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Biological Evaluation of *Azolla* in Ration of Commercial Chicken Broiler

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ABSTRACT

The study was conducted to evaluate feeding of dried *Azolla* (*Azolla piñnata)* and Azolla in combination with exogenous enzymes in commercial broiler poultry birds under complete randomized design. Total 110 day old vancobb broiler chicks were divided into five groups T_0,T_1, T_2, T_3 and T_4, further each group divided in 2 sub groups (n=11). Group T_1 and T_3 was given ration containing 2.5 and 5.0 percent dried *Azolla* without exogenous feed enzyme, where as group T_2 and T_4 was given ration containing 2.5 and 5.0 per cent dried *Azolla* with exogenous feed enzyme viz. *Phytase* and *Hizyme*. T_0 served as standard control diet. Overall growth performance of the dried *Azolla* fed groups (T_1, T_2, T_3, T_4) was found to be similar to the control group (T_0) during starter and finisher phase whereas, feed intake per unit gain in live weight was found to be significantly ($P<0.05$) higher for all the dried *Azolla* fed groups compared to control group. Further, group T_2 offered 2.5 percent substituted dried *Azolla* supplemented with enzymes exhibited lower feed conversion ratio compared to group T_3 offered 5 percent substituted dried *Azolla*. No effect was recorded in the plasma mineral viz. calcium and phosphorus levels of broilers. Based on the findings it can be concluded that dried *Azolla* can be safely administered in commercial poultry broiler ration up to 5 percent level without having any deleterious, however inclusion of 2.5 percent *Azolla* is more effective on the growth performance and biochemical parameters.

Keywords: *Azolla* feeding, broiler chicken, growth and production, exogenous enzymes and phytase

World animal protein inadequacy has led the nutritionists to explore the possibility of utilizing unconventional feed ingredients in animal feed formulations to feed the ever growing human population. In this context, chicken protein is considered to be the cheapest source of the animal protein available and is the second most consumed protein in the world after pork. Indian poultry sector is witnessing vast growth continuously over the last few decades and is one of the most money-spinning businesses of agriculture that bestows nutritious meat and eggs for human consumption within the shortest duration of time. But a key factor under-pinning India's poultry industry is the availability of cheap feed ingredients for rapid growth of commercial poultry and the nutritionists are continuously exploring new unconventional feed resources for economical feed production. This has led the nutritionists to explore the planktonic algae of both marine and fresh water origin which are considered to be potential sources of alternate feed items as a substitute for cereal grains in poultry ration as well as livestock. *Azolla* (*Azolla pinnata*), an aquatic fern, abundantly available in stagnant water in tropical and subtropical regions of the world, can be used as a suitable feed substitute. *Azolla* belongs to the family of Azollaceae (Kamalasanana Pillai *et al.,* 2002). *Azolla* forms a symbiotic relationship with the blue green algae, *Anabaena azollae* which fixes atmospheric nitrogen and convert to plant nitrogen. *Azolla* appears to have enormous potential as a livestock feed as having high content of proteins, essential amino acids, vitamins (vitamin-A, vitamin B_{12}, ß-Carotene), growth promoter intermediaries and minerals and proliferate without inorganic nitrogen fertilization (Kathirvelan *et al*., 2015). Shoukat Ara *et al.* (2015) reported 22.06 percent crude protein, 3.62 percent ether extract, 14.3 percent crude

fibre, 33.4 percent nitrogen free extract, 18.1 percent total ash, 2.04 percent calcium and 0.65 percent phosphorus in *Azolla* on dry matter basis.

It has been used for many years throughout Asia and parts of Africa to feed pigs, ducks, chickens, cattle, fish, sheep and goats and rabbits. Hence, *Azolla* has been considered to be the most promising unconventional feed stuff as it's easy to grow, higher productivity and good nutritive value. Thus, the present study focused to appraise the growth effect of inclusion of dried *Azolla* in poultry ration.

MATERIALS AND METHODS

Cultivation and harvesting of dried *Azolla* meal

Azolla was cultivated, multiplied and harvested in concrete (1 × 1 × 0.3) m and mud tanks (2 × 2 × 0.3) m constructed in the premises of metabolic stall CSK HPKV Palampur. Initial seed for cultivation of *Azolla* was available with the department. About 500 gram (g) to 1 Kilogram (Kg) of fresh and pure culture of *Azolla* was inoculated in the pit during summer with ambient temperature varying between 25 to 28 degree centigrade during day time and 18 to 20 degree centigrade during night. Provision of shade in the tanks using aluminium sheets was made to protect *Azolla* from direct sunlight. *Azolla* rapidly grew and filled the pit within 10 to 15 days and about 150 g/m square *Azolla* growth was recorded per day which was harvested thereafter. A mixture of 20 g of Single Super Phosphate (SSP) and about 1 Kg of cow dung was added once in 5 days in each tank. This was done to keep the *Azolla* in rapid multiplication phase and to maintain the yield. 0.25 percent of the water was replaced at an interval of 15 days. *Azolla* was harvested manually using aluminium mesh nets, sun dried in wire mesh racks for three days until they become crispy while retaining their greenish coloration. The dried leaves were then milled in hammer mill to produce dried *Azolla* meal, which was then stored in air tight sacs until used for feed manufacturing.

Table 1: Ingredient composition of experiment diets

Particulates	Starter phase					Finisher phase				
	T_0 (%)	T_1 (%)	T_2 (%)	T_3 (%)	T_4 (%)	T_0 (%)	T_1 (%)	T_2 (%)	T_3 (%)	T_4 (%)
Maize	47	47	47	47	47	60	60	60	60	60
Soybean	41	38.5	38.5	36	36	30	27.5	27.5	25	25
Fishmeal	5	5	5	5	5	3	3	3	3	3
Rice polish	3	3	3	3	3	5	5	5	5	5
Azolla dried	0	2.5	2.5	5	5	0	2.5	2.5	5	5
DCP	2	2	2	2	2	2	2	2	2	2
DL-Methionine	1	1	1	1	1	0.100	0.100	0.100	0.100	0.100
Lysine	1	1	1	1	1	0.100	0.100	0.100	0.100	0.100
Salt	0	0	0	0	0	0.240	0.240	0.240	0.240	0.240
+PREMIX	+	+	+	+	+	+	+	+	+	+
Phytase	-	-	+	-	+	-	-	+	-	+
#Hizyme	-	-	+	-	+	-	-	+	-	+
Total	100	100	100	100	100	100	100	100	100	100

HIZYME (15gm/quintal): (amylase, xylanase, ß-glucanate, pectinase, cellulose, protease, lipase, mannanose); *PHYTASE* (6gm/tonne): Activity 2000FPU/gm

+:Premix was prepared by mixing the following in 200 g maize flour VETRIMIX 20g (vitamin A-82,500 IU, B2-50 mg, D3-12,000 IU,K-10 mg/g).VENTRIBEE PLUS- 20 g (vitamin B1- 25 mg, B6 -35 mg, B12-250 µg,E-225mg, Pantothenate- 225 mg, Niacinamide-300mg, Folic acid -20 mg/5g. E-care Se forte - 25 gm (Vitamin E- 0.20g, Se-0.04mg/g). Super DOT 50 gm (Dinitro-o-TOULAMIDE-250 mg, Ethopabate-16 mg/g). Trace minerals- 100g (Ferrous oxide -2 g, Dicalcium phosphate -54 g, Copper sulphate -2g, Manganese sulphate- 3 g, Zinc sulphate -0.6g, Zinc oxide-1 g, Ferrous sulphate-10 g, Potassium iodide-2.5 g, Magnesium sulphate-25) Choline chloride: 150g

Experimental design and diet formulation

The research work was carried out in the experimental poultry house of the Department of Animal Nutrition, College of Veterinary and Animal Sciences, Palampur, Himachal Pradesh, India. For this purpose, day old vancobb broiler chicks (n=110) were divided into 5 main groups $T_{0,}$ T_1, T_2, T_3 and T_4 further divided in 2 sub groups or replicates with 11 chicks in each and were offered rations to contain dried *Azolla* at 0 percent, 2.5 percent and 2.5 percent with exogenous feed enzymes, 5 percent and 5 percent with exogenous feed enzymes respectively. All the diets (starter and finisher) were prepared as per BIS (1992). Group T_0 contain standard diet devoid of test ingredient, there by serving as control as presented in Table 1. Exogenous enzymes included *Phytase* (Saguna, India) and *Hizyme* (Vetline, India) (*Hizyme*, an enzyme based supplementation containing cocktail of enzymes namely α-amylase, xylanase, ß-glucanate, pectinase, cellulase, protease, lipase and mannanase). Ingredients and nutrient composition of experimental diet is presented in Table 1 and 2.

The difference in the physical composition of different ingredients in different treatments is due to fact that the chemical composition of the nutrients is to be kept similar in different treatments groups as per the experimental plan. Standard methods as reported in AOAC (2005) were followed for determination of proximate composition of *Azolla* as well as feed samples. The metabolisable energy (ME) contents of different test diets used under different experiments were calculated as per the equation proposed by Lodhi *et al*. (1976).

Experimental animals and management

One hundred and ten (110) day old unsexed chicks of vancobb strain procured from local hatchery were weighed using digital sensitive balance and there after randomly allotted to groups T_0, T_1, T_2, T_3 and T_4. Each group comprised of two replicates of eleven birds each. Total duration of the experiment was 42 days. For first 21 days broiler starter diet was fed while for the remaining period broiler finisher ration (Table 1 and 2) was offered to the respective groups. The digestibility of various nutrients was carried out in caged battery brooders. Five birds from each experiment unit, close to the average weight of the flock were selected and shifted to battery brooders and were given a three days acclimatization period before start of the trial from 23rd to 27th day of age. Total faecal collection method for five days was employed to estimate the digestibility during which the total intake of feed and faeces in voided in each group were recorded. Feed and faecal samples drawn were later analyzed for proximate principles as proposed by AOAC (2005).

The chicks were reared in deep litter system whose floor was covered with wood shavings at about 5 centimetre (cm) depth. Sufficient management conditions like floor space, light, temperature, ventilation and relative humidity were provided to each of the groups. During the

Table 2: Chemical composition (% DM) of experimental diets

Treatment	Starter phase					Finisher phase				
Nutrient	T_0 (%)	T_1 (%)	T_2 (%)	T_3 (%)	T_4 (%)	T_0 (%)	T_1 (%)	T_2 (%)	T_3 (%)	T_4 (%)
Dry matter	91.18	91.03	91.03	90.88	90.88	92.87	92.72	92.72	92.57	92.57
Crude Protein	23.33	22.89	22.89	22.45	22.45	19.34	18.90	18.90	18.46	18.46
Ether extract	2.64	2.65	2.65	2.65	2.65	2.78	2.78	2.78	2.79	2.79
Crude fiber	3.90	4.13	4.13	4.36	4.36	3.63	3.85	3.85	4.08	4.08
Total ash	6.36	6.54	6.54	6.72	6.72	5.12	5.30	5.30	5.48	5.48
Acid insoluble ash	2.43	2.12	2.12	2.17	2.17	2.88	3.64	3.64	3.48	3.48
Calcium	1.39	1.42	1.42	1.44	1.44	1.21	1.24	1.24	1.26	1.26
Total Phosphorus	1.00	0.98	0.98	0.97	0.97	0.93	0.91	0.91	0.90	0.90
NFE	54.94	54.80	54.80	59.83	59.83	62.00	61.86	61.86	61.73	61.73
ME(Kcal/kg)	2666	2657	2657	2649	2649	2837	2829	2829	2820	2820

experimental period, they were fed *adlibitum* on replicate basis and provided with clean and wholesome water. Data on feed intake by each replicate was recorded on weekly basis. Initial and final body weight gain was recorded. The data on feed intake and weight gain were recorded on regular basis to calculate feed conversion ratio.

Hematological analysis

Approximately 3-5 ml of blood from randomly chosen 5 birds per replicate was collected by wing vein, in a centrifuge tube containing anticoagulant heparin @ 0.1-0.2 mg/ml of blood, under aseptic conditions. The tubes were centrifuged at 5000 rpm for 15 minutes and thus plasma was separated and stored at -20^0 C for estimation of calcium, phosphorus and cholesterol by using (Blood chemistry analyser Photometer- 5010) using kits of Agappe.

Statistical Analysis

All the recorded and calculated data were subjected to analysis of variance (ANOVA) by Duncan Multiple Range Test using SAS software version 9.3 (Duncan, 1955).

RESULTS AND DISCUSSION

The growth performance of the broiler birds fed different levels of *Azolla* meal is presented in Table 3. Average final weight of the birds at 42nd day of age did not show any particular trend and were non-significant (P<0.05). Average value for the gain in live body weight in control group T_0 was 2340.46 g where as corresponding gain in live body weight in group T_1, T_2, T_3 and T_4 was 2161.24 g, 2244.14 g, 2207.08 g and 2119.68 g respectively. Highest value for gain in live body weight was again exhibited in control group T_0 2340.46g where as lowest value was recorded in group in group T_4 2119.68 g. A decreasing trend in live weight gain was recorded as the inclusion level of dried *Azolla* increased. Increased fibre contents owing to increasing levels of *Azolla* meal in the broiler diets could be the reason for this decreased gain, which is discussed below in the manuscript. Numerically lower weight gain of birds offered 2.5 percent and 5 percent dried *Azolla* could be attributed to higher fibre content and consequently reduced digestibility and lower metabolisable energy availability for growth.

Proximate analysis of *Azolla pinnata* used in the experiment exhibited 88.04 percent dry matter and 27.7 per cent crude protein (CP) which was similar to Singh (1977) who reported that crude protein might vary from 25 to 37.36 percent. Ether extract content was analyzed to be 2.76 per cent which was in accordance to Ali and Lesson (1995) and Querubin *et al*. (1986) who ranged it between 1.58 and 2.63 percent. The calculated and analyzed crude fiber was 17.52 per cent, which was according to Querubin *et al*. (1986) and Parthasarathy *et al*. (2001) who reported the crude fiber content of *Azolla* ranging from 13.19 to 16.54 per cent. The calculated total ash was 18.76 per cent, while the values were almost similar as given by Buckingham *et al.* (1978), Parthasarathy *et al*. (2001), Basak *et al.* (2002), Alalade and Iyayi (2006) compared to the present study. The calculated acid insoluble ash was 2.25 per cent, and 33.26 per cent was calculated nitrogen free extract which was in accordance to Parthasarathy *et al*. (2001) who reported nitrogen free extract ranging from 38.85 to 44.06 per cent. It contained 1.86 per cent calcium which was in close conformity with the report of Parthasarathy *et al*. (2001) and 0.41 per cent was calculated phosphorus which was nearer to the result of the present study by Ali and Leeson (1995) who found 0.31 per cent phosphorus in *Azolla* and its calculated metabolisable energy was 2180.6 kcal/kg.

Previous reports made by Basak *et al*. (2002) and Buckingham *et al*. (1978) have implicated high levels of ADF (30.08) and lignin (28.24) as the main factor limiting the efficient utilization of *Azolla* meal by monogastric animals. The results are in accordance with the findings of Parthasarthy *et al.* (2002) who reported no significant difference in body weight gain of broilers on basal and 5 percent *Azolla* supplementation.

Average value of feed intake (Table 3) in control group T_0 was 4003.86g, where as corresponding feed intake in group T_1, T_2, T_3 and T_4 was 4215.34 g, 4297.03 g, 4499.80 g and 4226.06 g respectively. Feed intake did not exhibit any significant (P<0.05) difference amongst control and *Azolla* meal fed groups with and without exogenous enzyme supplementation. Similar results were reported by Castillo *et al*. (1981) who reported that the inclusion of dried *Azolla* in broiler diet did not affect feed consumption up to 15 percent level of inclusion.

Table 3: Overall growth performance of broiler birds (3rd to 42nd day of age)

Particulars	Initial body weight(g)	Final body weight(g)	GIW(g)	FI(g)	FCR
T_0	77.43±1.84	2417.9±93.01	2340.46±92.88	4003.86±8.73	1.71±0.05[a]
T_1	74.48±1.36	2235.72±47.26	2161.24±47.23	4215.34±15.70	1.95±0.01[b]
T_2	73.61±1.89	2317.76±48.83	2244.14±49.29	4297.03±35.15	1.91±0.03[b]
T_3	77.94±0.89	2285.36±30.92	2207.08±30.75	4499.80±51.84	2.03±0.01[c]
T_4	73.21±1.27	2193.5±51.32	2119.68±50.92	4226.06±44.36	1.99±0.02[ab]

Figures with common super script in a column do not vary statistically (P<0.05); n=22; GIW: gain in weight; FCR: feed conversion ratio; FI: feed intake

Table 4: Digestibility coefficients of proximate principles in broilers supplemented with different levels of dried *Azolla* (per cent dry matter basis)

Particulars	DM	CP	CF	EE	Ca	P
T_0	72.02±4.99	67.34±5.52	24.02±8.49	79.95±3.56	75.95±4.95	46.48±8.49
T_1	65.94±1.80	63.50±4.15	23.64±9.16	69.10±3.10	71.53±3.07	48.73±5.82
T_2	71.13±3.52	67.16±3.89	30.23±8.15	78.27±3.08	73.44±2.90	47.82±6.05
T_3	69.16±5.54	65.53±6.52	22.36±4.21	72.03±5.89	75.79±4.09	46.96±8.65
T_4	69.88±3.57	66.27±4.22	28.24±7. 99	73.91±2.42	72.79±3.38	48.30±7.37

Feed conversion ratio (FCR) is a good indicator of economic importance in broiler farming. The results obtained regarding FCR in different groups are given in Table 3. The average value for FCR in control group T_0 was 1.71, whereas corresponding FCR in group T_1, T_2, T_3 and T_4 was 1.95, 1.91, 2.03 and 1.99. Experimental broilers offered standard feed to control group T_0 consumed least amount of feed to produce kilo gram of meat as exhibited by significantly (P<0.05) lowest value of average FCR 1.71, as compared to groups offered dried *Azolla* substituted feed. This confirms to the idea cited by Buckingham *et al*. (1978) that low growth rate is attributed to the high level of ADF (25- 30 per cent) (acid detergent fibre) and lignin (15-17 percent) contained by dried *Azolla*, which is the factor limiting the efficient utilization in monogastric animals. This also confirmed the findings of Castillo *et al*. (1981) that monogastric animals like broilers are inefficient in digesting cellulosic materials like the high fibre contents found in the dried *Azolla* meal. This notion was verified because experimental birds of group T_0 converted their feed more efficiently compared to experimental birds in the groups T_1, T_2, $T_3$and T_4 which were fed with 2.5 percent and 5 percent of dried *Azolla* in rations. However FCR of experimental birds in group T_2 given 2.5 percent dried *Azolla* with exogenous enzymes exhibited significantly (P<0.05) better FCR compared to group T_3 offered 5 percent *Azolla*. Results exhibited confirmed the notion regarding efficiency of exogenous enzymes at 2.5 percent substitution of dried *Azolla* but were ineffective at 5 percent level. This suggests that the inclusion rate of *Azolla* meal should not be higher than 2.5 percent in the diet of broilers similar with the findings of Alalade *et al*. (2006).

The digestibility trial was also conducted on 23rd day of experiment for five days. The digestibility coefficients of dry matter, crude protein, crude fibre, ether extract, nitrogen free extract and organic matter have been presented in Table 4.

Results exhibited no significant (P<0.05) difference amongst groups for dry matter digestibility and nitrogen balance. The average value of digestibility coefficient of crude fibre (CF) in groups T_0, T_1, T_2, T_3 and T_4 was 24.02 percent, 23.64 percent, 30.23 percent, 22.36 percent and 28.24 percent respectively, which exhibited no significant (P<0.05) difference though numerically group T_2 exhibited 6 percent higher CF digestibility than control T_0. High CF digestibility in group T_2 commensurate with the addition

of exogenous enzymes (*Hizyme*) which translated in higher breakdown of structural carbohydrates which might have increased the availability of metabolisable energy (ME) translating in better feed conversion at 2.5 percent dried *Azolla* substitution. The results were similar to Sharma (2014) who also reported that there was no significant (P<0.05) difference compared to control group for different nutrients during metabolism studies.

The results obtained regarding the blood plasma minerals are presented in Table 5. The blood calcium (Ca), phosphorus (P) and cholesterol level were not (P<0.05) influenced by the substitution of dried *Azolla* in the ration of experimental poultry birds. The blood calcium ranged from 8.00 to 9.40 mg/dl which was within normal range i.e. 8.0 to 13.0 mg/dl (Sakas, 2002).

Thus there was no significant effect of supplementation of dried *Azolla* in blood minerals. Mishra *et al*. (2016) also reported that Blood glucose, creatinine, cholesterol, total protein, albumin, uric acid and triglycerides were found similar in all control and dried *Azolla* fed groups and was within the normal physiological range for broiler chicken. Whereas in this experiment, also addition of dried *Azolla* in the poultry ration did not affect the mineral level of plasma of blood.

Table 5: Blood plasma estimation in broilers supplemented with different levels of dried *Azolla* (per cent dry matter basis)

Particulars	Ca (mg/dL)	P (mg/dL)	Cholesterol (mg/dL)
T_0	9.40±0.81	6.80±1.35	121.20±4.93
T_1	8.00±0.54	6.20±0.48	128.40±9.08
T_2	9.40±0.24	6.20±0.48	121.40±3.57
T_3	7.80±0.37	5.40±0.24	124.60±5.81
T_4	9.40±0.50	6.00±0.54	120.60±5.19

Figures with common super script in a column does not vary statistically (P<0.05)

CONCLUSION

Azolla substitution seems to affect the digestibility of the feed as indicated by high FCR but supplementation of exogenous enzyme at 2.5 percent dried *Azolla* was effective. It is therefore concluded that dried *Azolla* meal at 2.5 percent level can be used as alternative to soy bean in broiler chickens.

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