19.75	3.65%	
	Finisher	18.85	1.00 %	
D3 (8%DA)	Starter	20.06	6.26%	5.89%
	Grower	19.17	6.48%	
	Finisher	18.11	4.93%	
D4 (12%DA)	Starter	19.98	6.64%	6.78%
	Grower	18.94	7.61%	
	Finisher	17.89	6.10%	

- DA= Dried Azolla

**TABLE 2. Oligonucleotide primers for growth, immunological, and intestinal health genes used in real-time PCR, along with their sequence, accession number, annealing temperature, and product size.**

Gene	Isolation source	Primer	Product length (bp)	Annealing Temperature (°C)	Accession number
<i>GH</i>	Liver	F5'- GACATGGAGCTGCTTCGGTT -3' R5'- AACACTCTGTCTGAGGTGCC - 3'	116	58	NM_204359.2
<i>IGF-I</i>	Liver	F5'- TACCTTGGCCTGTGTTTGCT -3' R5'- CCCTTGTGGTGTAAGCGTCT - 3'	170	60	NM_001004384.3
<i>STAT5A</i>	Liver	F5'- TTCTGCAGCCGTCACCTAAGG -3' R5'- CGGAGGGGACAAAGGGGA - 3'	182	58	XM_046933285.1
<i>MYLK4</i>	Muscle	F5'- GCAGTGATGACTTGGGGGAA -3' R5'- TCTAGCTGAGTCTCTCTGCTGT - 3'	161	58	XM_040663935.2
<i>MEF2B</i>	Muscle	F5'- CCTCACTGGTTTCCCCTTCC -3' R5'- TGA CTTGATGCTGATGGCGT - 3'	211	60	XM_040692870.2
<i>TRPV1</i>	Muscle	F5'-GCCAGGAAGTGTATGTGGCT-3' R5'- GCTATTTGTGTCTGCCCT- 3'	233	60	NM_204572.1
<i>PGAM2</i>	Muscle	F5'- GGGGGATGCTCTCAAGCAAT -3' R5'- GGACTGGGCACCCATCTTAG - 3'	169	60	NM_001031556.3
<i>IL-4</i>	Spleen	F5'- TGGATATGGGTGGAGATAACAAC-3' R5'- GCTCGCTCTCCTGTCA TTT - 3'	96	58	NM_001398460.1
<i>IL-6</i>	Spleen	F5'-GCGAAAAGCAGAACGTCGAG-3' R5'- TTGGCGAGGAGGGATTCTG- 3'	130	58	NM_204628.2
<i>IL-8</i>	Spleen	F5'-AGATGTGAAGCTGACGCCAA-3' R5'- GAGCTGAGCCTTGCCATAA- 3'	145	60	HM179639.1
<i>IL-1β</i>	Spleen	F5'-CCTCCAGCCAGAAAGTGAGG-3' R5'- TTGTAGCCCTTGATGCCAG- 3'	109	60	HM179638.1
<i>AVBD9</i>	Spleen	F5'-ACCGTCAGGCATCTTCACAG-3' R5'- CTCTGCCTGCATGCTAAGGT- 3'	153	60	NM_001001611.3
<i>SOD</i>	Liver	F5'-ACTGGAATGCTGGACCTCG-3' R5'- TGCACACGGAAGAGCAAGTA-3'	140	60	NM_205064.2
<i>CAT</i>	Liver	F5'-CCTGACTATGGCGACGTAT-3' R5'- CAGACACACGAGAAGTGGCT-3'	106	58	NM_001031215.2
<i>GPX</i>	Liver	F5'-AACTGCAGGACGACATCGAG-3' R5'- CTTGAAAACATCGGGCGCA-3'	182	60	NM_001277853.3
<i>PRDX4</i>	Liver	F5'-GACATGCACTTAGGGGCCCTT-3' R5'- CAACCAGCAGGGCAA ACTTCA-3' F5'-CGAAGGATCTCCCATCAGGC-3'	160	58	XM_040658093.2
<i>PRDX6</i>	Liver	F5'- R5'- TGAGAGAGTGTGGGGGACAA- 3' F5'-	234	58	NM_001039329.3
<i>MUC2</i>	Intestine	ACAGAAGGA ACTTCTTCGTACA-3' R5'- GGTGGTGACATACTGCCAGA- 3'	173	58	XM_040673077.2
<i>Cath-B</i>	Intestine	F5'- TGAAGAACCTGATGTGGGGC-3' R5'- CAGGCCCTTCCCTAGGATCA-3'	196	58	NM_205371.3
<i>Gastrotrypin</i>	Intestine	F5'- TGAGGGTGATAGTGAGCTCGT-3' R5'- AATCCCCACACGACACCAAG-3'	205	60	NM_001277700.2
<i>GAPDH</i>		F5'- AGTCAACGGATTTGGCCGTA-3' R5'- ACAGTGCCCTTGAAGTGTCC -3'	159	60	NM_204305.2

TABLE 3. Impact of treated diets on feed intake and growth performance.

Group	FI (gm)	FCR	Total BWG (gm)	Final BW (gm)
	Mean± S. E	Mean± S. E	Mean± S. E	Mean± S. E
D1 (control)	3459.6± 6.76 <sup>a</sup>	1.71±0.01 <sup>a</sup>	2021.2±7.29 <sup>d</sup>	2064.9±6.13 <sup>d</sup>
D2 (4% DA)	3154.5± 10.09 <sup>b</sup>	1.51±0.003 <sup>b</sup>	2089.1±8.21 <sup>c</sup>	2132.8±7.19 <sup>c</sup>
D3 (8% DA)	3151.1± 36.58 <sup>b</sup>	1.47±0.05 <sup>b</sup>	2143.6±1.35 <sup>b</sup>	2187.5±2.59 <sup>b</sup>
D4 (12% DA)	3194.2±47.38 <sup>b</sup>	1.46±0.03 <sup>b</sup>	2187.7±12.43 <sup>a</sup>	2231.3±12.44 <sup>a</sup>

- BW= Body weight; BWG= Body weight gain; DA= Dried Azolla; FCR= Feed conversion rate; and FI= feed intake  
- a, b, c Means within the same row having different upper-case superscripts are significantly different at  $p \leq 0.05$ .

TABLE 4. Impact of treated diets on economic evaluation parameters.

Group	TC	TR	NR	GM	RGM	Economic efficiency
	Mean± S. E	Mean± S. E	Mean± S. E	Mean± S. E	Mean± S. E	Mean± S. E
D1 (control)	78.79±0.34 <sup>a</sup>	80.53±0.16 <sup>d</sup>	1.74±0.36 <sup>d</sup>	2.64±0.22 <sup>d</sup>	0	2.47%
D2 (4% DA)	72.67±0.41 <sup>b</sup>	83.17±0.69 <sup>c</sup>	10.50±0.23 <sup>c</sup>	13.54±0.19 <sup>c</sup>	5.12±0.13 <sup>c</sup>	16.81%
D3 (8% DA)	67.33±0.35 <sup>c</sup>	85.31±0.21 <sup>b</sup>	17.98±0.27 <sup>a</sup>	16.74±0.14 <sup>b</sup>	6.34±0.09 <sup>b</sup>	29.80%
D4 (12% DA)	68.98±0.39 <sup>c</sup>	87.02±0.52 <sup>a</sup>	18.04±0.38 <sup>a</sup>	18.94±0.31 <sup>a</sup>	7.17±0.15 <sup>a</sup>	29.82%

- DA= Dried Azolla; GM= Gross margin; NR= Net return; RGM= Relative gross margin; TC= Total costs; and TR= Total return;

- a, b, c, d Means within the same row having different upper-case superscripts are significantly different at  $p \leq 0.05$ .

#### Gene expression profile of growth, immune, and intestinal health makers

Treatments with various doses of Azolla in the diet significantly improved the expression profile of growth (*IGF-I*, *GH*, *MYLK4*, *MEF2B*, *TRPV1*, *STAT5A*, *PGAM2*), immune (*IL-4*, *IL-6*, *IL-8*, *IL-1 $\beta$* , and *AVBD9*), antioxidant (*SOD*, *CAT*, *GPX*, *PRDX2*, and *PRDX6*) and intestinal health (*MUC2*, *gastrotropin*, *Cath-B*) genes (Fig. 1-4). An enhanced gene expression profile of investigated makers was observed in broiler chicks fed diet containing 12% dried Azolla (DA) than other groups.

#### Discussion

The acquired results showed that, as compared to the control group, various levels of DA in the diet considerably improved the BWG and BW. According to Yadav and Chhipa [30], who stated that the broilers fed Azolla meal ingest lesser FI

than the control one, the outcomes of feed intake may be related to the reduced palatability of Azolla, which limits its utilisation. Additionally, these findings concurred with those of Wuthijaree et al. [31], who revealed a significant impact on the FI and FCR when azolla is added to the diet at a rate of 5 to 20%.

Regarding the BWG and BW results, this could be attributable to Azolla's high protein content as well as its superior protein utilization, which has a favourable impact on growth performance. Azolla is also upright foundation of vitamins, minerals, and important amino acids. These results support those of Samad et al. [32], who found that broilers fed 15% Azolla had considerably higher BW and BWG values than the other groups. Additionally, the grill fed 7.5% Azolla had a greater BW than the control one, according to Prabina and Kumar [33].

Results showed that employing DA at various levels in broiler diets has a substantial impact on the percentage reduction in food costs of various groups contrasted with the control group. As the level of DA in the diet increases, the cost savings increase. This outcome is consistent with Mishra et al. [34] who looked into why broilers fed Azolla meal had much lower feed costs per kg of live bird weight than the control group. Because of DA's nutritional profile, it was sufficient to be employed as a substitute ingredient in grill diets, especially instead of other ingredients of high prices as wheat bran, yellow corn and soybean. Our findings were in agreement with those of Dhumal et al. [13], who established that Azolla has a high nutritional composition and is a amusing basis of vitamins, minerals, and amino acids that could be employed in broiler feed rations.

The highest value of TC was recorded for the control group, and the lowermost were noted for D3 (8% DA) in the treatment diets of varied levels of DA in comparison to the control group. As feed accounts for more than 70% of the total production costs, it has a highly important impact on TC. The cause of TC was due to decreased FI in groups given treated diets as well as lower treated diet costs as compared to control groups. These findings were in line with those of Mishra et al. [34], who examined whether the cost of feed was significantly lower for broilers given Azolla meal than for controls.

Because growth performance, which includes the FI, FCR, BWG, and BW, has a direct impact on all of these economic parameters and because it was higher in all economic efficiency parameters for all groups fed DA-treated diets than the control diet, it is possible that the results of TR, NR, GM, and RGM are related to this. Additionally, DA-treated diets, particularly D4, had superior feed cost values than the control group, thus affecting the calculation of the economic evaluation criteria. These findings are in line with those of Chichilichi [14], who showed that broilers given Azolla at various levels had the highest profit margins when compared to controls.

In this situation, a significant improvement in the expression profile of growth (*IGF-I*, *GH*, *MYLK4*, *MEF2B*, *TRPV1*, *STAT5A*, *PGAM2*), immune (*IL-4*, *IL-6*, *IL-8*, *IL-1 $\beta$* , and *AVBD9*), antioxidant (*SOD*, *CAT*, *GPX*, *PRDX2*, and *PRDX6*) and intestinal health (*MUC2*, *gastrotropin*, *Cath-B*) genes was noticed a result of dietary treatments with different levels of

Azolla. An enhanced gene expression profile of investigated makers was observed in broiler chicks fed diet containing 12% dried Azolla (DA) than other groups.

The evident alteration in the expression profile of genes associated to growth, immunity, antioxidant defence, and intestinal health may be brought on by the high protein content of Azolla, notably the important amino acids. The performance of the growth was also positively impacted by a significant amount of vitamins and minerals, such as iron, calcium, potassium, magnesium, phosphorus, manganese, and magnesium [13]. Carotenoids and biopolymers, which are also present in azolla and are naturally occurring immunomodulators and antioxidants, are also present. These components play a substantial role in the improvement of animal health and productivity [35].

The alteration in the mRNA level of the investigated markers can be linked to the decrease in pro-inflammatory cytokines, the decrease in nitric oxide, and regarding the enhanced activity of glutathione peroxidase and glutathione S-transferase compared to rats treated with isoproterenol without prior Azolla treatment [36]. Azolla filiculoides enhanced oxidation status by cumulative GSH, GST, and GPx activity in a rat model of an induced ulcer [36]. The increase in intestinal villi length in the jejunum, the rise in the amount of neutral mucin in the duodenum, and the rise in neutral mucin in the jejunum brought on by the 10% Azolla supplementation, according to Abdelatty et al. [37].

## **Conclusion**

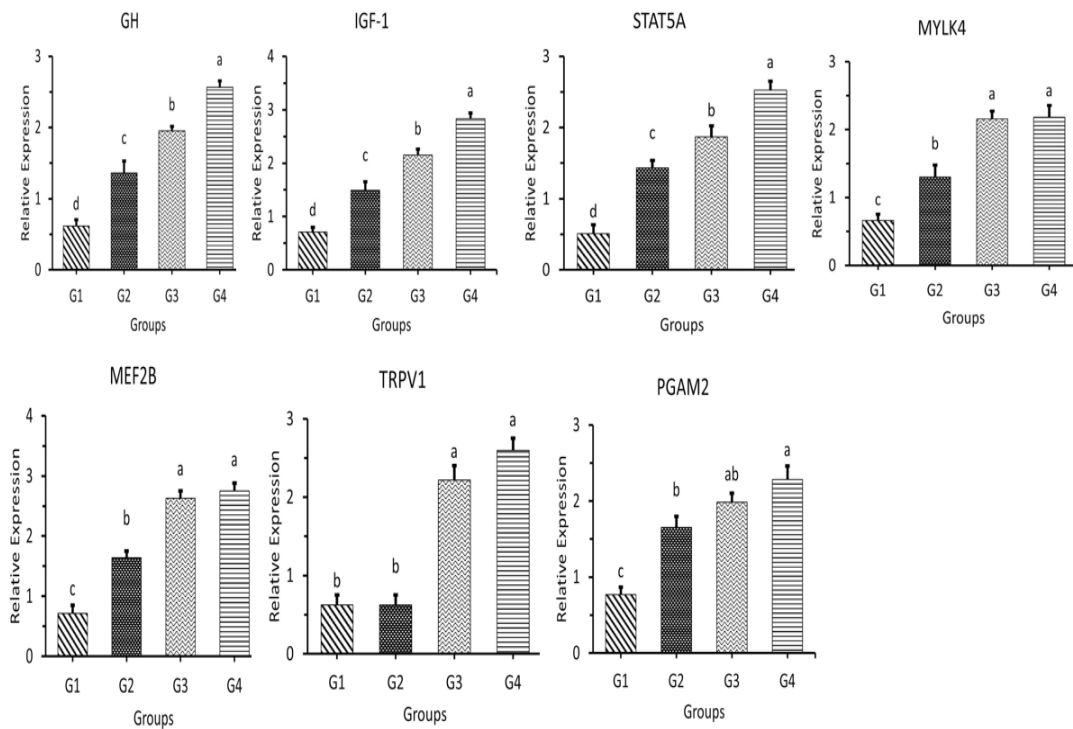
Feeding Azolla could improve growth performance and mRNA levels of growth, immune, antioxidant and intestinal health markers. Azolla plays also an significant part in refining productive, economic competence of broiler, and declaring that the best diet from the economic point of view is D4 (12% DA).

## **Acknowledgements**

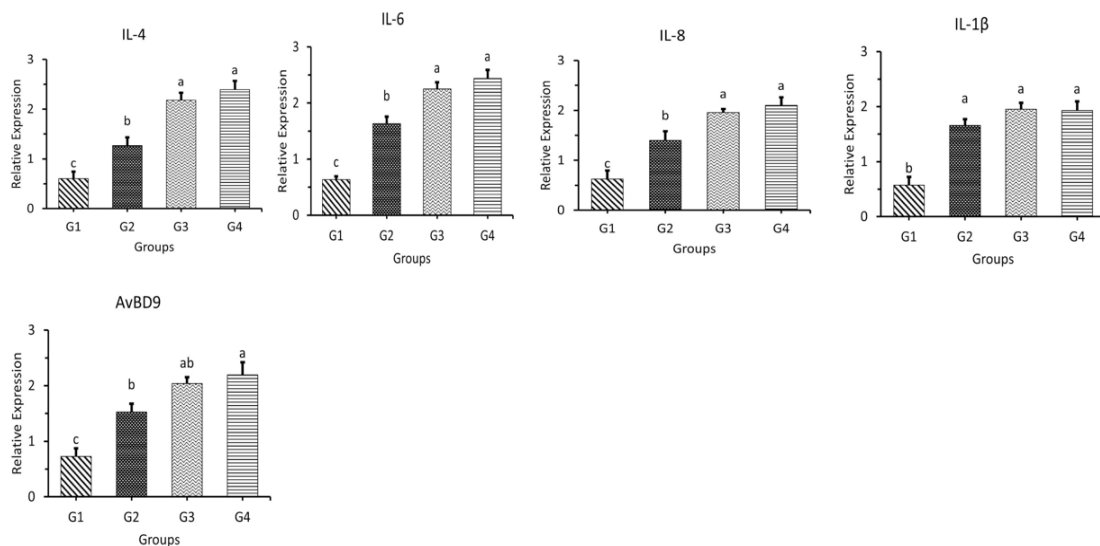
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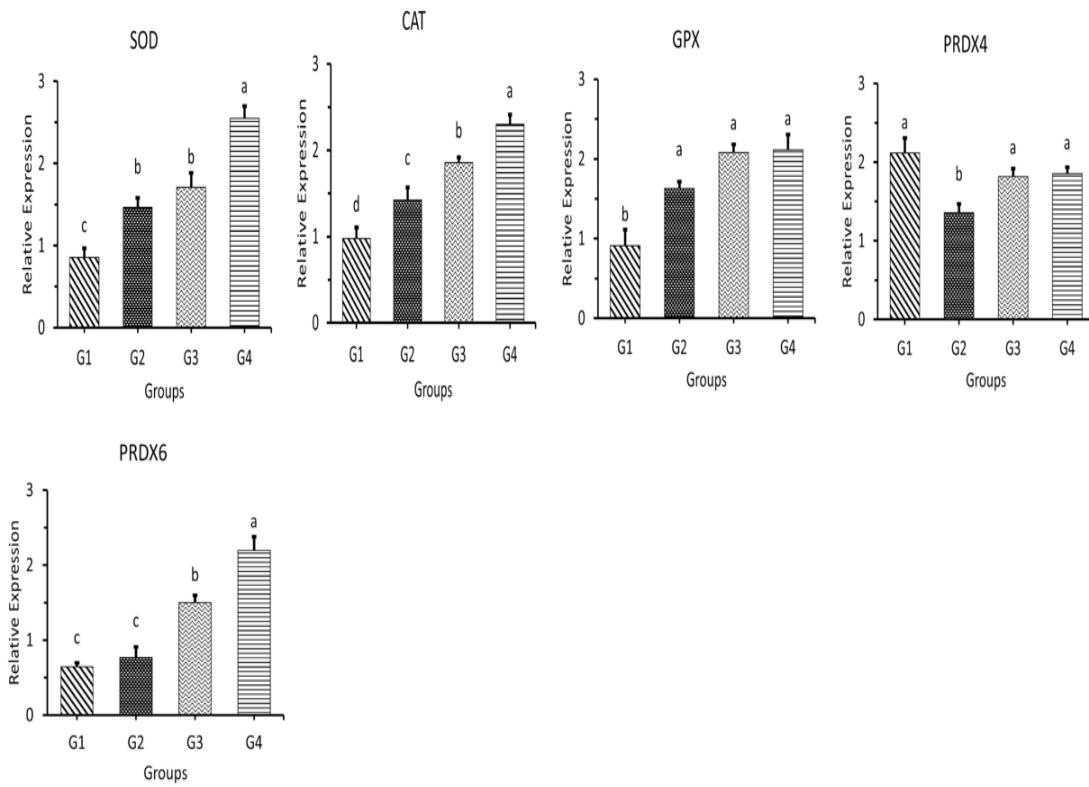


**Fig. 1.** mRNA level of growth related genes in broiler chicks fed different diets containing 0% (G1), 4% (G2), 8% (G3), and 12% (G4) dried Azolla (DA).

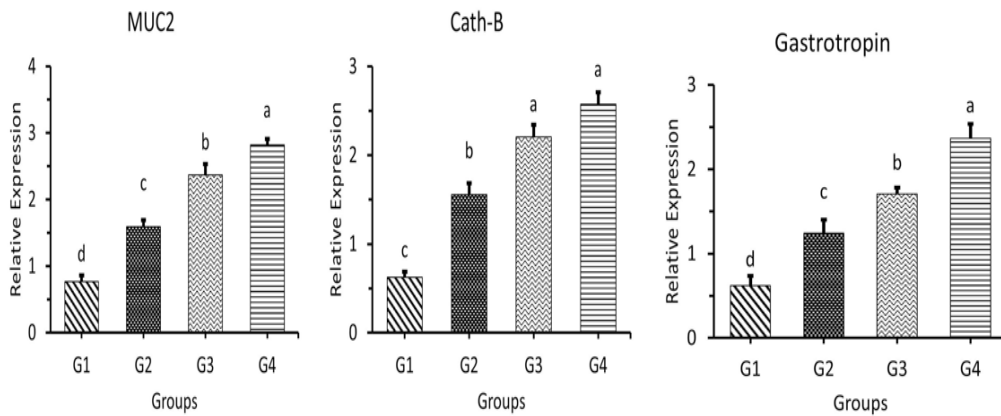


**Fig. 2.** mRNA level of immune genes in broiler chicks fed different diets containing 0% (G1), 4% (G2), 8% (G3), and 12% (G4) dried Azolla (DA).





**Fig. 3.** mRNA level of antioxidant genes in broiler chicks fed different diets containing 0% (G1), 4% (G2), 8% (G3), and 12% (G4) dried Azolla (DA).



**Fig.4.** mRNA level of intestinal health genes in broiler chicks fed different diets containing 0% (G1), 4% (G2), 8% (G3), and 12% (G4) dried Azolla (DA).

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## التقييم الاقتصادي لاستخدام الأزولا في أداء نمو دجاج التسمين: تأثير التعبير الجيني

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هدفت هذه الدراسة إلى استكشاف تأثير تغذية الأزولا على أداء النمو والتعبير الجيني وتأثيره الاقتصادي على دجاج التسمين. تم تقسيم 120 كنبوت عمرها يوم واحد (من سلالة كوب 500) إجمالاً إلى أربع مجموعات عشوائياً لمدة 42 يوماً، تم تغذية دجاج اللحم بالعديد من العلائق التي تحتوي على 0%، 4%، 8%، و 12% أزولا مجفف. مجموعة الدجاج التي تم تغذيتها بـ 12% أزولا مجفف كان لها أعلى متوسط معنوي لوزن الجسم النهائي وزيادة وزن الجسم. أدت العلاجات الغذائية من Azolla بجرعات مختلفة إلى تحسن ملحوظ في ملف التعبير الجيني لدلائل النمو (IGF-I، GH، MYLK4، MEF2B، TRPV1، STAT5A، PGAM2)، والمناعة (IL-1β، IL-8، IL-6، IL-4، AVBD9) ومضادات الأكسدة (SOD و CAT و GPX و PRDX2 و PRDX6) وجينات صحة الأمعاء (MUC2 و gastrotropin و Cath-B). تم العثور على أعلى عائد صافي لكل كجم عائد اقتصادي، وقيم كفاءة اقتصادية للمجموعة التي تغذى 12% أزولا. عند الاستنتاج، يمكن أن تعزز تغذية الأزولا المجفف حتى 12% من أداء النمو، وخصائص مضادات الأكسدة دون التأثير سلباً على الحالة الصحية لكتاكيت التسمين، وتزيد من ربحية دجاج التسمين.

**الكلمات الدالة:** التقييم الاقتصادي، الأزولا، أداء النمو، البداري، التعبير الدينيوص والبوري، العوامل البكتيرية، محافظة الاسماعيلية.