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Blood profile and carcass traits of Kadaknath broilers fed on various levels of azolla (*Azolla pinnata*)

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Abstract

*The present study examined the blood composition and carcass traits of Kadaknath broilers raised in the Poultry Research Centre, School of Agriculture, ITM University, Gwalior, between September and November 2023 for a total of 56 days. They were fed varied amounts of azolla (*Azolla pinnata*) powder. A total of 120 Kadaknath day-old-chicks were divided into 30 flocks. The flocks were randomly allotted to five treatments with six replications. They were housed in a deep litter system with eight hours of darkness and sixteen hours of light. Starter (week 1; 23% CP and 3000 kcal/kg ME), grower (weeks 2–6; 22% and 3100 kcal/kg), and finisher (weeks 7-8; 20% CP and 3200 kcal/kg ME) feeds were provided to the broilers in the control group (Az_{00}). The sun-dried azolla powder replaced the ration in the test groups at 2.5, 5.0, 7.5, and 10.0 per cent in $Az_{2.5}$, $Az_{5.0}$, $Az_{7.5}$, and $Az_{10.0}$, respectively. On the final day of the experiment, the haemeto-biochemical profiles of the blood and carcass characteristics were compared. The blood parameters in $Az_{5.0}$ were higher than the others: PCV (43.40±1.31%), TLC (66.83±0.47 n/µl), TEC (1.40±0.03 n/µl), basophils (1.69±0.04%), heterophils (18.72±0.18%), and haemoglobin (13.77±0.10 g/dl). $Az_{5.0}$ had higher live weight (1151±9 g), dressed weight (888±17 g), eviscerated weight (831±36 g), eviscerated weight (73.1±2.6%), and heart weight (1.65±0.1 g). The study concluded that the broiler ration may be replaced at a 5 per cent level with sun-dried azolla powder to increase the productivity of the Kadaknath broilers.*

Keywords: Azolla, Blood, Broiler, Carcass, Kadaknath.

Introduction

Azolla ferns (*Azolla pinnata*) are rich not only in protein and the necessary amino acid contents but also in minerals and vitamins. The application of azolla to increase poultry production varies (El-Ghany, 2020). Since azolla is an

inexpensive and abundant alternative plant protein source that enhances feed conversion ratio (FCR), energy efficiency, and performance without having a negative impact on chickens, adding it to the diet reduces production cost (Alalade and Lyayi, 2006; Namra *et al*., 2010). The majority of the data indicate improvements in the production of chicken-fed diets containing azolla, despite contradictory findings. According to Sarria and Preston (1995), broiler growth increased when azolla was used in place of soybean protein, up to a 15% level. The body weight at 4, 6, and 8 weeks of age was considerably increased when dietary azolla protein was added to the broiler chicken diet at a level of 6% (Sundararaju *et al*., 1995). The improved FCR have also been reported that following azolla feeding in broiler rations at 5–15% (Ardakani *et al*., 1996) and 5% (Seth *et al*., 2013; Nagshi *et al*., 2014) azolla levels. The growth rate, feed conversion ratio, and energy efficiency of 2 to 6-week-old broilers were enhanced when azolla was added to their diet up to 5% without having a negative impact on the palatability or mortality rates (Basak *et al*., 2002). Because commercial feed might enhance the weight of chickens, it was advised that 20% fresh azolla be used in their meals (Subudhi and Singh, 1978). When Azolla was increased up to 15% (Querubin *et al*., 1986) and 30% (Dhumal *et al*., 2009), a notable variation in feed intake was observed. Adding up to 10% of azolla meal to growing pullet rations is safe and does not pose any health risks (Alalade *et al*., 2007). The group that received supplements containing up to 5% azolla alone experienced increase in body weight gain; however, high concentrations of azolla's fiber severely impact the birds' hunger, which in turn slows down their gain in growth (Saikia *et al*., 2014). Following the addition of azolla to the diet at levels of 5% and 10% (Acharya *et al*., 2015), 7.5% (Kumar *et al*., 2018), and 15% (Samad *et al*., 2020), improvements in body weight gain and FCR were observed. The addition of Azolla at any level significantly decreased the production's feed costs. Azolla supplementation at a modest level of inclusion has been shown to benefit chicken (Bacerra *et al*., 1995), and studies on broilers have shown positive economic returns (Parthasarathy *et al*., 2002). The highest amount of economic advantage was demonstrated by the 10% inclusion of azolla. Due to lower FCR, mortalities, and production costs and higher net profit, it was advised that adding azolla at levels of 5% or 7% is suitable for safe and successful broiler production (Islam and Nishibor, 2017). Contradictorily, the feeding of broilers on azolla resulted in similar body weight and growth as those kept on a maize-soybean meal (Ali and Leeson, 1995) and a decrease in the body weights of broilers (Parthasarathy *et al*., 2002) because of decreased feed intake (Alalade and Lyayi, 2006). A linear reduction was found in feed intake with increasing azolla levels in the chicken broiler diets (Ara *et al*., 2015).

Haematological and biochemical testing may be helpful for diagnosing health, observing how ill birds respond to treatment regimens, and determining the prognosis for specific poultry diseases, even if they have not been widely utilized in avian medicine. By calculating the normal values for hematological and biochemical variables, a comprehensive database for the blood profiles of domestic (Elagib *et al*., 2012) and genetically approved industrial (Talebi *et al*., 2005) poultry species was created. Blood parameter analyses employ standard values for multiple purposes, such as evaluating

health disorders (Harper and Lowe, 1998), predicting potential resistance to environmental conditions (Silversides *et al*., 1997), estimating future body weight (Singh *et al*., 1998), diagnosing diseases (Prameela Rani *et al*., 2011), and assessing poultry immune status (Seiser *et al*., 2000). These data could be used in breeding programs to enhance the genetic composition of native hens, in addition to being beneficial for diagnosis and care (Alewi *et al*., 2012). Comprehending the normal physiological parameters in a given environment is crucial for efficient breeding, nutrition, illness prevention, and treatment.

The Kadaknath chicken is mostly raised by the tribal people who live in the western districts of Madhya Pradesh (Jhabua and Dhar) and the neighboring areas of Gujarat and Rajasthan. This breed yields meat that is dark in color and has a flavorful flesh (Panda and Mahapatra, 1989). Eggs and meat from Kadaknath are considered to be rich sources of protein and iron.

In view of the above, the objective of this investigation was to determine the blood composition and carcass characteristics of Kadaknath chickens fed varying levels of azolla (*Azolla pinnata*).

Materials and Methods

The present work was carried out at the poultry farm of the School of Agriculture, ITM University, Gwalior, situated at 26.140°N and 78.196°E longitude at an altitude of 197 m MSL. The soil is sandy loam in texture with uniform topography. It has a subtropical climate with an average annual rainfall of 700 mm. The summer temperature goes up to 48 °C, and in winter it remains as low as 3°C.

The day-old Kadaknath chicks in a total of 120 were randomly selected and divided into 30 flocks of 4 birds each. The flocks were further randomly allotted to five treatments with six replications. They were tagged with the help of wing banding. The chicks were maintained in a deep litter system of housing with 16 hours of light and 8 hours of darkness. The chicks were vaccinated against Marek's, Ranikhet, and infectious bronchitis diseases on 0, 7, and 14 days, respectively. The experiment was carried out during September and November 2023 and lasted for 56 days.

The broilers in the control group (Az_{00}) were offered a ration containing maize, soybean meal, de-oiled rice bran, stone grit, dicalcium phosphate, NaCl salt, DL-methionine, L-lysine HCl, trace minerals, vitamins A, D_3, K, and B complex, toxin binder, tyrosine, and coccidiostat, maintaining levels of 23% and 3000 kcal/kg during the starter phase (week 1), 22% and 3100 kcal/kg during the grower phase (weeks 2–6), and 20% crude protein and 3200 kcal/kg metabolizable energy during the finisher phase (weeks 7-8), respectively (BIS, 2022). The ration was replaced in test groups with sun-dried azolla powder at a level of 2.5, 5.0, 7.5, and 10.0 percent in $Az_{2.5}$, $Az_{5.0}$, $Az_{7.5}$, and $Az_{10.0}$, respectively.

The heparinized blood samples from one chicken selected randomly from each flock were drawn on the last day of the experiment. The hematological parameters RBCs (million/mm³ or million/µl), WBCs (thousand/mm³ or thousand/µl), DLC (%), Hb (g/dl), PCV (%), MCV (µ³), MCH (pg/cell), and MCHC (g/dl) were recorded in the blood samples. Blood drawn was subjected to estimation of hematological parameters by using an auto-hematology analyzer. Differential WBC counts were

made on monolayer blood films and stained using Giemsa stain after fixing them with methyl alcohol.

Biochemical parameters were recorded from serum isolated from the blood sample collected from six birds in each group. The blood samples were collected in a sterile vial and kept in a slating position for 30 minutes, followed by centrifugation at 2000 rpm for 15 minutes. The biochemical parameters included total protein, albumin, globulin, AG ratio, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), cholesterol, bilirubin, blood urea nitrogen (BUN), and creatinine by using a standard diagnostic kit (Erba Pvt. Ltd.).

One bird from each flock was randomly selected to be sacrificed for finding out the carcass traits of Kadaknath broilers on the last day of the experiment.

The data obtained were analyzed using the analysis of variance (ANOVA) single factor following the procedure of Snedecor and Cochran (1994). The data analysis pack of MS Office Excel 2016 was used for this purpose (UQ Library, 2016).

Results and Discussion

Table 1 shows the impact of azolla levels on the haemato-biochemical profile of Kadaknath broilers. Blood haemoglobin levels in Kadaknath broilers were found to be significantly higher ($P<0.01$) in $_{5.0}$ and lower in Az_{00}, with the values of 11.30±0.17 in Az_{00}, 12.40±0.15 in $Az_{2.5}$, 13.77±0.10 in $Az_{5.0}$, 11.86±0.12 in $Az_{7.5}$, and 11.68±0.17 g/dl in $Az_{10.0}$. The findings suggested that azolla can replace poultry ration up to a 5 per cent level, with an increasing trend followed by a declining one. The same trends were observed in the levels of monocytes (1.96±0.05 in Az_{00}; 3.29±0.09 in $Az_{2.5}$; 5.67±0.15 in $Az_{5.0}$; 3.64±0.10 in $Az_{7.5}$; 3.65±0.10% in $Az_{10.0}$), basophils (1.56±0.04 in Az_{00}; 1.48±0.04 in $Az_{2.5}$; 1.69±0.04 in $Az_{5.0}$; 1.52±0.07 in $Az_{7.5}$; 1.54±0.08% in $Az_{10.0}$), heterophils (15.72±0.15 in Az_{00}; 16.37±0.16 in $Az_{2.5}$; 18.72±0.18 in $Az_{5.0}$; 18.36±0.18 in $Az_{7.5}$; 18.34±0.18% in $Az_{10.0}$), TLC (58.53±0.41 in Az_{00}; 60.63±0.43 in $Az_{2.5}$; 66.83±0.47 in $Az_{5.0}$; 61.61±0.43 in $Az_{7.5}$; 61.59±0.43 n/µl in $Az_{10.0}$) and TEC (0.98±0.03 in Az_{00}; 1.21±0.03 in $Az_{2.5}$; 1.40±0.03 in $Az_{5.0}$; 1.15±0.03 in $Az_{7.5}$; 1.13±0.03 n/µl in $Az_{10.0}$) in the blood of Kadaknath broiler. Blood basophil level in the blood of Kadaknath broilers (1.56±0.04 in Az_{00}, 1.48±0.04 in $Az_{2.5}$, 1.69±0.04 in $Az_{5.0}$, 1.52±0.07 in $Az_{7.5}$, and 1.54±0.08% in $Az_{10.0}$) was found to be significantly higher ($P<0.01$) in $Az_{5.0}$ and lower in $Az_{7.5}$. The results reported that azolla replacement up to a level of 5% of the concentrate increased basophil levels, and then the same decreased with no specific trend. Blood eosinophil levels in Kadaknath broilers were found to be significantly higher ($P<0.01$) in $Az_{7.5}$ and lower in Az_{00}, with values of 1.62±0.05 in Az_{00}, 1.96±0.06 in $Az_{2.5}$, 2.00±0.06 in $Az_{5.0}$, 2.31±0.07 in $Az_{7.5}$, and 2.32±0.07 per cent in $Az_{10.0}$. The findings showed that azolla can replace poultry rations up to a 7.5 per cent level, with an increasing trend followed by a declining one. Blood PCV level in the Kadaknath broilers (39.23±1.18 in Az_{00}, 40.31±1.22 in $Az_{2.5}$, 43.40±1.31 in $Az_{5.0}$, 37.45±1.13 in $Az_{7.5}$, and 37.42±1.13% in $Az_{10.0}$) was found to be significantly higher ($P<0.01$) in $Az_{5.0}$ and lower in $Az_{10.0}$. The results reported that azolla replacement up to a level of 5% of the concentrate increased PCV levels and then declined. The H-L ratio in the Kadaknath blood was higher in $Az_{10.0}$ and in Az_{00}, with the values

0.15±0.03, 0.17±0.01, 0.22±0.01, 0.22±0.01, and 0.25±0.01 in Az_{00}, $Az_{2.5}$, $Az_{5.0}$, $Az_{7.5}$, and $Az_{10.0}$, respectively. It can be presumed that the increased level of replacement of ration with azolla caused an increase in the H-L ratio in the blood of Kadaknath broilers.

The current study's results closely align with those of Kamel and Hamad (2021), who found that, when compared to the control group, dietary dried Azolla significantly improved several haemato-biochemical parameters at different levels, but the PCV value showed no significant difference. When compared to the control group, all groups fed DA had higher Hb and TLC levels. The high phenolic and flavonoid content of Azolla may be the cause of this increased value. However, Mishra *et al*. (2016) reported that birds given Azolla at levels of 5 and 7.5% had greater values of lymphocytes and heterophils than the control birds. According to Thavasi *et al*. (2020), the PCV and RBC of the birds given a meal supplemented with 5% Azolla showed substantial increases. These outcomes concur with those of Kumar *et al*. (2018), who found no differences in the levels of HB, heterophils, and TLC when DA was supplemented at 2.5, 5, 7.5, and 10%.

Table 1. Effect of Azolla levels on haemato-bichemical profile of Kadaknath broilers.

Parameter	Azolla (*Azolla pinnata*) levels					
	Az_{00}	$Az_{2.5}$	$Az_{5.0}$	$Az_{7.5}$	$Az_{10.0}$	*P-Value*
Heamoglobin (g/dl)	11.30±0.17^{d}	12.40±0.15^{b}	13.77±0.10^{a}	11.86±0.12^{c}	11.68±0.17^{c}	**
Eosinophils (%)	1.62±0.05^{c}	1.96±0.06^{b}	2.00±0.06^{b}	2.31±0.07^{a}	2.32±0.07^{a}	**
Monocytes (%)	1.96±0.05^{d}	3.29±0.09^{c}	5.67±0.15^{a}	3.64±0.10^{b}	3.65±0.10^{b}	**
Basophils (%)	1.56±0.04^{ab}	1.48±0.04^{b}	1.69±0.04^{a}	1.52±0.07^{b}	1.54±0.08^{b}	**
Heterophils (%)	15.72±0.15^{b}	16.37±0.16^{b}	18.72±0.18^{a}	18.36±0.18^{a}	18.34±0.18^{a}	**
PCV (%)	39.23±1.18^{ab}	40.31±1.22^{ab}	43.40±1.31^{a}	37.45±1.13^{b}	37.42±1.13^{b}	**
TLC (n/µl)	58.53±0.41^{c}	60.63±0.43^{b}	66.83±0.47^{a}	61.61±0.43^{b}	61.59±0.43^{b}	**
TEC (n/µl)	0.98±0.03^{c}	1.21±0.03^{b}	1.40±0.03^{a}	1.15±0.03^{b}	1.13±0.03^{b}	**
HL ratio	0.15±0.03^{c}	0.17±0.01^{c}	0.22±0.01^{b}	0.22±0.01^{b}	0.25±0.01^{a}	**

^{a,b,c} *Values bearing different supersriptes within the row differed significantly P<0.05.*

Carcass Traits: The effect of azolla levels on carcass traits of Kadaknath broilers has been shown in Table 2. The live weight (1001±21 in Az_{00}; 1026±24 in $Az_{2.5}$; 1151±9 in $Az_{5.0}$; 1078±23 in $Az_{7.5}$; 1077±28 g in $Az_{10.0}$), dressed weight (733±9 in Az_{00}; 751±22 in $Az_{2.5}$; 888±17 in $Az_{5.0}$; 800±16 in $Az_{7.5}$; 801±23 g in $Az_{10.0}$), eviscerated weight (567±15 in Az_{00}; 600±45 in $Az_{2.5}$; 831±36 in $Az_{5.0}$; 681±33 in $Az_{7.5}$; 664±41 g in $Az_{10.0}$), and Eviscerated portion (57.3±0.7 in Az_{00}; 58.6±2.3 in $Az_{2.5}$; 73.1±2.6 in $Az_{5.0}$; 63.2±1.6 in $Az_{7.5}$; 63.4±2.2 per cent in $Az_{10.0}$) were recorded higher in the group $Az_{5.0}$, whereas lower in Az_{00}. The findings indicated that the above carcass traits improved with the increased level of azolla replacement up to 5 per cent of the ration and then declined. The thigh portion (1.44±0.02 in Az_{00}; 1.42±0.02 in $Az_{2.5}$; 1.37±0.03 in $Az_{5.0}$; 1.32±0.01 in $Az_{7.5}$; 1.33±0.01% in $Az_{10.0}$) in the Kadaknath carcass was higher in Az_{00} and lower in

$Az_{10.0}$, pointing out that increasing replacement of ration with azolla caused a decrease in the thigh portion of the carcass. The heart weight (1.26±0.07 in Az_{00}; 1.36±0.08 in $Az_{2.5}$; 1.65±0.1 in $Az_{5.0}$; 1.35±0.08 in $Az_{7.5}$; 1.19±0.07 g in $Az_{10.0}$) was also higher in $Az_{5.0}$ but lower in $Az_{10.0}$, indicating that this carcass traits improved with the increased level of azolla replacement up to 5 per cent of the ration and then declined. The remaining traits of the Kadaknath broiler carcass, such as dressing (73.2±0.8-77.1±1.5%), thigh weight (14.23±0.28-15.74±0.3g), drumstick weight (14.96±0.34-16.19±0.41g), and portion (1.39±0.05-1.54±0.04%), liver weight (2.07±0.08-2.90±0.24g), gizzard weight (3.12±0.17-3.74±0.15g), and portion (0.31±0.02-0.33±0.02%) and heart portion (0.11±0.01-0.14±0.01%) were all recorded as nonsignificant (P>0.05). As a result, the findings showed that the above-mentioned carcass traits remained unaffected by the replacement of ration with different levels of azolla in Kadaknath broiler carcasses.

Table 2. Effect of Azolla levels on carcass traits of Kadaknath broilers.

Parameter	Azolla (*Azolla pinnata*) levels					
	Az_{00}	$Az_{2.5}$	$Az_{5.0}$	$Az_{7.5}$	$Az_{10.0}$	*P-Value*
Live weight (g)	1001±21^{c}	1026±24^{c}	1151±9^{a}	1078±23^{b}	1077±28^{b}	**
Dressed weight (g)	733±9^{c}	751±22^{c}	888±17^{a}	800±16^{b}	801±23^{b}	**
Dressed weight (%)	73.4±1.0	73.2±0.8	77.1±1.5	74.2±0.8	74.3±0.8	NS
Eviscerated weight (g)	567±15^{c}	600±45^{c}	831±36^{a}	681±33^{b}	664±41^{b}	**
Eviscerated weight (%)	57.3±0.7^{c}	58.6±2.3^{c}	73.1±2.6^{a}	63.2±1.6^{b}	63.4±2.2^{b}	**
Thigh weight (g)	14.39±0.17	14.56±0.44	15.74±0.3	14.23±0.28	14.34±0.42	NS
Thigh weight (%)	1.44±0.02^{a}	1.42±0.02^{a}	1.37±0.03^{c}	1.32±0.01^{d}	1.33±0.01^{d}	**
Drumstick weight (g)	15.41±0.2	15.14±0.51	16.19±0.41	14.96±0.34	15.53±0.39	NS
Drumstick weight (%)	1.54±0.04	1.47±0.02	1.41±0.04	1.39±0.05	1.45±0.04	NS
Liver weight (g)	2.07±0.08	2.34±0.27	2.9±0.24	2.42±0.16	2.36±0.11	NS
Liver weight (%)	0.21±0.01	0.23±0.03	0.25±0.02	0.22±0.01	0.22±0.01	NS
Gizzard weight (g)	3.12±0.17	3.37±0.16	3.74±0.15	3.5±0.18	3.31±0.18	NS
Gizzard weight (%)	0.31±0.02	0.33±0.02	0.32±0.01	0.32±0.01	0.31±0.02	NS
Heart weight (g)	1.26±0.07^{bc}	1.36±0.08^{b}	1.65±0.1^{a}	1.35±0.08^{b}	1.19±0.07^{c}	**
Heart weight (%)	0.13±0.01	0.13±0.01	0.14±0.01	0.13±0.01	0.11±0.01	NS

^{a,b,c} *Values bearing different supersriptes within the row differed significantly P<0.05.*

The results of this study are consistent with those of Naghshi *et al*. (2014), who found that adding 5% Azolla powder to the diet raised the percentage of carcass efficiency. The data from this study was further supported by the fact that there were no significant differences between the treatments for the liver and gizzard. According to Bhattacharya *et al*. (2018), adding 4.50% of Azolla meal to the broiler diet increased the target percentage significantly, but the other carcass attributes did not differ significantly across treatments. Furthermore, Mishra *et al*. (2016) discovered that the only differences in the cut-up sections (thigh, drumstick, neck, and back) and carcass quality criteria (dressing percentage and ready to cook yield) were in the wings and liver weight percentage. The giblet percent was higher

in the 3% Azolla meal fed group compared to the control and 6% Azolla fed group, according to *Lakshmi et al. (2019)* and Varadharajan *et al*. (2019). According to Thavasi *et al*. (2020), birds fed 0, 3, 6, 9, and 12% Azolla meal had average dressing percentages of 64.11, 66.14, 69.07, 67.12, and 68.62. Azolla meal feeding resulted in numerically higher dressing percentages at the 6% and 9% levels, respectively, demonstrating the advantageous effects of herbals like Azolla meal feeding on dressing percentage.

Conclusion

It can be concluded on the basis of present investigation that the broiler ration may be replaced at a 5 per cent level with sun-dried azolla powder to increase the productivity of the Kadaknath broilers.

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