



Effect of Azolla Supplementation on Growth, Immunocompetence and Carcass Characteristics of Commercial Broilers

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ABSTRACT

One hundred and twenty, one week old, Cobb 400 broiler chickens were randomly distributed into three dietary treatments having four replicates each with ten birds. The birds of the control group (T1) were fed a basal diet (23.16% CP 1-3 weeks & 19.68% CP 3-6 weeks) while the other two groups were offered the treatment diets (T2& T3) replacing 4.50% or 5.50%, of the dry matter of the basal diet with dry *Azolla pinnata* powder on dry matter basis, respectively. Feeding azolla meal did not significantly ($p>0.05$) affect the weekly body weight gain of the birds during the experimental period. Total immunoglobulins and mercaptoethanol sensitive (IgM) antibody titer (\log_2) values in response to sheep red blood cells (SRBC) was significantly higher ($P<0.05$) in the birds fed T3 diet compared to the other two dietary treatments at 6 weeks of age. Cell mediated immune response i.e. *in vivo* cutaneous basophilic hypersensitivity response to lectin phytohaemagglutinin from *Phaseolus vulgaris* (PHA-P) determined as foot web index was significantly ($p<0.05$) higher in the T3 birds compared to the other two dietary treatments. Dressing percentage was significantly higher ($P<0.05$) in T2 compared to the other two treatment groups. However, there was no significant difference among the treatment groups in the other carcass traits. Thus, it may be inferred that replacement of basal diet with dry *Azolla pinnata* meal on dry matter basis did not adversely affect the growth and carcass characteristics. Moreover, *Azolla pinnata* possesses promising immunomodulatory potential in commercial broilers.

Keywords: Growth, immunity, azolla, broilers

Over the past few decades, there has been a dramatic rise in poultry production globally. This rise has resulted in competition with the conventional human food ingredients thereby leading to shortage and increasing the cost of conventional feed ingredients (CAST, 2013). Thus, studies on utilization of unconventional feed stuff as poultry feed ingredient have drawn the attention of scientists throughout the world. FAO programs focus on increasing the feed base production systems to locally available feed resources in developing countries (Sansoucy, 1993). Among the different sources of proteins, plant originates are less costly than animal protein. The water fern *Azolla* (*Azolla pinnata*) is an unconventional feed ingredient. *Azolla* is a free floating fresh water fern belonging to the family Azollaceae and order Pteridophyta. There are six species of *Azolla*. It is commonly found in tropics and

sub-tropics. It grows naturally in stagnant water of drains, canals, ponds, rivers and marshy lands. *Anabaena-azollae*, living in the cavity of *Azolla* leaf, can fix high amount of atmospheric nitrogen due to presence of symbiotic algae in the leaves (Becking, 1979). *Azolla* is rich in protein having total protein around 25-30%. Other constituents in *Azolla* are minerals, chlorophyll, carotenoids, amino acids, vitamins etc. It is also a potential source of nitrogen and is a potential feed ingredient for livestock (Lumpkin, 1984; Pannerker, 1988). Considering its nutrient content (Alalade *et al.*, 2006; Balaji *et al.*, 2009), some studies have been undertaken on growth and feeding value of *azolla* as a feed ingredient in broilers and egg-type chicks. However, a comprehensive study is necessary to assess the efficacy of *azolla* as an unconventional feed stuff in broilers. Hence, an experiment was designed to study



effect of *Azolla pinnata* meal on growth performance and immunocompetence traits of commercial broilers.

MATERIALS AND METHODS

Preparation of azolla meal

Fresh *Azolla Pinnata* was collected from Azolla production unit, Instructional Livestock Farm Complex, College of Veterinary Science and Animal Husbandry, Mathura (India) and dried overnight in hot air oven at 100±2°C. The powder was packed in an airtight container.

Birds and experimental design

A total of 120, a week old Cobb 400 broiler chickens were randomly distributed into three dietary treatments having four replicates each with ten birds. The birds of the control group (T1) were fed a basal diet (broiler starter till 3 weeks and broiler finisher after 3 weeks as per BIS, 1992) while the other two groups were offered the treatment diets (T2 & T3) replacing 4.50% or 5.50%, of the dry matter of the basal diet with dry *Azolla pinnata* powder on dry matter basis, respectively.

Experimental procedure and analyses

Weekly body weight was recorded till 6th week of age. After 6 weeks of age, general immune response was studied by taking 10 birds from each treatment group and measuring important immunocompetence traits such

as antibody response (log₂ titer) to sheep red blood cells (SRBC) (Siegel and Gross 1980; Van der Zijpp, 1983), 2-mercaptoethanol resistant antibodies (MER or IgG) and mercaptoethanol sensitive antibodies (MES or IgM) against SRBC (Martin *et al.*, 1989) and Cell mediated immune response to PHA-P (Corrier and DeLoach, 1990). After 42 days of the experimental trial, six representative birds (other than those used for immunological studies) from each treatment group was randomly selected and slaughtered to study the gastrointestinal tract development (proventriculus, gizzard, small intestine, large intestine & caeca) and various slaughter traits viz. preslaughter fasting shrinkage in live weight, dressing yield, eviscerated yield, ready to cook yield, giblet yield (heart, liver & gizzard), yield of individual cut-up parts (thighs, drumsticks, breast, back, neck, wings) as a percentage of live weight.

Statistical analyses

Data obtained from above experiment was analyzed as per the standard statistical procedure (Snedecor and Cochran, 1980). Significant differences among treatment means were calculated as per Duncan’s multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Chemical composition of experimental diet

The chemical composition of *Azolla* meal and experimental diet has been tabulated in Table 1. The proximate

Table 1: Nutrient contents of experimental diet (g/kg as dry matter basis)

Attributes	Starter			Finisher			Azolla meal
	C	T1	T2	C	T1	T2	
DM	886.5	891.0	873.8	864.5	881.9	883.4	918.8
ASH	151.8	154.8	155.4	153.6	154.7	155.4	216.7
CP	231.6	232.7	233.0	196.8	232.7	233.0	256.4
EE	43.2	42.7	42.6	56.2	42.7	42.6	31.5
CF	40.1	46.0	47.4	43.9	46.1	47.4	172.9
NFE	533.3	523.8	521.6	549.5	523.8	521.6	322.5
NDF	303.0	312.4	314.5	356.7	312.4	314.5	511.5
ADF	112.7	127.1	130.4	122.2	127.1	130.4	433.8
Ca	13.8	18.2	19.2	10.2	18.2	19.2	111.2
P	9.7	11.9	12.4	9.0	11.9	12.4	59.1

Table 2: Effect of azolla feeding on the average weekly body weight (g) of commercial broilers during 1-6 week period

Treatment	1 st wk	2 nd wk	3 rd wk	4 th wk	5 th wk	6 th wk
T1	128.65	274.20	430.35	677.70	846.40	971.40
T2	132.10	275.55	404.55	663.90	767.63	938.40
T3	128.15	299.15	483.45	742.35	858.72	1022.95
Pooled SEM	1.20	5.87	16.49	19.43	21.02	20.17
Significance	NS	NS	NS	NS	NS	NS

Means bearing different superscripts within a column differ significantly ($P < 0.05$); NS: Non significant ($P > 0.05$) SEM: Standard error of means

principles, i.e., DM, organic matter, total ash, CP, EE, CF and nitrogen free extract (NFE) of *Azolla* meal were found to be 9.88%, 78.33%, 21.67%, 25.64%, 3.15%, 17.29% and 32.25%, respectively. The cell wall constituents, i.e. neutral detergent fiber and acid detergent fiber and Ca and P contents of *Azolla* meal were found to be 51.15%, 43.38% and 11.12%, 5.91%, respectively. The chemical composition of *Azolla* was comparable with the previous observations of different scientists (Bolka, 2011; Kumar *et al.*, 2012; Kumarasinghe *et al.*, 2012; Cheryl *et al.*, 2014; Veerabahu *et al.*, 2015; Bhaskaran and Kannapan, 2015; Parashuramulu *et al.*, 2013).

Growth Performance

Feeding Azolla powder did not significantly ($P < 0.05$) affect the weekly body weight of the birds during the experimental period. Further, T₃ group birds had apparently higher weekly body weight compared to the other two treatments throughout the experiment. This may be attributed to the beneficial effect of Azolla, possibly due to better utilization of protein. The improvement in body weight is similar to the finding of Basak *et al.* (2002) who observed significant ($P < 0.01$) improvement in live weight of broiler chicks than control diet when they were fed with 5 per cent Azolla meal replacing sesame meal, while lower live weights were recorded in 10 and 15 per cent Azolla incorporated diets.

Similarly, Naghshi *et al.* (2014) reported chickens fed diets containing 5% Azolla powder significantly ($P < 0.01$) improved daily weight gain compared to control. Further, Balaji *et al.* (2009) reported that dietary inclusion of dried Azolla up to 4.5% level did not have any adverse effect on production performance of broiler chicken.

Immuno competence traits

Total immunoglobulins and mercaptoethanol sensitive (IgM) antibody titer (log 2) values in response to sheep red blood cells (SRBC) was significantly higher ($P < 0.05$) in the birds fed T3 diet compared to the other two dietary groups at 6 weeks of age.

Table 3: Effect of azolla feeding on the humoral immune responses [antibody titer (log 2) values to SRBC] and cell mediated immune response (response to PHA-P) (foot web index) at 6 weeks of age

Treatment	HA	IgG	IgM	Foot web index
T1	5.50 ^a	2.00	3.25 ^a	0.10 ^a
T2	6.25 ^a	2.25	4.25 ^a	0.11 ^a
T3	8.00 ^b	2.80	5.20 ^b	0.27 ^b
Pooled SEM	0.08	0.38	0.06	0.03
Significance level	$P < 0.05$	NS	$P < 0.05$	$P < 0.05$

Means bearing different superscripts within a column differ significantly ($P < 0.05$); NS: Non significant ($P > 0.05$) SEM: Standard error of means

In addition, the above antibody titer values were apparently higher in the birds fed with T₂ compared to the control. It is noted that mercaptoethanol resistant (IgG) (log 2) antibody titer in response to sheep red blood cells (SRBC) was apparently higher in T3 and T2 compared to T1. Dhumal *et al.* (2009) reported feeding *Azolla* meal in broiler improved the antibody titer values as compared to control group at 35th days of age in commercial broilers. Cell mediated immune response i.e. *in vivo* cutaneous basophilic hypersensitivity response to lectin phytohaemagglutinin from *Phaseolus vulgaris* (PHA-P) determined as foot web index was significantly

**Table 4:** Effect of azolla feeding at different levels on the carcass quality characteristics of commercial broilers at 6 weeks of age

Treatment	Shrinkage (%)	Dressing (%)	Eviscerated wt (%)	Heart wt (%)	Liver wt (%)	Gizzard wt (%)	Total ready to cook yield (%)
T1	4.19	74.76 ^a	58.00	0.36	2.44	2.17	62.99
T2	5.55	89.74 ^b	60.71	0.41	3.54	2.27	66.93
T3	4.69	75.43 ^a	58.25	0.41	2.79	1.88	63.33
Pooled SEM	0.88	2.19	0.89	0.03	0.20	0.10	1.10
Significance level	NS	P<0.001	NS	NS	NS	NS	NS

Means bearing different superscripts within a column differ significantly (P<0.05); NS: Non-significant (P>0.05) SEM: Standard error of means.

Table 5: Effect of azolla feeding at different levels on the cut up-parts of commercial broilers at 6 weeks of age

Treatment	Thighs (%)	Drumstick (%)	Breast (%)	Back (%)	Neck (%)	Wings (%)
T1	16.49 ^{ab}	16.36	30.14	19.77	6.32	10.91
T2	15.62 ^a	15.87	30.07	20.10	6.54	10.90
T3	17.95 ^b	14.78	30.79	19.66	6.01	10.81
Pooled SEM	0.40	0.38	0.47	0.43	0.19	0.24
Significance level	P<0.05	NS	NS	NS	NS	NS

Means bearing different superscripts within a column differ significantly (P<0.05); NS: Non-significant (P>0.05)SEM: Standard error of means

(p<0.05) higher in the birds fed T₃ compared to the other two dietary treatments. It was also observed that the foot web index was apparently higher in T₂ compared to T₁. Similar observation was reported by Mishra *et al.* (2016) on replacing a part of the basal diet of chabro chicken with azolla meal.

Carcass characteristics and yield of cut up parts

Dressing percentage was significantly higher (P<0.001) in T2 compared to the other two treatment groups. Basak *et al.* (2002) and Parthasarathy *et al.* (2002) reported highest dressing percentage in the birds fed with 5 per cent level of Azolla than control. However, in the present study, there was no significant difference among the treatment groups in the other carcass traits. Similarly, Dhumal *et al.* (2009) reported non-significant differences among the means of various carcass traits signifying the non-influence of Azolla meal on carcass quality. Percent yield of thigh was significantly higher (P<0.05) in T3 than T2 and apparently higher than control. Similarly, Naghshi *et*

al. (2014) reported supplementation of 5% Azolla powder significantly increased (P<0.05) thigh relative percentage. However, there was no significant difference among the treatment groups in the other cut up parts.

CONCLUSION

Thus, it may be concluded that replacement of basal diet with dry *Azolla pinnata* meal on dry matter basis did not adversely affect the growth and carcass characteristics of commercial broiler. Moreover, *Azolla pinnata* possesses promising immunomodulatory potential in commercial broilers. Hence, replacement of 5.5% of basal diet with *Azolla pinnata* meal on dry matter basis may elicit higher immunity in commercial broilers.

REFERENCES

- Alalade, O.A. and Iyayi, E.A. 2006. Chemical composition and the feeding value of *Azolla* (*Azolla pinnata*) meal for egg-type chicks. *Int. J. Poult. Sci.*, **5**: 137-141.

- Balaji, K., Jalaludeen, A., Churchil, R.R., Peethambaran, P.A. and Senthilkumar, S. 2009. Effect of dietary inclusion of Azolla (*Azolla pinnata*) on production performance of broiler chicken. *Indian J. Poult. Sci.*, **44**: 195-198.
- Basak, B., Pramanik, A.H., Rahmnan, M.S., Taradar, S.U. and Roy, B.C. 2002. Azolla (*Azolla pinnata*) as a feed ingredient in broiler ration. *Int. J. Poult. Sci.*, **1**: 29-32.
- Bhaskaran, S.K. and Kannapan, P. 2015. Nutritional composition of four different species of Azolla. *Eur. J. Exp. Biol.*, **5**: 6-12.
- Becking, J.H. 1979. Environmental requirements of Azolla for the use of tropical rice production. In: *Nitrogen and Rice*. International Rice Research Institute, Los Banos. Leguna, Philippines, pp. 345-374.
- Bolka, P.C. 2011. Nutritional evaluation of azolla (*Azollapinnata*) in broilers and layers. Ph.D Thesis, Karnataka Veterinary Animal and Fisheries Sciences University, Bidar.
- Corrier, D.E. and Deloach, J.R. 1990. Evaluation of cell mediated, cutaneous basophil hypersensitivity in young chickens by interdigital skin test. *Poult. Sci.*, **69**: 403-408.
- CAST. 2013. Animal Feed vs. Human Food: Challenges and Opportunities in Sustaining Animal Agriculture toward 2050. Issue Paper, 53. CAST, Ames, Iowa.
- Cherryl, D.M., Prasad, R.M.V., Jagadeeswara, R.S., Jayalaxmi, P. and Srinivas, K.D. 2014. A study on the nutritive value of Azolla pinnata. *Livestock Res. Int.*, **2**: 13-15.
- Dhumal, M.V., Siddiqui, M.V., Siddiqui, M.B.A. and Avai, P.E. 2009. Performance of broilers fed on different levels of Azolla meal. *Indian J. Poult. Sci.*, **44**: 65-68.
- Duncan, D.B. 1955. Multiple range and multiple F tests. *Biometrics*, **11**: 1-42.
- Kumar, D.S., Prasad, R.M.V., Kishore, K.R. and Rao, E.R. 2012. Effect of azolla (*Azollapinnata*) based concentrate mixture on nutrient utilization in buffalo bulls. *Indian J. Anim. Res.*, **46**: 268-271.
- Kumarasinghe, K.S. and Eskew, D.L. 2012. Isotopic Studies of Azolla and Nitrogen Fertilization of Rice. Springer Science and Business Media, Berlin, Germany, pp. 24-31.
- Lumpkin, T.A. 1984. Assessing the potential for Azolla use in the humid tropics. *Int. Rice Commission News*, **33**: 30-33.
- Martin, A., Gross, W.B. and Siegel, P.B. 1989. IgG and IgM responses in high and low antibody selected lines of chickens. *J. Hered.*, **80**: 249-252.
- Mishra, D.B., Roy, D., Kumar, V., Bhattacharyya, A., Kumar, M., Kushwaha, R. and Vaswani, S. 2016. Effect of feeding different levels of Azolla pinnata on blood biochemicals, hematology and immunocompetence traits of Chabro chicken. *Vet. World.*, **9**: 192-198.
- Naghshi, H., Khojasteh, S. and Jafari, M. 2014. Investigation the effect of different levels of Azolla (*Azolla pinnata*) on performance and carcass characteristics of Cobb broiler chicks. *Int. J. Farm Alli. Sci.*, **3**: 45-49.
- Pannaerker, S. 1988. Azolla as a livestock and poultry feed. *Livestock Adviser*, **13**: 22-26.
- Parashuramulu, S., Swain, P.S. and Nagalakshmi, D. 2013. Protein fractionation and *in vitro* digestibility of Azolla in ruminants. *Online J. Anim. Feed Res.*, **3**: 129-132.
- Parthasarathy, R., Kadriavel, R. and Kathaperumal, V. 2002. Azolla as a partial replacement for fishmeal in broiler rations. *Indian Vet. J.*, **79**: 144-146.
- Sansoucy, R. 1993. The FAO program for better utilization of local feed resources in developing countries. In: Proceedings of 7th World conference Animal Production, Edmonton, Canada. pp. 77-80.
- Siegel, P.B. and Gross W.B. 1980. Production and persistency of antibodies in chickens to sheep erythrocytes. 1. Directional selection. *Poult. Sci.*, **59**: 1-5.
- Snedecor, G.W. and Cochran, W.G. 1980. Statistical Methods. 6th ed. Iowa State University Press, Ames, Iowa.
- Van der zijpp, A.J. 1983. The effect of genetic origin, source of antigen and dose of antigen on the immune response of cockerels. *Poult. Sci.*, **62**: 205-11.
- Veerabahu, C., Radhika, D., Mohaideen, A., Indrani, S. and Priya, R. 2015. Phytochemical and biochemical profiles of *Azollamicrophylla* cultured with organic manure. *Int. J. Curr. Agric. Res.*, **4**: 131-133.

