



Effect of inclusion of Azolla on performance, haematobiochemical parameters and economics in Japanese quail broilers

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Received: 4 January 2024; Accepted: 31 July 2024

ABSTRACT

An experiment was conducted to study the effect of dietary supplementation of Azolla on the performance of Japanese quail broilers. Day old Japanese quails (135) were divided into three treatment groups with three replicates per treatment and 15 quails in each replicate. The feeding of birds was done in two phases from 1-3 weeks and 4-5 weeks. Azolla was included at 0%, 5% and 10% levels in T₀ (control), T₁ and T₂ groups, respectively. Azolla inclusion did not show any significant effect on the feed intake up to 5 weeks of age. Body weight gain and PCV%, total protein and albumin were significantly higher at 5% level whereas no significant difference was observed at 10% level of Azolla inclusion. Azolla at both 5% and 10% level significantly reduced serum cholesterol concentration and the highest net profit was in 10% Azolla supplemented group. So, Azolla can be included effectively at 10% level in diet of Japanese quail broiler.

Keywords: Azolla, Biochemical, Growth, Haematology, Japanese quail broiler

The growing demand for food among humans because of the expanding population cannot be completely fulfilled by plant-based foods. The livestock and poultry products may play a significant role in filling the deficit of food supply. In the above perspective, the poultry industry plays a substantial role in supplying affordable animal protein sources and ensuring food security. Products made from poultry hold the promise of offering higher-quality protein at lower prices. Due to their small size, low feed consumption, and short marketing age, poultry broilers have attracted the interest of farmers and entrepreneurs. The increase in poultry population by 16.8% in the 20th livestock census (851.81 million) of the country over the previous census, demonstrates the growing demand for poultry production. The availability of poultry meat is only 2.8 kg per person against the recommended need of 11 kg (ICMR 2019). The expensive feed ingredients used in poultry rearing are the biggest limiting factors for profit determination as they make up to 60–70% of the entire cost of production. Increased population of both human and livestock has provoked a competition for food and feed which has fairly contributed to their increased cost. The gap between demand and supply of feed and the rising price of ingredients have prompted a quest for unconventional feed

ingredients, and inclusion of these ingredients in poultry diet could hopefully reduce feed cost and subsequently increase the profit. Azolla, the aquatic fern is well-recognized as an alternative feed resource for use in animal feed. It has been tested to be rich in protein, essential amino acids, vitamins and minerals. It easily grows in the wild and can grow under controlled conditions also and biomass doubles in about 6 days (Arora and Singh 2003). Its suitability for inclusion in the diet of broiler chicks (Rana *et al.* 2017 and Samad *et al.* 2020) has been tested. Quail rearing is gaining interest among farmers due to their smaller size, lower feed intake, easy rearing and most relevantly the increased consumer demand due to high-quality meat protein (Sathiya *et al.* 2019). Scarce research (Rathod *et al.* 2013) has been conducted for inclusion of Azolla in the diet of Japanese quail broiler. Therefore, the present study was undertaken to see the effect of inclusion of azolla in the diet on the growth and haematobiochemical parameters of Japanese quail broilers.

MATERIALS AND METHODS

A feeding experiment of 5 weeks duration was conducted in 135 day-old Japanese quail chicks. There were three treatment groups and each treatment group had three replicates with 15 birds in each. For all treatment groups, feeding was done in two phases, i.e. growth phase I (1-3 weeks) and growth phase II (4-5 weeks), based on ICAR (2013) standard for the nutrient requirement of poultry. The energy and protein content of diet was 2900 Kcal ME/kg and 2950 Kcal ME/kg and 25% and

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Table 1. Ingredient composition of quail feed

Ingredient	0-3 weeks			4-5 weeks		
	T ₀	T ₁	T ₂	T ₀	T ₁	T ₂
Maize	48	44.5	42	59.65	56.09	52.41
Soybean meal	46	43.9	41.5	36.42	34.3	32.2
Oil	2.5	3.1	3.3	1.14	1.82	2.6
Azolla	0	5	10	0	5	10
Minerals, Vitamins, Toxin binder and salt	1.25	1.25	1.20	0.53	0.53	0.53

21.5% CP up to 0-3 weeks and 4-5 weeks old birds, respectively. Three different types of diets were formulated for each growth phase (Table 1). Diet 1 served as the control (T₀) without Azolla, while diets 2 and 3 contained 5% (T₁) and 10% (T₂) Azolla in place of other ingredients on DM basis.

The weekly body weight gain was calculated by subtracting the initial weight of every week from the final weight at the end of the respective week. A measured quantity of feed was offered daily, and the quantity of left-over feed was recorded at the end of the week. Weekly feed consumption was calculated in each treatment group by subtracting the left-over feed from the total feed offered during the week. The input cost was calculated by taking into consideration the cost of chick, the cost of feed consumed by birds during the experiment, supplementation cost and other miscellaneous expenditures during the experiment. The selling price of the birds was supposed to be according to the minimum local market value of the bird based on the unit of bird and the net profit was calculated.

Data was evaluated using a one-way Analysis of Variance (ANOVA) with a Completely Randomized Design (CRD) in accordance with Snedecor and Cochran (1994). Duncan's Multiple Range Test (Duncan 1955) was used to evaluate the significance of difference and interaction (DMRT).

RESULTS AND DISCUSSION

Body weight gain and feed intake: The average body weight of day-old chicks was similar between the groups at the beginning of the experiment. The body weight at 7th day was non-significantly different among groups and ranged between 20.89 g to 24.42 g (Table 2). There was no significant difference ($p>0.05$) in feed intake (28.45, 30.95 g) among different treatment groups in 1st week. The body weight in the control group (T₀) was similar to that of the Azolla supplemented groups up to

2nd week. But, in 2nd week, feed intake was significantly ($p<0.05$) higher in T₀ (81.21 g) compared to T₁ (70.17 g) and T₂ (66.25 g) group, possibly due to fast adaptability of the basal diet compared to Azolla supplemented diet. The better intake in control group during 2nd week was reflected in significantly better body weight of birds in control group (101.79 g) then the Azolla supplemented groups (98.41 g and 90.64 g for 5% and 10%, respectively) during the third week. During 3rd week, there was no difference in feed intake (114.42-122.22 g) among the groups that indicated similar acceptance of Azolla supplemented and basal diets.

In the second phase of growth during the 4th week, 5% Azolla supplemented group performed better ($p<0.05$) compared to control or 10% Azolla supplemented group, though there was no variation in the diet intake (164.50-175.12 g) between the groups, possibly due to better weight response to Azolla supplementation at 5% level (Table 3). Several previous observations indicated a depression in nutrient utilization when Azolla was included more than 5% in broiler ration (Basak *et al.* 2002, Parthasarathy *et al.* 2002). At the end of 5th week also, 5% Azolla supplemented group performed better ($p<0.05$) whereas the feed intake was lower in T₂ (182.11 g) compared to T₀ (195.63 g) and T₁ (195.51 g). Alike current findings, better body weight gain in Vencobb broiler birds fed on 5% Azolla based diet compared to birds fed on unsupplemented, 10% and 15% Azolla supplemented diets replacing sesame meal was observed, despite no significant difference in feed intake (Basak *et al.* 2002). Parvez *et al.* (2018) when fed Azolla from 14 days age observed better live weight in quails at 42 days on Azolla supplementation and the highest live weight (128.20 g) was in 5% Azolla group compared to 3% (123.20) and 7% (119.00) groups. Kumar *et al.* (2018a) observed better body weight gain in 5% (2173.10 g) Azolla supplemented group compared to 10% (2132.67 g) and highest weight gain (2182.07 g) was observed at level

Table 2. Effect of different dietary inclusion levels of Azolla on body weight of growing quail (0-5 weeks)

Age (days)	T ₀	T ₁	T ₂	p-value
0 day	7.62±0.33	7.97±0.25	7.44±0.18	0.421
7 th day	24.42±1.00	23.36±0.47	20.89±1.83	0.196
14 th day	60.85±0.56	62.42±2.05	59.44±1.50	0.425
21 st day	101.79 ^a ±3.48	98.41 ^{ab} ±0.28	90.64 ^b ±2.55	0.048
28 th day	135.17 ^b ±3.47	143.48 ^a ±0.82	128.6 ^b ±1.0	0.008
35 th day	170.05 ^b ±3.26	182.85 ^a ±3.70	163.35 ^b ±0.52	0.005

*, Means in the same row with different superscripts a, b, c are significantly different ($p<0.05$).

Table 3. Effect of dietary inclusion of varying levels of Azolla on feed intake (g) in growing quails (0-35 d)

Age (Week)	T ₀	T ₁	T ₂	p-value
1 st	30.95±2.10	28.45±1.30	25.58±1.12	0.130
2 nd	81.21 ^a ±2.02	70.17 ^b ±3.32	66.25 ^b ±3.27	0.027
3 rd	120.91±2.03	122.22±4.98	114.42±2.39	0.295
4 th	164.50±1.57	164.58±6.86	175.12±7.27	0.394
5 th	195.63 ^a ±3.39	195.51 ^a ±4.49	182 ^b .11±0.33	0.041
Total	593.22±3.40	580.94±10.53	579.23±9.88	0.122

*, Means in the same row with different superscripts a, b, c are significantly different (p<0.05).

of 7.5%.

But Samad *et al.* (2020) found no significant difference (p>0.05) in feed consumption in broiler chickens with Azolla supplementation up to 15% level. Parthasarathy *et al.* (2002) observed no significant difference in feed consumption of broiler birds fed 0, 5, 10, 15 and 20% of Azolla in their diets. Alalade *et al.* (2007) also observed non-significant difference in weekly feed intake among control and Azolla fed (10% and 15% level) broilers. In contrast, Alalade and Iyayi (2006) observed a decrease in average weekly feed intake (p<0.05) with Azolla inclusion (5%, 10% and 15%) in diet of broiler.

Haematological profile: Estimation of blood parameters aids in assessing the health status of birds. The changes in the haematological parameters may be inferred for some nutritional, environmental, or pathological stress. Hb and PCV values are indicators of anaemic status (Turkson and Ganyo 2015). The Hb concentration was 13.00, 14.39 and 14.45 g/dl in group T₀, T₁ and T₂, respectively (Table 4). The variation between the groups was statistically non-significant. The Hb values in present study fall in the normal range reported by Boda *et al.* (1996) and Agina *et al.* (2017). They reported Hb % varying from 11.76-13.65% and 8.61-20.57%, respectively. Parvez *et al.* (2018) observed no significant (p>0.05) effect of Azolla inclusion up to 7% level on the Hb concentration in Japanese quail, however, reported lower value (8.03-8.10%) compared to current study. Mishra *et al.* (2016a) reported no effect of Azolla supplementation at 5 and 10% level on Hb% in broiler birds. Kumar *et al.* (2018b) and Alalade *et al.* (2007) reported no effect of Azolla on Hb level in Nera brown pullets up to 10% level of inclusion. But, Kamel and Hamed (2021) reported better Hb concentration in Azolla supplemented birds (4, 8 and 12%) than the basal diet fed group. The PCV values were 32.17%, 36.15% and

Table 4. Effect of different dietary inclusion levels of Azolla on haematological parameters in growing quails (0-35 d)

Parameter	T ₀ (control)	T ₁ (5%)	T ₂ (10%)	p-value
Hb (g/dl)	13.00 ±0.53	14.39±0.30	14.45±0.28	0.063
PCV%	32.17 ^b ±0.58	36.15 ^a ±0.48	33.35 ^b ±0.25	0.002

*, Means in the same row with different superscripts a, b, c are significantly different (p<0.05).

33.35% for 0, 5% and 10% Azolla supplemented birds. The effect of dietary supplementation of dried Azolla on PCV (%) was significant (p<0.05) at 5% level. Absorption of minerals like iron, copper, Zn, Co and few heavy metals by Azolla have been reported (Padmavathiamma and Li 2007). The improved mineral concentration could possibly be responsible for increased PCV in 5% Azolla group. The present finding is supported by reports of improved PCV in broiler birds on Cu and Fe supplementation (Habib *et al.* 2020). No further improvement in PCV beyond 5% level of Azolla could possibly be due to excess of mineral like Cu. The finding could be well corroborated with the result of decrease in PCV with increased Cu supplementation (Samanta *et al.* 2011).

Unlike present findings, Alalade *et al.* (2007), Parvez *et al.* (2018) and Kamel and Hamed (2021) reported no significant effect (P>0.05) of Azolla supplementation on PCV% of Nera brown pullets, quail broiler and Cobb-500 broiler, respectively. Alalade *et al.* (2007), Parvez *et al.* (2018), Kamel and Hamed (2021) reported PCV% in Nera brown pullets, quail broiler and Cobb-500 broiler ranging from 26.328.3%, 35.03-35.33% and 26.6-29.65%, respectively when Azolla was included up 15%, 7% and 12% level, respectively in their diet.

Serum biochemical profile: Biochemical parameters indicate the health of organs, tissues, and metabolic status of the animal. Liver is involved in synthesis of metabolites

Table 5. Effect of different dietary inclusion levels of Azolla on serum biochemical parameters in growing quails (0-35 d)

AST (IU/L)	154.17±1.01	180.98±11.85	157.07±1.71	0.064
ALT (IU/L)	13.16±1.42	17.81±0.41	13.54±1.48	0.062
Total Protein (g/dl)	3.62 ^b ±0.27	4.49 ^a ±0.25	3.64 ^b ±0.05	0.047
Albumin (g/dl)	1.19 ^b ±0.05	1.62 ^a ±0.08	1.19 ^b ±0.05	0.004
Globulin (g/dl)	2.44±0.25	2.86±0.18	2.45±0.10	0.25
Cholesterol (mg/dl)	174.60 ^a ±1.64	157.97 ^b ±2.16	152.68 ^b ±2.65	0.001
Uric acid	11.32 ^a ±0.37	8.30 ^b ±0.12	10.86 ^a ±0.47	0.002

*, Means in the same row with different superscripts a, b, c are significantly different (p<0.05).

and excretion of metabolic end products. Any impairment in its function or hepatocellular damage due to any metabolite in unconventional feed could be inferred from decrease in albumin concentration and increase in activities of ALT and AST (Soyinka *et al.* 2007). Non-significant ($P>0.05$) effect of dietary levels of Azolla on the activity of aspartate aminotransferase enzyme (IU/L) was observed in present study (Table 5). The value was 154.07 IU/L in control, 180.98 IU/L in 5% Azolla supplemented and 157.07 IU/L in 10% Azolla supplemented group. No significant effect of different dietary levels of dried Azolla on the activity of alanine aminotransferase was observed in the present study. The ALT value (IU/L) ranged between 13.16 (T_0) to 17.81 (T_1) in the quails. Agina *et al.* (2017) reported AST value of adult Japanese quail in the range of 30.70 to 90.70 IU/L in male and 23.85 to 85.00 IU/L in female. They reported average ALT (IU/L) value of 23.20 ± 1.34 IU/L in male and 18.99 ± 1.13 in female Japanese quail. The present findings are in corroboration with the reports of Sherif *et al.* (2022) who observed no significant shift in serum AST and ALT activity due to Azolla supplementation up to 16% level in the diet of Japanese quail broilers. The AST and ALT values due to Azolla supplementation ranged from 99.17-101.00 IU/L and 17.43-19.10 IU/L, respectively. Kumar (2016) also reported no significant difference in levels of AST and ALT due to varying levels (0, 2.5, 5, 7.5 and 10%) of Azolla in the diet of broiler chicken. Likewise, Shukla *et al.* (2018) observed no-significant difference in concentration of AST and ALT in 5% and chose Azolla supplemented group compared to control group of turkey. Mishra *et al.* (2016b) reported that liver enzymes of Chabro bird was not affected by any treatment except the higher AST in 10% replacement group than control, but the value was within normal range. Significant ($p<0.05$) effect of Azolla inclusion was observed on total protein and albumin, the higher value being recorded in 5% supplemented group (T_1). The serum total protein content (g/dl) and albumin content (g/dl) were 3.62, 4.49 and 3.64 g/dl and 1.19, 1.62 and 1.19 in group T_0 , T_1 and T_2 , respectively. The high protein content in the blood is reflected in protein deposition in the meat (Adriani *et al.* 2021). In present study, higher body weight was reported in 5% Azolla supplemented group in response to higher plasm levels of protein. There was no significant effect of inclusion of Azolla on the serum globulin concentration (2.44-2.86 g/dl). Sherif *et al.* (2022) observed significant effect of Azolla on the serum total protein, albumin and globulin concentration when supplemented at graded levels (0, 4, 8, 12 and 16%) in the diet of Japanese quail broilers. There was an increase ($p<0.05$) in concentration of total protein from 3.09 to 3.27 g/dl, albumin from 2.29 to 2.39 g/dl and globulin from 0.80 to 0.89 g/dl with increase in concentration of Azolla from 0 to 16% in the quail diet. Kamel and Hamed (2021) reported significant improvement in total protein and albumin concentration beyond 4%, i.e at 8% level of Azolla inclusion whereas at 4% level of inclusion no significant effect was observed in

broiler chickens. At 12% level of inclusion total protein was reduced to become comparable with control group whereas albumin was reduced below the level of control group; they also reported no effect of Azolla supplementation in diet (0, 4, 8 and 12%) on serum globulin concentration (9.19-13.45 g/dl) in broilers. No effect ($p>0.05$) of Azolla supplementation (0, 5, 7.5 and 10% level) was observed on the serum total protein (3.40-3.68 g/dl) and albumin (1.67-1.73 g/dl) concentration in the serum of 8 weeks old broiler chicken (Mishra *et al.* 2016b).

Higher serum cholesterol level is an indication of higher fat, lower protein level in the diet of birds (Masouleh *et al.* 2021). Significant ($p<0.001$) decrease in serum cholesterol was observed in Azolla supplemented groups. The values were 174.60, 157.97 and 152.68 mg/dl in group T_0 , T_1 and T_2 , respectively. Rich presence of secondary metabolites along with unsaturated fatty acids, those are implicated with serum cholesterol lowering effects have been reported in Azolla (Sreenath *et al.* 2016). Also, cholesterol-lowering properties of phytochemicals by virtue of their effects on cholesterol metabolism have been established (Leng *et al.* 2018). Decrease in cholesterol with increase in Azolla levels (8%, 12% and 16%) was observed in Japanese quail broiler and the values ranged from 161.06 to 182.30 mg/dl (Sherif *et al.* 2022). Similar findings have been reported by Kamel and Hamed (2021) in broiler chickens. Non-significant effect of Azolla on the serum cholesterol concentration was observed at 5% level and up to 10% level in the diet in turkey (Shukla *et al.* 2018) and broiler chicken (Kumar 2016).

Azolla at 5% level in diet significantly reduced serum uric acid concentration whereas at 10% level uric acid concentration resembled to that of control group. Mishra *et al.* (2016b) reported no significant effect of Azolla supplementation (0, 5, 7.5 and 10% level) on the concentration of serum uric acid of 8 weeks old broiler chicken. But Shukla *et al.* (2018) reported a decrease ($p<0.01$) in serum uric acid concentration at 5% level of Azolla in the diet of turkey. Kamel and Hamed (2021) reported effectiveness of Azolla in reducing uric acid concentration when supplemented in the diet (4%, 8% and 12% level) of broiler chicken.

Cost economy: The birds sale price was kept at a minimum local market value per unit of bird, the Table 6. Effect of different dietary inclusion levels of Azolla on cost economy in growing quails (0-35 d)

Particular	T_0	T_1	T_2
Chick cost (₹/bird)	10	10	10
Feed intake (g/bird)	593.23	580.94	563.49
Feed cost (₹/bird)	19.45	18.48	17.35
Miscellaneous cost (₹/bird)	2	2	2
Net cost of production (₹/bird)	31.45	30.48	29.35
Body weight at 5 th week (g)	170.05	182.85	163.35
Sale price/bird as per market price during the experiment	40	40	40
Net profit (₹/bird)	8.55	9.52	10.65

net profit from sale of a bird in control, 5% and 10% Azolla supplemented group was ₹8.55, 9.52 and 10.65, respectively (Table 6). There was an increase in 11.34 and 24.56% of net profit from sale per bird from T_1 and T_2 group compared to T_0 group. Shamna *et al.* (2013), observed improved net profit from Japanese quail broiler at 5% level of Azolla. Ara *et al.* (2015), Islam and Nishibori (2017) reported more benefit in Azolla supplemented (5 and 7%) broiler birds with the highest benefit in 5% Azolla fed group. Mishra *et al.* (2016a) reported decrease in feed cost per kg weight gain in Chabro chicks with inclusion of Azolla up to 10% levels. Hassen *et al.* (2019) when substituted soybean meal with Azolla meal in the diet of Cobb-500 broiler birds observed better net profit in 5% Azolla-supplemented chickens whereas 2.5% and 7.5% Azolla-fed group showed a decrease in profit compared to control. Kamel and Hamed (2021) and Borkar *et al.* (2021) observed a direct relationship of Azolla supplementation up to 12% and 7.5% levels with net profit in broiler chicken and Kadaknath birds, respectively.

It can be concluded from the study that Azolla inclusion at 10% level could be recommended safely to maintain the body weight of birds without altering feed intake, haematological and plasma biochemical parameters. It also lowered the cholesterol levels and there was improvement in net profit.

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