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Research Article

Study on blood biochemistry and histomorphology of various organs in broiler chicken fed duckweed (*Lemna minor*) based diet with or without enzyme supplementation

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Abstract

An experiment was taken up to study the impact of (DW) duckweed (*Lemna minor*) based diet on blood biochemistry and histomorphology of liver, small intestine and kidneys of broiler chicken with or without enzyme supplementation. One hundred fifty one week old broiler chicks were weighed and distributed in a completely randomized design into 5 treatments (each replicated 3 times) viz. TC (Control-basal diet), T5 and T5E containing 5% DW with or without the use of enzyme respectively, T10 and T10E containing 10% DW with or without the use of enzyme respectively. The blood biochemistry like total protein, albumin, AST, ALT and BUN was similar in each treatment except glucose which significantly ($p<0.05$) increased with enzyme supplementation. Histomorphology of the liver, intestine and kidneys of birds fed various levels of DW with or without the use of enzyme supplementation showed no variation from normal structure. In conclusion DW upto 10% level can be used in the feed of broiler birds without any harmful effect on the blood biochemistry and histomorphological parameters of the liver, small intestine and kidneys.

Key words: Duckweed, Blood biochemistry, Broiler chicken, Enzyme, Histomorphology

Highlights

- Duckweed, with or without enzyme supplementation, was used in the diet of broiler chicken.
- The proximate analysis revealed that duckweed is a good source of protein (20.33%).
- Blood total protein and albumin were similar in each treatment except for glucose which increased within enzyme supplemented groups.
- Liver and kidney function tests like AST, ALT and BUN values were normal in each treatment group.
- The histomorphology of the liver, intestine and kidneys of birds showed no variation from the normal structure with the incorporation of duckweed or enzyme.

INTRODUCTION

The Indian poultry sector has evolved into a vibrant agribusiness growing at around 8-10% annually over the last decade with annual growth rates of 5.57% and 11.44% in egg and broiler production respectively (Wakchaure *et al*., 2016). Indian poultry sector accounts for about one percent of India's GDP and 11.70 percent of the GDP from the livestock sector providing direct or indirect employment to 6.5 million people (Rajendran *et al*., 2014; Sasidhar and Murari, 2015). In the Kashmir division of

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Jammu and Kashmir Union territory, India has high market demand for poultry meat and eggs because of the likeness for non-vegetarian food and tourist influx. However, there is a lack of good quality feed ingredients in Kashmir. Compared to other parts of the country, the cost of feed is more in Kashmir because of additional transportation cost incurred for importation from neighbouring states. Every year huge amount of money is spent on the purchase of ingredients of feed for poultry production. Therefore, it is imperative to explore other local cheap feed resources that are easily available and that too in sufficient quantity. Incorporation of non-conventional feed resources in the diet of poultry like aquatic macrophytes could prove a good alternative which may decrease the production cost of poultry in Kashmir.

Duckweed (DW) in this regard has some potential. It belongs to the family *Lemnaceae* and is a monocotyledon species. It is a small floating aquatic plant which thrives mostly on stagnant ponds, lakes, natural ponds and flooded rice fields throughout tropical countries (Leng *et al*., 1995). It has the properties of recycling nutrients from wastewater, besides provides an excellent protein source and also used in the production of animal feed and fuel ethanol (Cheng and Stomp, 2009). This plant grows quickly, producing high yields with high protein and mineral content and less fibre content. It is a rich source of proteins and amino acids, and also has many macro- and micro-nutrients besides vitamins and carotenoids (Soñta *et al*., 2019). It contains 28% crude protein, 3.7% crude fat, 33.8% ash,11.5% fiber and 42.6% carbohydrates (Tania *et al*., 2009). Even though the moisture content of DW could be the major limiting factor for chickens, it can play an important role in poultry feeding and has been postulated to be a better ingredient for feeding of poultry (Ahammad *et al*., 2003; Khandaker *et al*., 2007). In previous trials, DW has replaced protein sources such as sesame oil cake (Ahammad *et al*., 2003) and fishmeal (Effiong *et al*., 2009) at graded levels in animal feed.

Since some aquatic weeds are harmful, a research trial was taken to see whether a DW-based diet has any negative impact on blood biochemical profile and histomorphology of liver, small intestine and kidneys of broiler chicken.

MATERIALS AND METHODS

Experimental site and material: The experiment was carried out in the Instructional Poultry farm of the Division of LPM, FVSc and AH, SKUAST-K, Shuhama, Srinagar. The birds (Cobb strain) and feed were purchased from a local dealer. The DW was collected from the local water bodies, dried and properly stored until use.

Experimental diets: The starter diet until 21 days and a finisher diet until 42 days of age were offered. The diets were iso-nitrogenous and iso-caloric as per recommendations of the Bureau of Indian Standards (BIS, 1992). The ingredient composition of all the experimental diets is presented in Table 1. The percentage of crude protein, total ash, ether extract, crude fibre and total ash content of the experimental diets and dried samples of DW were determined as per the methods of the Association of Official Analytical Chemists (AOAC, 2005) and given in Table 2 and 3. Each diet was prepared from a uniform batch of ingredients and had the same composition. One hundred fifty one week old broiler birds were weighed and randomly distributed into 5 treatments (each replicated 3 times) viz, TC (Control-basal diet), T5 and T5E containing 5% DW with or without the use of enzyme respectively, T10 and T10E containing 10% DW with or without the use of enzyme respectively. The cocktail enzyme was added to the feed at the rate of 40 g per 100 kg feed. The enzyme contained cellulase 180000000 U, amylase 125000 U, xylanase 1800000 U, phytase 200000 U, protease 16000 U, lipase 40000 U and pectinase 7000 U.

The feed and water were provided *ad Lib.* to birds throughout the experiment with the use of recommended lighting schedule. Vaccination against prevalent diseases was done on

Table 1. Percent ingredient composition of experimental diets

Ingredients(kg)	TC	T5	T5E	T10	T10E
Maize	60.60	55	55	52	52
Soyabean meal	35.40	34	34	31	31
Duckweed	0	5	5	10	10
Vegetable oil	1	2	2	2	2
Limestone powder	1	1	1	1	1
Di calcium phosphate	1.25	1.25	1.25	1.25	1.25
Common salt	0.35	0.35	0.35	0.35	0.35
DL Methionine	0.10	0.10	0.10	0.10	0.10
Vitamin and mineral mixture	0.35	0.35	0.35	0.35	0.35
Total (kg)	**100**	**100**	**100**	**100**	**100**

Vitamin premix contained (units/kg) : Vitamin A 12375 IU, Riboflavin 7.5 mg, Cholecalciferol 1800 IU, Vitamin K 1.5 mg, Thiamine 1.2 mg, Pyridoxine 2.5mg, Cynacobalamine 12 mcg, Vitamin E 12 mg, Pantothenic acid 12 mg, Niacin 18 mg

Table 2. Chemical composition of experimental diets

Attributes (%)	C	T5	T5E	T10	T10E
Moisture	9.80	10.90	10.90	11.10	11.10
Crude protein	21.56	21.61	21.61	22.50	22.50
Crude fibre	5.18	5.24	5.24	5.60	5.60
Ether extract	4.17	4.09	4.09	4.06	4.06
Total ash	7.24	8.17	8.17	8.17	8.17
Nitrogen free extract	51.25	49.90	49.90	47.64	47.64
Metabolizable energy (Kcal/kg)*	2954	2902	2902	2852	2852

*Calculated value

Table 3. Chemical composition of duckweed

Attributes	Mean
Dry matter (%)	89.85
Moisture (%)	10.15
Crude protein (%)	20.33
Ether extract (%)	3.10
Crude fibre (%)	18.06
Total ash (%)	30.35
Acid Insoluble Ash (%)	7.00
Nitrogen Free Extract (%)	18.52
Calcium (%)	2.80
Phosphorus (%)	1.10
Tannins (%)	0.00
Phytins (%)	0.93
Metabolizable energy (Kcal/ kg)	1660.70

recommended days. Chicks were checked twice daily for mortality, if any. Similar managerial, hygienic and environmental conditions were followed for all the birds.

Blood biochemistry: At 42 days of age, two birds per each replicate were selected randomly for determination of blood biochemistry profile of birds after integration of DW with or without the use of enzyme supplementation in their ration. The blood sample was collected in sterilised anticoagulant added tubes and kept in a slanting position. The tubes were then incubated at 37°C for 1 hour. Afterwards, the clots of blood were broken, and tubes were centrifuged at 3000 rpm for 30 minutes. The resultant serum was then pipetted into small tubes and stored in the deep freezer (at -20°C) until required. Glucose, total protein, albumin, blood urea nitrogen (BUN), aspartate transaminase (AST) and alanine transaminases (ALT) were estimated from the serum samples using auto analyzer equipment by using respective biochemical kits from Accurex Biomedical Pvt Ltd, India.

Histomorphological study: At 42 days of age, 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 at room temperature until processing to prevent autolytic changes. The samples of tissues were cut in the thickness of 2-3 mm and prior to dehydrating in ascending grades of alcohol, the samples were washed with water for a few hours and thereafter cleared in benzene and then paraffin embedding was done. Tissue sections of 4-5 μm thickness were stained using the Harri's Haematoxilin and Eosin method to observe any patho-morphological changes in the affected tissues (Lillie, 1965).

Statistical analysis: The analysis of data regarding blood biochemistry values was done using one-way analysis of variance (ANOVA) as per Snedecor and Cochran (1994) using the statistical package SPSS version 21.0. The difference within the means was estimated using Duncan's multiple range test (Duncan, 1955) by considering the differences at a significant level ($p<0.05$).

RESULTS

Blood biochemistry: The values of blood biochemistry in birds fed various levels of DW with or without the use of enzyme supplementation are presented in Table 4. The serum glucose levels (mg/dL) in various treatments ranged from 140.16±3.53 in TC to 170.50±4.44 in T5E group. The average value of serum glucose significantly ($p<0.05$) increased in birds in which feed was supplemented with enzymes (T5E and T10E) compared to other treatments (TC, T5 and T10).

The values for the total protein varied between 4.03±0.32 for TC and 4.52±0.54 for T10E treatment group without any statistical significance among different treatments. The values for the albumin (g/dL) level ranged from 1.48±0.56 for T5 to 1.55±0.06 for T10 treatment group.

The values for the BUN (mg/dL) were recorded as 4.12±0.07, 4.26±0.13, 4.19±0.07, 4.28±0.09 and 4.22±0.06 for TC, T5, T5E, T10 and T10E respectively without any significant difference among various treatments. The serum AST(U/L) values ranged from 228.33±7.93 and 257.83±10.30 U/L without any statistical significance between different treatments. The serum ALT (U/L) values ranged between 13.70±0.15 and 14.35±0.02 U/L again without any statistical significance between different treatments.

Histomorphological study: The histomorphological structure of the liver in various treatments is presented in Fig. 1 to 5. The liver histomorphology of birds in each treatment had normal structure, the lobulae being undelimited by conjunctive septa, being identified on the basis of centrolobular venula and the Kiernan spaces near it. Hepatic cells had a polygonal shape, with 1 or 2 nuclei, with the nucleolus.

Table 4. Blood biochemical parameters of broiler chicken fed duckweed with or without enzyme supplementation

Parameters	Treatment				
	TC	T5	T5E	T10	T10E
Glucose (mg/dL)	140.16[a]±3.53	142.16[a]±4.14	170.50[b]±4.44	144.50[a]±4.64	167.16[b]±4.86
Total protein (g/dL)	4.03±0.32	4.13±0.27	4.25±0.29	4.41±0.23	4.52±0.54
Albumin (g/dL)	1.52±0.07	1.48±0.56	1.49±0.06	1.55±0.06	1.53±0.05
BUN (mg/dL)	4.12±0.07	4.26±0.13	4.19±0.07	4.28±0.09	4.22±0.06
AST (U/L)	228.33±7.93	245.33±6.65	241.83±6.88	257.83±10.30	251.00±6.80
ALT (U/L)	13.7±0.15	14.2±0.26	14.08±0.19	14.35±0.02	14.30±0.20

Means across rows bearing different superscripts differ significantly (P≤0.05)

The liver in each treatment showed normal structure of central vein, hepatic sinusoids, endothelium, portal vein, sinusoids and kupffer cells.

The histomorphological structure of the small intestine in various treatments is presented in Fig. 6 to 10. No variation in histomorphology of intestine among all the treatment groups. Columnar epithelial cells and enterocytes lined the villi with enterocytes having apical microvilli (brush border), epithelium of villi was intact with no degeneration or denudation and there was no infiltration of inflammatory cells.

The histomorphological structure of kidneys in various treatments is presented in Fig. 11 to 15. The histomorphology of kidneys in birds from each treatment showed normal histomorphology. The vast area was made up by cortex with medulla being only a small portion. The cortex and the medulla were organized in cones of various lengths distributed in a random fashion within the kidney. The renal corpuscle had an outer Bowman's capsule that was separated from a centrally located glomerulus by Bowman's space. The glomeruli had central core of mesangial cells as tightly packed, surrounded by capillary loops. The cuboidal epithelium made up the proximal and distal tubules. The proximal tubules' luminal surface area was found to be enhanced by a thick microvilli layer forming a brush border. It was concluded that the cortex, medulla, the renal corpuscle, Bowman's space, the glomeruli and the proximal and distal tubules showed normal structure.

DISCUSSION

Blood biochemistry: The mean values of serum glucose significantly ($p<0.05$) increased in chicken in which enzyme supplementation was done (T5E and T10E) compared to other treatments (TC, T5 and T10). The increased serum glucose values were found in enzyme supplemented groups. In previous studies, exogenous enzymes used in the feed of young chicks were found to be very beneficial in improving nutrient digestibility, thus making more nutrients and energy available for the growth of the chicks (Olukosi *et al.*, 2007). This could have been the reason for increased blood glucose concentration at later stage in birds due to enzyme supplementation in the present study. Similar findings were observed by Balamurugan and Chandrasekaran (2010), who found a significant or numerical increase in the serum glucose levels in enzyme supplemented groups compared to the control group.

The total protein values showed no statistical significance among different treatments. Similar findings were also documented by Thrall *et al.* (2004) and Cafe *et al.* (2012) with serum albumin values as 2.5-4.5 g/dL and 1.58 g/dL respectively for birds at 42 days. However, Aderemi *et al.* (2018) found that total protein and albumin values significantly improved with an increased level of DW supplementation meal in broiler ration.

The values for the BUN (mg/dL) did not differ statistically between different treatment groups. Yuan *et al.* (2008) also observed that enzyme supplementation did not affect the blood urea nitrogen level in broiler chicken. The serum AST (U/L) and ALT (U/L) levels showed no statistical significance between different treatments. The normal AST and ALT values indicated that no impairment of hepatic function occurred as a result of DW based diets up to 10% level.

Histomorphological study: Histomorphology of the liver in birds from each treatment showed the normal structure of the central vein, hepatic sinusoids, endothelium, portal vein, sinusoids and kupffer cells. Normal liver histomorphology with the incorporation of other weeds has already been documented by other workers. The histomorphological structure of the small intestine in various treatments showed no harmful effect. No harmful effect on small intestine histomorphology has been found with dietary incorporation of other weeds like Azolla in chicken (Ara *et al.*, 2012). The histomorphological structure of kidneys in various treatments showed normal histomorphology. The cortex, medulla, the renal corpuscle, Bowman's space, the glomeruli and the proximal and distal tubules showed normal structure.

So, the addition of neither duckweed nor enzyme had a detrimental impact on the histomorphology of these organs. Our findings are in concordance with the reports of Attia *et al.* (2021), who found that enzyme supplementation had no effect on intestinal histomorphological parameters. Wickramasuriya *et al.* (2019) also found no change in intestinal histomorphology by adding enzymes in the feed of birds.

On the basis of our study, it is concluded that DW upto 10% level with or without the use of enzyme supplementation can be supplemented in the feed of broiler chicken without any harmful effect on blood biochemistry and histomorphology of various internal organs.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: BZ: Carried out the experiment; IUS and MTB: Conceptualized the idea; SA, HAA and SB: Helped during the trial and processing of samples; SSN and UM: Helped in collection of samples.

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