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Effect of Aquatic Fern, *Azolla cristata* in Diet on Growth, Serum Biochemistry and Laying Performance of Chicken

Shoukat Ara[1], Sheikh Adil[2,*] and Manzoor Ahmad Khan[3]

[1]Division of Environmental Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar-190006, India
[2]Division of Livestock Production and Management, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar-190006, India
[3]Division of Veterinary Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar-190006, India

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SA designed the study and provided guidelines during the work. SA carried out the experiments and wrote the manuscript. MAK helped during the trial and in the processing of samples.

Key words
***Azolla*, Serum biochemistry, Egg quality and layer chicken.**

ABSTRACT

To determine the effect of dietary supplementation of aquatic fern *Azolla cristata* on growth, serum biochemistry and laying performance layer chicken, a study was conducted on 240 key stone golden layer chicks which were randomly assigned into five groups having three replicates of 16 each. Birds in control (T_1) group were fed the basal diet, where as other groups the groundnut cake was replaced with 5 (T_2), 10 (T_3), 15 (T_4) and 20% (T_5) *Azolla*. The results revealed that inclusion of *Azolla* showed a non-significant increase ($p>0.05$) on the body weight gain of grower birds. Further, the SGPT and SGOT levels of birds did not differ among various treatments at 19 weeks of age. A non-significant ($p>0.05$) improvement was observed in the egg weight, shell thickness and weight in all the groups fed *Azolla* in the diet (T2, T3, T4 and T5) when compared with the control group (T1). The differences in the total number of eggs laid, % hen-day-egg production, various external and internal egg parameters under different treatments were statistically non-significant ($p>0.05$). In conclusion, *Azolla* is beneficial at lower levels in improving the performance of grower chicken but had no effect on the number of eggs laid. Further, *Azolla* inclusion in the diet showed an improvement in the shell quality of eggs which could prove beneficial in decreasing the incidence of egg breakage and thereby maximizing profits. The *Azolla* inclusion in the diet of layer chicken had no effect on SGPT and SGOT levels indicating that *Azolla* supplementation had no toxic effect in layer chicken even up to 20% levels.

INTRODUCTION

The poultry industry in India has made remarkable progress during last 3 decades, but the rising cost and unavailability of the feed ingredients are the major hindrances in achieving the target production. The higher price and non-availability of feed ingredients are the major limitations to the growth and profitability of poultry enterprise (Islam *et al*., 2010). Feed is the most expensive of all inputs and about 70% of production cost is accounted for feed alone (Parthasarathy *et al*., 2001). This has led to the poultry nutritionists to look for alternative cost effective, non-conventional feed ingredients for economic poultry production (Mishra *et al*., 2016).

Aquatic weeds are one such alternative and they have been proposed to be used as feed ingredients (Pirie, 1980). They are believed to offer a fairly cheap feedstuff for poultry and can partly substitute the conventional and expensive dietary protein sources of layer chicken (Haustein *et al*., 1990). Aquatic plant species, because of their growth habit, appear not to accumulate secondary plant compounds and therefore offer a greater potential than tree leaves as a source of protein for monogastric animals (Bacerra *et al*., 1995). There has been an increased emphasis in the use of aquatic plants in poultry ration because the protein and other nutrient content in them are comparable to certain leguminous plants. Of these, the water fern *Azolla* is perhaps the most promising, being free crop of high nutritive value (Lumpkin and Plucknett, 1982).

Azolla is a free floating fresh water fern belonging to the family Azollaceae and order Pteridophyta. It is commonly found in tropics and sub-tropics and grows naturally in stagnant water of drains, canals, ponds, rivers, haors-baors and marshy lands. Anabaena-Azollae, living in the cavity of *Azolla* leaf, can fix high amount of atmospheric dinitrogen due to presence of symbolic algae in the leaves (Becking, 1979). With its sudden appearance in 2002, *Azolla* has now spread widely in aquatic ecosystems

* Corresponding author: aadilsheikh5@gmail.com
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of Kashmir valley. The *Azolla* species present in aquatic ecosystem of Kashmir has been identified as *Azolla cristsa* on the basis of presence of bicelled trichomes, hook shaped, multiseptate glochidia, and a 3-float megaspore apparatus with a granular perine surface (Ahad *et al*., 2012).

Azolla is a potential feed ingredient for poultry and livestock (Singh and Subudhi, 1978; Pannaerker, 1988). Use of *Azolla* meal up to 5% in the broiler ration has been reported to improve the performance in broiler diets as a safe level (Basak *et al*., 2002; Ara and Adil, 2012). Since, there is a very scanty literature available regarding the use of *Azolla* in layer chicken, the present study was undertaken to evaluate the effect of *Azolla cristata* in grower chicken and its subsequent effect on the layer performance.

Table I.- Ingredient composition of experimental diets.

Ingredients	T1	T2	T3	T4	T5
Maize	51.0	51.0	51.0	51.0	51.0
Soya-Full fat	14.0	14.0	14.0	14.0	14.0
Groundnut cake	10.0	9.5	9.0	8.5	8.0
Azolla cristata	-	0.5	1.0	1.5	2.0
De oiled rice bran	8.0	8.0	8.0	8.0	8.0
Wheat bran	7.0	7.0	7.0	7.0	7.0
Fish meal	4.0	4.0	4.0	4.0	4.0
Shell grit	3.0	3.0	3.0	3.0	3.0
Salt	0.5	0.5	0.5	0.5	0.5
Mineral mixture	2.5	2.5	2.5	2.5	2.5
	100.0	100.0	100.0	100.0	100.0
Chemical composition*					
Crude protein (%)	17.94	17.84	17.75	17.65	17.56
Metabolizable energy (Kcal/kg)	2659.7	2658.1	2656.7	2655.2	2653.8

Vitamin premix was added @ 10 g/quintal (Each g contained vitamin A, 82500 IU; vitamin D3, 12000 IU; vitamin B2, 50 mg; vitamin K, 10 m), Spectrimix-BE, 10 g/q (Each g contained vitamin B1, 80 mg; vitamin B6,16 mg; niacin, 120 mg; vitamin B12, 80 mg; calcium pantothenate, 80 mg; vitamin E, 160 mg; L-lysine HCl, 10 mg; DL methionine, 10 mg; calcium, 260 mg). *Calculated values.

MATERIALS AND METHODS

Bird husbandry and experimental protocol

To achieve the envisaged objectives, 240 key stone golden chicks at two weeks of age were individually weighed and randomly assigned into five groups having three replicates of 16 chicks each. The birds were reared and vaccinated as per the routine recommended procedures. The birds in the control (T_1) group were fed the basal diet, where as in other groups groundnut cake was replaced with 5 (T_2), 10 (T_3), 15 (T_4) and 20% (T_5) *Azolla*. The basal diets were formulated to meet the recommendations of Bureau of Indian Standards (1992) and shown in Table I. Up to 8 weeks of age, the birds were fed starter diet having crude protein (CP) 18 % and metabolisable energy (ME) 2750 Kcal/kg of feed. From 8 to 20 weeks, grower diet with 16 % CP and ME 2600 Kcal/kg feed was used. Thereafter, from 20th week onwards, layer diet was used having 18 % CP and ME 2700 Kcal/kg feed. The chemical analysis of *Azolla* (Table II) was done as per standard procedures of AOAC (1996).

Table II.- Analyzed chemical composition of *Azolla cristata.*

Constituent	% DM basis
Crude protein (CP)	22.06
Ether extract (EE)	3.62
Crude fibre (CF)	14.3
Nitrogen free extract (NFE)	33.4
Total ash	18.1
Calcium	2.04
Phosphorus	0.65

Collection, drying and mixing of Azolla

Azolla cristata has engulfed most of the water bodies in Kashmir, from where it was collected and then dried in the sunlight. After sun drying, it was ground and stored in the plastic bags until used for feeding. *Azolla* was mixed thoroughly in aforesaid quantities to small amount of feed (1 kg) in a premixer. The resultant mixture was then mixed with the rest of the feed in a mechanical blender until a thorough and consistent mixture was obtained.

Parameters recorded

The body weight and feed consumption of pullets was recorded on individual basis at bi-weekly intervals. At the age of 19 weeks, serum samples were collected from 4 birds per replicate for the determination of SGPT (Alanine Aminotransferase) and SGOT (Aspartate Aminotransferase) by means of auto analyzer using commercially available kits purchased from the Accurex biomedical company. The egg production was determined on daily basis while as egg quality parameters like egg weight, yolk weight, albumin height and width, shell weight and thickness were estimated from randomly collected 4 eggs per replicate on weekly basis from 33 to 40 weeks. Egg weight was estimated by means of a digital weighing balance. Each egg was carefully broken at the equator with a spatula and contents emptied on a dry, smooth and flat surface. The albumin height and width

were measured by means of a spherometer and vernier caliper respectively. The yolk was carefully separated from albumin and its weight was measured by digital balance. For determination of shell quality, the shells were washed and dried at room temperature for the determination of shell weight and thickness. The shell weight was measured by digital balance and thickness was measured without membranes by taking the mean of 3 pieces (from the 2 ends and the middle) using a micrometer.

Statistical analysis

The data obtained were statistically assessed by the Analysis of Variance (ANOVA) through Statistical Analysis System (SAS, 1996) considering replicates as experimental units. Duncan's multiple range test (Duncan, 1955) was used to test the significance of difference between means by considering the differences significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

The results of chemical analysis of *Azolla* (Table II) revealed presence of 22.06 % Crude protein (CP), 3.62% Ether extract (EE), 14.3% Crude fiber (CF), 33.4% Nitrogen free extract (NFE), 18.1% Total ash, 2.04% Calcium and 0.65% Phosphorus on dry matter basis. The values of CP content of *Azolla* estimated are in close concordance with the findings of Alalade and Iyayi (2006) and Raseena (2006). Further, Basak *et al*. (2002) reported that EE content of *Azolla* varied between 3.0 to 3.5%, in present study more or less similar value (3.62%) was obtained. The CF content of 14.3% is in agreement with the findings of Querubin *et al*. (1986). Parthasarathy *et al*. (2001) reported that NFE content of the fern varies between 38.85 to 44.06%, however, in our study NFE value of only 33.4% was recorded. The total ash content of 18.1% was recorded which corroborates with the findings of Basak *et al*. (2002) and Alalade and Iyayi (2006). *Azolla* contains calcium content of 2.11% (Parthasarathy *et al*., 2001) which is in close conformity with the value recorded in present study. Ali and Leeson (1995) reported 0.31% phosphorus in *Azolla* but in our study a value of 0.65% phosphorus was recorded. The variations in the nutrient composition of *Azolla* could be due to species difference and habitat variation of the taxon.

The performance parameters of grower chicken have been shown in Table III. The body weights of grower birds improved non-significantly ($p>0.05$) in the group fed *Azolla* (T_2) in the diet when compared with the control group, thus confirming the results of Querubin *et al*. (1986) and Basak *et al*. (2002) who reported an increase in the body weight of broiler chicken as a result of *Azolla* supplementation. However, among the other *Azolla* fed groups, a proportional decrease in the body weights was observed, thus confirming the results of Parthasarthy *et al*. (2002). Higher *Azolla* levels (10, 15 and 20%) result in decreased body weight than low levels which might be due to higher level of (Neutral Detergent Fibre) NDF (Buckingham *et al*., 1978) and lignin (Tamany *et al*., 1992) in *Azolla* meal which are the main limiting factors for its efficient utilization. Alalade and Iyayi (2006) attributed poorer growth rate of birds fed high levels of *Azolla* to the lower feed intake and consequently a reduced metabolizable energy intake. There was no significant ($p>0.05$) effect on feed consumption between control and 5% *Azolla* supplemented group (T_2); however a significantly lower feed consumption was observed in higher *Azolla* supplemented groups (10, 15 and 20%) compared to control and T_1. Lowest feed consumption was observed in the group fed 20% *Azolla* in the diet (T_5), confirming the results of Alalade and Iyayi (2006) who reported decrease in feed consumption with increase in the level of *Azolla* in the diet of poultry birds. The decrease in the feed consumption has been attributed to reduced palatability (Bested and Morento, 1985) and increased bulkiness of *Azolla* (Bacerra *et al*., 1995) which reduces its utilization. The feed conversion ratio of chicks fed diets

Table III.- Effect of *Azolla cristata* on the growth performance and liner function enzymes of grower chicken (Mean±SE).

Parameters	Treatments				
	T1	T2	T3	T4	T5
Body weight gain (9 to 19 wk in g)	847.3±7.35^{b}	869.5±7.58^{b}	830.7±9.24^{b}	825.6±6.33^{b}	799.3±8.76^{c}
Feed consumption (kg)	4.72±2.51^{c}	4.79±1.84^{c}	4.64±1.16^{b}	4.62±3.01^{b}	4.52±2.37^{a}
Feed conversion ratio	5.6	5.5	5.6	5.6	5.7
SGPT(µ/l)	13.4±0.13	13.7±0.09	12.1±0.25	12.8±0.06	13.1±0.17
SGOT(µ/l)	55.9±0.48	53.4±0.23	55.4±0.37	56.0±0.59	52.6±0.33

Means within the same row with different superscripts are significantly different ($p<0.05$); each value is the mean of 3 replicates. SGPT, alanine aminotransferase; SGOT, aspartate aminotransferase.

Table IV.- Effect of *Azolla cristata* on laying hen performance (33 to 40 week of age) (Mean±SE).

Parameters	Treatments				
	T1	T2	T3	T4	T5
No. of eggs laid	62.37±5.93	60.91±7.68	63.16±4.35	61.51±8.14	62.13±3.47
Egg weight (g)	57.19±1.93	58.16±2.37	58.20±3.14	57.54±2.63	57.82±3.06
Hen day egg production (%) (%)	74.82±4.36	72.61±6.53	75.67±4.76	72.55±7.81	73.14±3.69
Feed/bird/day (g)	148.0	148.0	148.0	147.0	147.0
Yolk weight (g)	13.36±0.46	13.57±0.38	13.14±0.41	13.35±0.27	13.52±0.55
Albumen height (mm)	6.37±0.15	6.36±0.09	6.34±0.22	6.38±0.18	6.35±0.11
Albumen width (mm)	62.49±4.54	61.98±5.39	62.74±7.21	61.57±6.08	62.51±5.36
Eggshell weight (g)	6.32±0.24	6.39±0.21	6.38±0.19	6.36±0.20	6.35±0.87
Eggshell thickness (mm)	0.41±0.07	0.43±0.08	0.43±0.01	0.42±0.04	0.43±0.10

Each value is the mean of 3 replicates.

wherein groundnut cake was replaced with *Azolla* showed no significant ($p<0.05$) effect among various groups including the control. Best FCR was noticed in the group fed 5% *Azolla* in the diet. Improvement in the FCR as a result of *Azolla* feeding was also reported by Ardakani *et al*. (1996) who found better FCR in birds fed low levels of *Azolla* compared to control. The mean values of serum constituents in chicken fed *Azolla* supplemented diets are shown in Table III. Supplementation of *Azolla* showed no significant ($p>0.05$) difference in the concentration of SGPT and SGOT levels between the chicks fed diets supplemented with *Azolla* and the control group reflecting that *Azolla* could be used up to the level of 20% in the diet of layer chicken without causing any adverse effect on the health of birds.

The results on laying performance (33 to 40 week of age) of chicken fed *Azolla* based diets are shown in Table IV. The differences in the total number of eggs laid, % hen-day-egg production, various external and internal egg parameters under different treatments were statistically non-significant ($p>0.05$). However, a non-significant ($p>0.05$) improvement was observed in the egg weight, shell thickness and weight was observed in the groups fed *Azolla* in the diet. This improvement in the strength of eggs could be attributed to the presence of good amount of calcium in *Azolla*, as it has been reported that the eggshell consists primarily of mineral matter most of which is calcium carbonate deposited in organic matrix (Austic and Nesheim, 1990). The thickness of the shell affects the quality of the egg which contributes to eggshell breakage (Rodriguez, 2013). Eggshell quality is one of the most important issues in the poultry industry, influencing the economic profitability as high breaking strength of eggshell and absence of shell defects are essential for protection against the penetration of pathogenic bacteria such as *Salmonella* spp. into eggs (Swiatkiewicz *et al*., 2010).

In conclusion, dietary inclusion of *Azolla cristata* at lower levels was beneficial in improving the performance of grower chicken but had no effect on the number of eggs laid in layer phase. Further, *Azolla* inclusion in the diet showed an improvement in the shell thickness of eggs which could prove beneficial in decreasing the incidence of egg breakage and thereby maximizing profits. The *Azolla* inclusion in the diet of layer chicken had no abnormal effect on SGPT and SGOT levels indicating that *Azolla* supplementation had no hepatotoxic effect in layer chicken even up to 20% levels.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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