



Original Research

Effect of Supplementation of Azolla on the Hematology, Immunity and Gastrointestinal Profile of Broilers

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Abstract

A feeding trial was carried out to determine the effect of Azolla supplementation on haematology, immunity, gastrointestinal profile and the economics of broiler chicks. Five dietary treatment groups designated as C, T₁, T₂, T₃ and T₄ were formulated by incorporating 0.0, 2.5, 5.0, 7.5 and 10.0% levels of dried Azolla. All the rations formulated for various treatments were made iso-caloric and iso-nitrogenous. The result indicated that level of inclusion of Azolla in the diet had no effect on means of percent haemoglobin, PCV, TEC, TLC, DLC, H/L ratio, weight of lymphoid organs for various treatment groups. Azolla in diet had a significant effect on pH of the crop, highly significant ($P < 0.01$) effect on pH of the proventriculus, caecum and liver and no significant effect on pH of gizzard, duodenum, jejunum and ileum. No effect was observed on relative length of different segments of the small intestine and caecum. Highly significant effect was observed on body weight gain and feed consumption. The haematology, immunity, gastrointestinal profile of broiler chicks suggested that feeding of Azolla did not adversely affect the immune status of broilers and can be included in the ration for improving performance and reduce cost of production.

Key words: Azolla, Economics, Gastrointestinal, Haematology, Immunity

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Introduction

In latest era, poultry production has been a steep rise throughout the world. In India, the poultry industry has transformed into one of the most dynamic and self-sustaining sector of livestock production. This increase has resulted in competition with the conventional human food ingredients leading to shortage and increased the cost of conventional feed ingredients. Hence, several attempts are being constantly made to seek the alternative feed resources for the economic poultry production. Azolla can be used as



unconventional high potential feed source in monetary poultry production. Azolla is a free floating freshwater fern belonging to the family *Azollaceae* and order *Pteridophyta*. Azolla grows symbiotically with blue-green algae, *Anabaena azollae*. The higher crude protein content (above 20 %) and presence of essential amino acids (high lysine content), vitamin A precursor beta-carotene and B₁₂ and minerals like iron calcium, phosphorous, potassium and magnesium made Azolla useful feed supplement for livestock, poultry and fish. It furthermore contains probiotics and biopolymers Pillai *et al.* (2002). Azolla seems to be a potential source of nutrients. Therefore, the present study was carried out to study the effect of inclusion of sun dried Azolla supplementation on haematology parameter, immunity and gastrointestinal profile of broiler chicks.

Materials and Methods

One hundred and fifty day-old, unsexed, apparently healthy broiler chicks were individually weighed and randomly divided into five groups of 30 chicks each having almost similar average body weight. Each group of 30 chicks was further subdivided into three replicates having 10 chicks each. Identical to standard management practices were followed for each group. Five dietary treatment groups designated as C, T₁, T₂, T₃ and T₄ were formulated by incorporating 0.0, 2.5, 5.0, 7.5 and 10.0% levels of dried Azolla, respectively. As Azolla contain a higher content of protein; parts of soybean meal and maize were replaced with graded level of Azolla incorporation in the diet. Proximate composition of feed ingredients is presented in Table 1.

Table 1: Proximate composition of feed ingredients (% DM basis)

Ingredients	DM	CP	CF	EE	NFE	TA
Maize	91	10	3.17	2.78	79.38	4.67
Soya meal	90.4	43.8	10.5	1	39.84	4.86
Azolla	91.78	22.25	11.19	2.45	38.61	25.5
Premix	95.15	40.12	5	5.74	12.71	36.43

*Premix contained (g/100g): Lysine-2.85g, DL-Methionine-2.12g, Cystine-0.65g, Calcium-9.20g, Phosphorus-4g, Chloride-2.30g, Sodium-1.30g.

The crude protein content of starter and finisher ration was 22 percent and 19 percent, respectively. All the rations formulated for various treatments were made iso-caloric and iso-nitrogenous. The nutrient compositions of experimental starter and finisher rations have been presented in Table 2. The chicks were weighed individually at the start of the experiment and subsequently at weekly intervals for six weeks. The live weight gain was calculated from the difference in body weight attained at the end and the start of the concerned period. Feed offered and leftover were measured for each replication. Blood samples were collected from six birds of each treatment on the 42nd day from wing vein with all aseptic precaution. Haematological parameters were investigated at the end of experiment to judge the physiological status of broiler chicks.

Table 2: Ingredient composition of experimental ration (kg/100kg feed)

Ingredients	C	T ₁	T ₂	T ₃	T ₄
Starter Ration (0-3 weeks)					
Maize	63	61.4	59.8	58.18	56.59
Soyabean meal	27	26.1	25.2	24.32	23.41
Azolla	-	2.5	5	7.5	10
Premix*	10	10	10	10	10
Soya Oil	-	-	-	-	-
Finisher Ration (4-6 week)					
Maize	70.03	68.23	66.4	64.74	63
Soya bean meal	18.94	18.24	17.57	16.84	16.13
Azolla	-	2.5	5	7.5	10
Premix*	10	10	10	10	10
Soya Oil	1.03	1.03	1.03	0.92	0.86

*Premix contained (g/100g): Lysine-2.85g, DL-Methionine-2.12g, Cystine-0.65g, Calcium-9.20g, Phosphorus-4g, Chloride-2.30g, Sodium-1.30g.

To study the effect of different treatments on gastrointestinal organ length and pH, lymphoid organ, two representative birds having live weight similar to the mean live weight of the population concerned from each replicate were sacrificed. The pH measurements were performed by inserting the probe of the digital pH meter into the distal sections of the crop, proventriculus, gizzard, duodenum, jejunum, ileum, caecum and liver. The data were analyzed using one way ANOVA (Snedecor and Cochran, 1994) and the significance of mean differences was tested by Duncan's New Multiple Range Test (Duncan, 1955). The comparative economics were compared using Standard formula used for calculating the cost of production/Kg live broiler (Narhari and Rajini, 2005).

Result

Haematology Parameter and Lymphoid Organ

The results indicate that level of inclusion of Azolla in the diet had no effect on means of percent Hb, PCV, TEC, TLC, lymphocyte, monocyte, heterophil, eosinophil, basophil and H/L ratio for various treatment groups (Table 3).

Table 3: Effect of feeding different levels of Azolla meal on haematology parameter

Parameter	C	T ₁	T ₂	T ₃	T ₄	SEM
Hb%	8	7.87	8	8	7.93	0.15
PCV%	24.6	23.93	24.73	25.23	24.2	0.83
TEC*10 ⁶ µl	1.7	1.93	1.93	1.74	1.87	0.05
TLC*10 ³ µl	37.92	41.74	32.56	37.48	44.96	2.2
Lymphocyte	69	67.67	69	68.67	70	0.43
Monocyte	6.33	10	6	6.67	8	0.53
Heterophil	19	17.33	20.33	19	16.67	0.59
Eosinophil	5	4.33	4	5	4.33	0.26
Basophil	0.67	0.67	0.67	0.67	1	0.12
H/L ratio	0.28	0.26	0.3	0.28	0.24	0.01



The values of haematology indices obtained in this study were within the normal ranges. In poultry the H: L ratio is a sensitive haematology indicator and biomarker of stress response and immune function. Mishra *et al.* (2016) reported similar H: L ratio in control and Azolla fed birds. The weight of the spleen, bursa, thymus and lymphoid organs or immune organs of all the treated groups did not differ significantly (Table 4).

Table 4: Effect of feeding different levels of Azolla meal on lymphoid organ (g/100g bw)

Parameter	C	T ₁	T ₂	T ₃	T ₄	SEM
Spleen	0.127	0.133	0.113	0.117	0.117	0.008
Bursa	0.057	0.047	0.043	0.05	0.047	0.002
Thymus	0.137	0.097	0.097	0.113	0.1	0.01
Immune organ	0.317	0.273	0.253	0.283	0.26	0.015

Chichilichi *et al.* (2015) also reported no significant difference on lymphoid organs or immune organs on supplementation of Azolla. Supplement of dietary protein from Azolla up to 10.0% level had no significant effect on the hematology indices, H:L ratio and weight of lymphoid organs indicating that the birds were adequately nourished, represented similar immune response and not showing any sign of disease, stress, infection or parasitic problems.

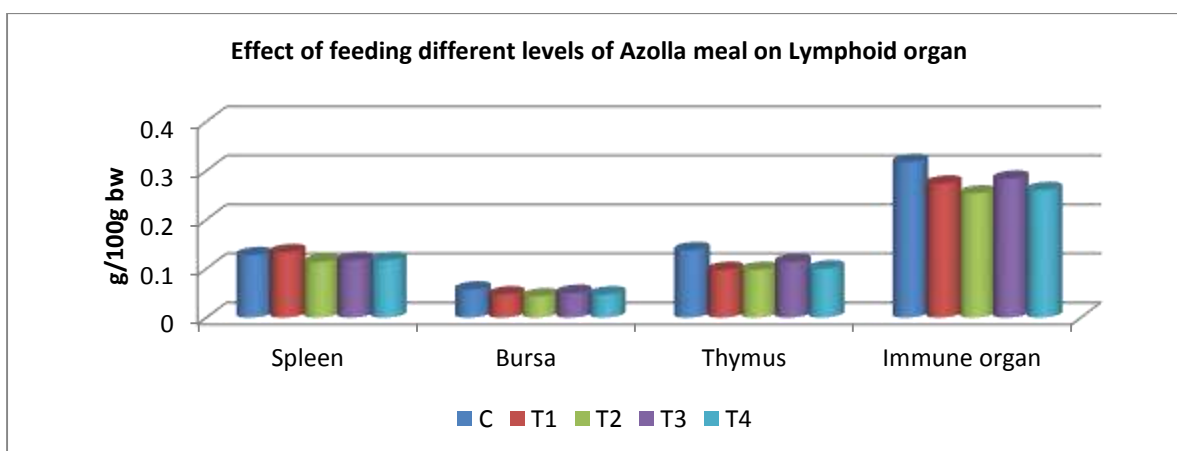


Fig.1: Effect of feeding different levels of Azolla meal on lymphoid Organ

Gastrointestinal Organ pH and Length

The pH of the gastrointestinal tract was presented in Table 5. The results indicate that level of inclusion of Azolla in diet had a significant effect on pH of crop and highly significant effect on pH of proventriculus. In crop the lowest pH value was recorded in T₄ which was comparable with T₁ group. Highest pH value was observed in T₂ group which was comparable with T₃ and control. Fonseca *et al.* (2010) observed significant ($P < 0.05$) effect of probiotic supplementation on crop pH at the ages of 1, 7 and 18 days and non-significant effect on 12, 23 and 28 days. In birds, pathogenic bacteria attain the digestive tract after

overcoming the barrier of the crop. It is important to have an environment with low pH to prevent the colonization by pathogens in the digestive tract.

Table 5: Effect of feeding different levels of Azolla meal on gastrointestinal organ pH

Parameter	C	T ₁	T ₂	T ₃	T ₄	SEM
Crop	4.27 ^{ab}	4.17 ^{bc}	4.47 ^a	4.27 ^{ab}	3.97 ^c	0.05*
Proventriculus	4.37 ^b	4.37 ^b	4.50 ^a	4.20 ^c	4.40 ^{ab}	0.03**
Gizzard	3.4	3.53	3.43	3.43	3.43	0.02
Duodenum	5.37	5.27	5.47	5.43	5.5	0.04
Jejunum	5.8	5.8	5.8	5.73	5.77	0.02
Ileum	7	6.97	7.03	6.93	6.87	0.03
Caecum	6.40 ^b	6.77 ^a	6.40 ^b	6.90 ^a	6.47 ^b	0.06**
Liver	6.03 ^a	6.03 ^a	5.83 ^b	5.67 ^c	5.93 ^{ab}	0.04**

^{abc} Means with different superscript in a row differ significantly (* $P < 0.05$, ** $P < 0.01$)

In proventriculus the lowest pH was recorded in T₃ and highest pH was observed in T₂, which was comparable with T₄ but higher than control and T₁. Nkukwana *et al.* (2015) studied the effects of *Moringa oleifera* leaf meal on GIT pH in broiler chickens, and reported no significant effect on pH of the proventriculus. Azolla in the diet had no-significant effect on pH of gizzard. Digesta pH drops gradually as digesta reach the proventriculus or glandular stomach, where hydrochloric acid and pepsinogen are secreted and mixed with digesta through muscular movements in the gizzard (Svihus, 2014). There was no significant effect of Azolla in the diet on the pH of duodenum, jejunum and ileum. Nonetheless, the ileum, the last segment of the small intestine, which ends at the ileo-ceco-colic junction, had slightly higher pH levels than the jejunum. The duodenal pH across treatments was lower than in the jejune and ileal digesta. Khalaji *et al.* (2011) studied the effects of dried *Artemisia sieberi* leaves and Nkukwana *et al.* (2015) studied the effects of and *Moringa oleifera* leaves on digesta pH in broiler chickens, and reported a significant increase in pH of jejune contents.

Azolla in diet had highly significant ($P < 0.01$) effect on caecum pH. The highest pH was observed in T₃ group which was comparable with T₁ group but higher than control, T₂ and T₃. The pH in control, T₂ and T₃ was comparable with each other. Nkukwana *et al.* (2015) reported significant effects on caecum pH on supplementation *Moringa oleifera* leaf meal in broiler. The functionality of the caeca is affected largely by diet, and the caeca enlarges because of an increased amount of fermentable material in the diet Svihus (2014), which may explain the rise in pH towards alkalinity. Gastric acidity can be detrimental to some of the microflora that inhabits the hindgut. Thus, maintaining caeca pH is important in promoting gut health (Ricke, 2003). Inclusion of Azolla in diet had highly significant ($P < 0.01$) effect on pH of the liver. The pH of liver in T₁, T₄ and control group was comparable with each other, but higher than T₂ and T₃. Lowest liver pH was observed in T₃ group.

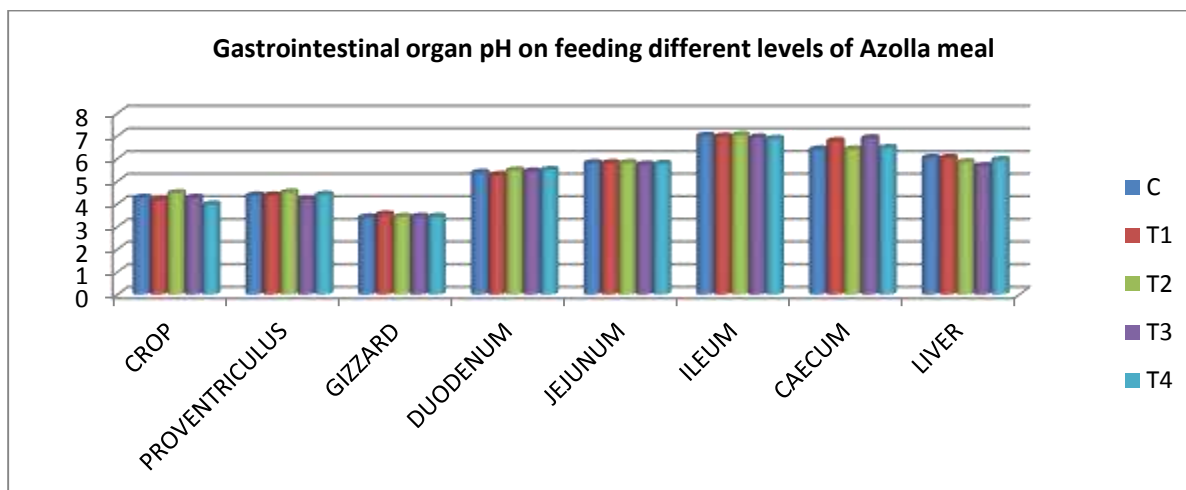


Fig.2: Gastrointestinal organ pH on feeding different levels of Azolla meal

No effects of treatments were detected on the relative length of different segments of the small intestine, duodenum, jejunum, ileum and caecum (Table 6). Similar finding is also reported by Sadeghi *et al.* (2016) on supplementation of *Ferulago angulate* (Schlecht.) Boiss powder in broiler chicks.

Table 6: Effect of feeding different levels of Azolla meal on gastrointestinal organ length (cm.)

Parameter	C	T ₁	T ₂	T ₃	T ₄	SEM
Small intestine	184.33	178	197	183.67	178.67	2.41
Duodenum	26.67	25.33	29.67	24.33	25	0.82
Jejunum	81.67	70.33	78	75	75	1.32
Ileum	59.67	66.67	72.67	68	62.67	1.69
Caecum	16.33	15.67	16.67	16.33	16	0.17

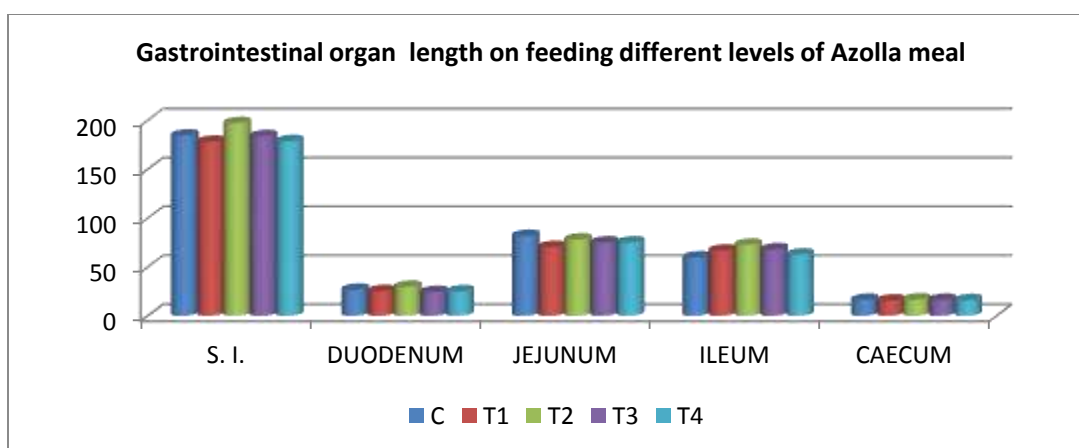


Fig.3: Gastrointestinal organ length on feeding different levels of Azolla meal

Overall Weight, Weight Gain, Feed Consumption and Economics

The weight gain and economics were presented in Table 7. There was a highly significant improvement in body weight gain due to supplementation of Azolla. The mean body weight gain in T₃, T₂ and T₄ were comparable but higher than in C and T₁. C had lowest body weight gain. Basak *et al.* (2002) observed highly significant improvement in live body weight of broiler chicks fed diet with 5 per cent Azolla meal. While diet containing AZM at higher levels (10%) resulted in significant reduction in body weight gain. Rathod *et al.* (2013) also observed significant ($P < 0.01$) improvement in live weight of quail fed diet with 7.5 per cent AZM. There was highly significant effect of Azolla incorporation on feed consumption which was highest in T₃ followed by T₄, C and T₂. Lowest feed consumption was in T₁ (Table 7). There was a reduction in feed cost and total cost of production per kg live weight. Group T₂ had a lowest mean value of feed cost and total cost of production per kg live weight followed by T₃, T₄, T₁ and Control.

Table 7: Effect of feeding different levels of Azolla meal on weight gain and economics

Parameter	C	T ₁	T ₂	T ₃	T ₄	SEM
Overall weight gain (g)	2033.30 ^a	2075.37 ^{ab}	2173.10 ^c	2182.07 ^c	2132.67 ^{bc}	67.61 ^{**}
Feed consumption (g)	3734.02 ^c	3576.33 ^a	3634.56 ^b	3900.98 ^e	3851.30 ^d	0.19 ^{**}
Feed cost/ kg broiler (in Rs.)	52.6	48.41	46.43	47.59	47.94	4.32
Total cost/ kg broiler (in Rs.)	71.85	67.16	64.93	66.23	66.63	4.84

^{abc} Means with different superscript in a row differ significantly ($*P < 0.05$, $**P < 0.01$)

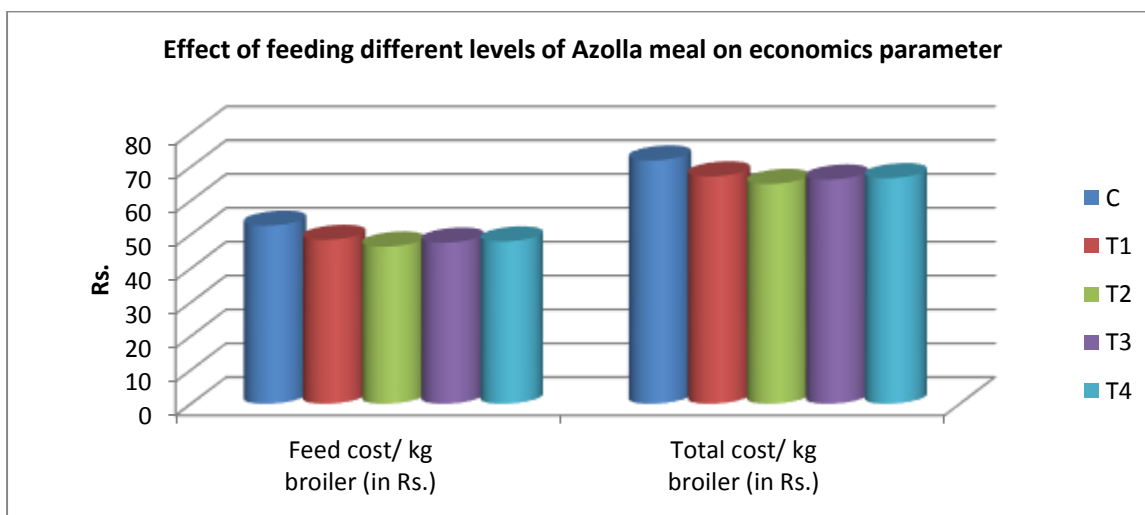


Fig.4: Effect of feeding different levels of Azolla meal on economics parameter

Conclusion

The values of haematology indices, H:L ratio and weight of spleen, bursa, thymus and lymphoid organs or immune organs are normal and within range. The haematology, immunity profile of broiler chicks suggesting that feeding of Azolla did not adversely affect the immune status of broilers and can be included in the ration for improving performance and reducing cost of production.



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