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Effect of *Azolla cristata* with or without enzyme supplementation on blood biochemistry and intestinal histomorphology of broiler chicken

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Feed is the major component in the production of poultry, as it constitutes 70 to 80% of total production cost. The conventional feedstuffs used in poultry are maize and soybean meal, but the declining food resources around the world have led to hike in their price, hence becoming a major constraint in poultry production. A possible and perhaps the most viable proposition is to exploit the locally available non-conventional feed resources (NCFR) in livestock and poultry production systems, but only a narrow range of the raw materials are used for poultry feed formulation due to lack of reliable data on their nutrient composition, presence of toxic/anti-nutritional factors, feeding value and effective level of inclusion. Among the feed proteins, plant originates are less costly than animal proteins.

Of late, Azolla (AZ) species have been tried as alternative feed sources for livestock (Shital *et al.* 2012, Meena *et al.* 2017). The water fern Azolla (*Azolla cristata*) belonging to the family Azollaceae and order Pteridophyta is a fresh water free floating fern naturally seen in tropics and sub-tropics. It is a good source of protein (leucine and alanine) and minerals (calcium, magnesium and iron) (Bhaskaran and Kannapan 2015, Ara *et al.* 2018). Aflatoxin content in AZ species has been reported to be 0.01 µg/kg, so its inclusion in the diet of livestock has no adverse effect on their health and performance (Rana *et al.* 2017).

In view of the above benefits, the present study was conducted with the objectives to evaluate the effect of partial replacement of soybean meal with AZ (either alone or in combination with enzyme supplementation) on blood biochemistry and intestinal histomorphology of broiler chicken.

The experiment was conducted on commercial broilers during February to March, 2021 at Experimental Poultry Farm, Department of Livestock Production and Management, Faculty of Veterinary Science, SKUAST-Kashmir, Jammu and Kashmir, India. Azolla was collected from famous Dal lake of Srinagar, Kashmir. After collection, it was dried in the sun, ground and packed in plastic bags and used thereafter in the trial.

Day-old commercial broiler chicks (210) were procured from a reputed source. Chicks were reared in battery cages until 7 days of age. On eighth day, the chicks were individually weighed, distributed into 7 treatment groups of 3 replicates with 10 chicks in each group, in a completely randomized design, so that the treatment means differ as little as possible. The experiment was done for a period of 6 weeks under deep litter system. The temperature was controlled and gradually reduced from 32 to 20°C on day 42. The birds were provided 24 hrs light throughout the experiment along with proper ventilation with the help of exhaust fans. The birds were vaccinated against New castle and Gumboro's diseases. Fresh feed and water was provided daily *ad lib*. The feeding programme consisted of a starter diet from 7-21 days and a finisher diet until 42 days of age. The birds in the control group were given a diet without Azolla (T_1). The other six treatment groups were given the same diet as fed to the control group, but soybean meal was replaced with 5 (T_2), 10 (T_3) and 15% (T_4) Azolla, whereas the other three treatment groups, i.e. T_5, T_6 and T_7 contained 5, 10 and 15% Azolla with cocktail enzyme respectively @ 50 g/100 kg of feed. Table 1 shows the composition (ingredients and nutrient) of experimental diets.

At the end of trial, 2 birds per replicate were utilized for ascertaining the blood biochemistry associated with incorporation of DW with or without enzyme supplementation. The blood sample was collected in sterile test tubes, without the addition of anticoagulant and kept in slanting position. The tubes containing blood were incubated at 37°C for 1 hr. Blood clots were broken and tubes were centrifuged at 3000 rpm for 30 min. The serum was pipette out in small tubes which were stored under deep freeze (at -20°C) until further examination. Glucose, cholesterol, total protein, albumin, SGPT and SGOT values were estimated from the serum samples with the aid of auto analyzer using respective biochemical kits.

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Table 1. Ingredients and nutrient composition of broiler chicken diets

Ingredient	Starter (7-21 days)				Finisher (22-42 days)			
	T_1	T_2/T_5	T_3/T_6	T_4/T_7	T_1	T_2/T_5	T_3/T_6	T_4/T_7
Maize	56.48	55.06	56.17	55.22	60	59.30	59.56	59.56
Soyabean	35.06	35.24	33.40	33.00	30.00	30.00	30.00	30.00
Azolla	0.00	1.76	3.34	4.95	0.00	1.50	3.00	4.50
Fish meal	2.0	1.5	2.2	2.0	3.0	2.6	1.7	1.0
Oil	1.8	1.8	1	1	2.69	2.5	1.8	1.8
Limestone	1.2	1.2	1.2	1.2	1	1	0.9	0.9
DCP	1.8	1.8	1.7	1.7	1.5	1.5	1.7	1.7
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
DL-Meth	0.15	0.14	0.13	0.11	0.17	0.14	0.14	0.1
lysine	0.15	0.14	0.2	0.16	0.15	0.15	0.14	0.15
TM. Premix[1]	0.8	0.8	0.1	0.1	0.8	0.8	0.1	0.1
Vit. Premix[2]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
B complex	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
Ch. Chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Toxin binder	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Enzyme**	-	-/p	-/p	-/p	-	-/p	-/p	-/p
Nutrient Composition								
[3]Crude Protein	21.30	21.39	21.31	21.28	19.82	19.88	19.81	19.81
Crude Fibre	4.16	4.38	4.55	4.73	4.0	4.20	4.41	4.63
*ME (Kcal/kg)	2942	2939	2941	2943	3033	3028	3029	3030
[3]Calcium	1.18	1.17	1.16	1.16	1.06	1.04	1.03	1.01
Available P	0.48	0.48	0.48	0.48	0.44	0.44	0.46	0.45
Lysine	1.26	1.26	1.30	1.27	1.17	1.17	1.15	1.16
Methionine	0.51	0.51	0.52	0.51	0.52	0.50	0.51	0.47
Threonine	0.88	0.90	0.90	0.91	0.79	0.81	0.84	0.87

*ME, Metabolizable energy calculated; [3], Analysed value; [1], Trace mineral premix supplied mg/kg diet: Mg, 300; Mn, 55; I, 0.4; Fe, 56; Zn, 30; Cu, 4; [2], Vitamin premix supplied per kg diet: Vit.A, 8250 IU; Vit.D_3, 1200 ICU; Vit.K, 1 mg; Vit.E, 40 IU; Vit. B_1, 2 mg; Vit. B_2, 4 mg; Vit.B_{12},10 µg; niacin, 60 mg; pantothenic acid, 10 mg; choline, 500 mg.**, Cocktail enzyme @50 g/100 kg diet.

For histomorphological changes, the representative samples of liver, small intestine and kidneys were collected from 2 birds per replicate into sterile containers containing 10% buffered formalin for fixation in order to prevent autolytic changes. The tissues were kept in capsules with proper labelling and then washing was done to drain out formalin. Dehydration was achieved by treating tissues with different grades of alcohol. Afterwards, the tissue samples were kept in benzene for the firmness in order to facilitate section cutting. The Casting of blocks was done with the help of L- molds and then section cutting was achieved by rotatory microtome. Afterwards, deparafinization was done with the help of xylene and then the tissue was stained with haematoxylin and eosin to observe pathomorphological changes in the affected tissues (Lillie 1965).

The analysis of data regarding blood biochemical parameters was carried out byfor one way analysis of variance (ANOVA) using statistical package SPSS version 21.0. The difference within the means were estimated using Duncan's multiple range test (Duncan 1955) by considering the differences at significant level ($p<0.05$).

Blood biochemistry: The values of different blood biochemical parameters in broiler chicken fed different levels of AZ with or without enzyme supplementation are presented in Table 2. All the blood biochemical parameters were well within the normal range. The glucose levels (mg/dl) in different treatment groups ranged from 171.66±2.07 to 176.07±2.58 with no statistical difference ($p>0.05$). The cholesterol levels (mg/dl) did not differ ($p>0.05$) among different treatment groups and ranged from 192.85±4.43 to 198.44±1.69. The values for the total protein (gm/dl) ranged between 4.53±0.41 for TC and 4.83±0.16 with no statistical significance among various dietary treatment groups. The SGPT (ranged from 91.62±3.28 and 94.37±3.11) and SGOT (18.84±1.96 and 20.22±1.69) levels (µ/l) were also at par in all the treatment groups with no statistical significance ($p>0.05$)

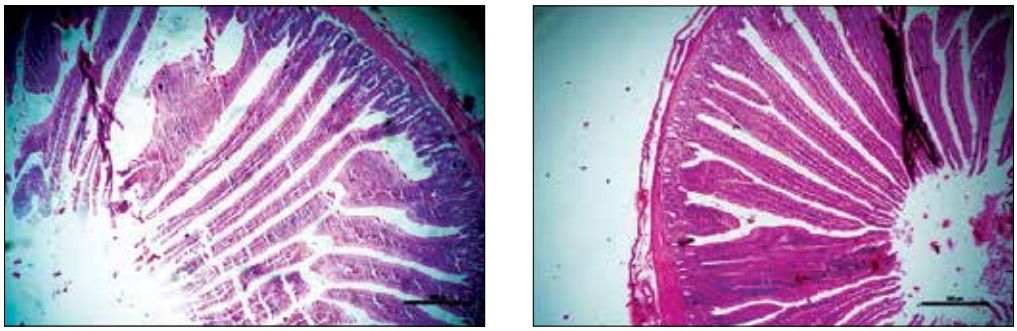
Intestinal histomorphology: The effect of azolla in feed on histomorphology of small intestine of broiler chicken has been depicted in Table 3 and Fig. 1. There was a no significant ($p>0.05$) effect in villus height (V), crypt depth (C) and V/C ratio among various treatment groups when compared to control. Highest villus height was observed in the control group (T_1) and there was reduction in villus height in all the groups wherein AZ was included in the diet either alone or with enzyme supplementation. These results are in agreement with our earlier results wherein it was found that higher levels of AZ in the diet of broiler chicken

Table 2. Effect of azolla in feed on blood biochemical parameters of broiler chicken (mean±SE)

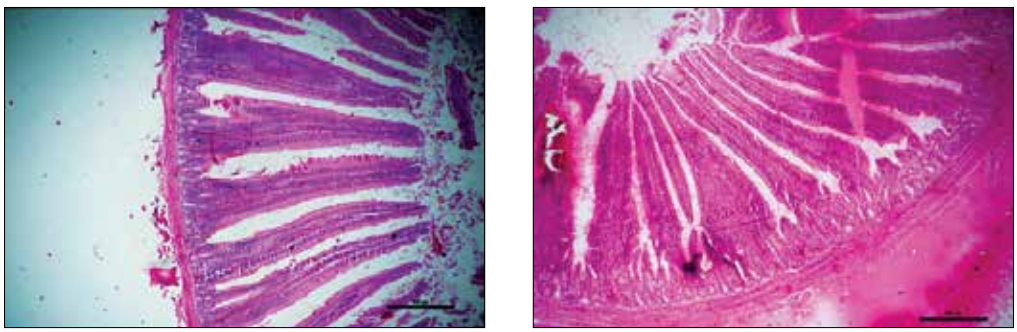
Parameter	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Glucose (mg/dl)	175.70±1.23	174.05±1.04	173.43±1.27	174.51±2.41	171.66±2.07	173.81±1.12	176.07±2.58
Cholesterol (mg/dl)	198.44±1.69	196.57±3.01	198.25±2.70	195.19±2.93	196.13±3.64	195.45±3.02	192.85±4.43
Protein gm/dl	4.83±0.16	4.71±0.21	4.60±0.27	4.63±0.32	4.61±0.33	4.53±0.41	4.58±0.45
SGOT (µ/l)	93.44±1.73	92.38±2.42	93.18±2.23	91.62±3.28	94.37±3.11	92.93±2.38	94.08±2.75
SGPT (µ/l)	19.81±1.71	18.84±1.96	20.11±1.93	20.42±1.77	19.58±2.18	20.22±1.69	19.96±1.73

Table 3. Effect of azolla in feed on histomorphology of small intestine of broiler chicken (mean±SE)

Parameter	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Villus ht. of duodenum (µm)	1471.9±14.2	1452.0±9.44	1434.4±13.6	1430.2±9.11	1438.6±6.26	1414.6±10.6	1415.7±8.48
Crypt depth of duodenum (µm)	212.3±3.28	207.6±2.50	217.9±4.22	215.7±5.50	211.9±4.87	210.7±1.29	204.8±6.52
V/C ratio	6.93±0.33	6.99±0.12	6.58±0.18	6.63±0.16	6.79±0.26	6.71±0.08	6.91±0.31



T_1 (Control) T_2 (5% Azolla)



T_3 (10% Azolla) T_4 (15% Azolla)

Fig 1. Histomorphology of small intestine in broiler chicken fed Azolla in the diet (40×). Note: T_1 showing higher villus height than T_2, T_3 and T_4.

resulted in reduction of small intestinal villus height (Ara *et al*. 2012). Decreased villus height as a result of increase in the inclusion level of AZ in the diet of broiler chicken justifies the fact of decrease in the performance of birds at higher AZ inclusion levels (Ara *et al*. 2012). Higher levels of AZ have also been reported to decrease the performance of layer chicken (Ara *et al*. 2018). Parthasarthy *et al*. (2002) also reported reduction in the body weights of broiler chicken due to replacement of 10 and 20% protein source with AZ in ration. Increased villi height increases surface area for greater absorption of nutrients (Awad *et al*. 2008), hence, enhancement in growth performance of birds. So, the poorer weight gains due to higher AZ levels in ration as discussed above by various researchers could be attributed to reduction in the villus height in small intestine which might have decreased the absorption of available nutrients.

SUMMARY

A study was undertaken to determine the effect of partially replacing soybean protein with Azolla (AZ) with or without enzyme on blood biochemistry and intestinal histomorphology of broiler chicken. The experiment was performed on 210 Cobb broiler chicks randomly assigned to 7 treatment groups each replicated 3 times with 10 birds in each replicate. Basal diet was offered to the control (T_1) group, whereas soybean protein was replaced with 5 (T_2), 10 (T_3) and 15% (T_4) AZ and the other three groups, i.e. T_5, T_6 and T_7 contained the respective inclusion levels of AZ, but were supplemented with cocktail enzyme. The results revealed that there was no statistical difference on blood

glucose, cholesterol, protein, SGPT and SGOT levels among various treatment groups including control. Also there was a no significant effect in villus height (V), crypt depth (C) and V/C ratio among various treatment groups when compared to control. Highest villus height was observed in the control group (T_1) and there was reduction in villus height in all the groups wherein AZ was included in the diet either alone or with enzyme supplementation. It is thus concluded that dietary inclusion of AZ had no adverse effect on the blood biochemistry and intestinal histomorphology when provided alone or in combination with enzyme supplementation, indicating that AZ could be included up to 15% level in the diet of broiler chicken without any adverse effect.

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