



Nutritional Evaluation of *Azolla pinnata* Meal in Broiler Chicken Diets

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ABSTRACT

Background: This study was carried out to evaluate the nutritional value of *Azolla pinnata* meal (AZM) in broiler chickens' diets.

Methods: The experiment was laid in a completely randomized design comprising a total of one hundred and sixty Cobb 500 strain of day-old broiler chicks which were randomly assigned into 4 dietary treatment groups with 4 replications, comprising 10 birds per replicate for a six-week duration. The iso-caloric and iso-nitrogenous diets were tested at 0, 10, 20 and 30% levels of AZM inclusion designated as T₁, T₂, T₃ and T₄ respectively. The experimental diets were offered *ad libitum* throughout the trial. At the end of the feeding trial, one bird per replicate was selected for carcass evaluation. Proximate compositions of AZM and meat samples were determined. Data generated were statistically assessed by Analysis of Variance.

Result: Proximate analysis of AZM revealed crude protein, crude fibre, ether extract and total ash values of 22.5, 7.37, 3.59 and 19.46, respectively. Results also showed no significant difference (P>0.05) in live weight gain but, feed intake significantly differed (P<0.05) with T₁ having the highest value of 2.91 kg/bird and T₄ recorded the least value of 1.80 kg/bird. The feed conversion ratio was also found to be statistically (P<0.05) lower in T₄ and highest in T₁ group. The inclusion of AZM in the diets did not (P>0.05) influence the carcass and internal organs characteristics except for empty gizzard. However, the crude protein content of broiler meat was improved significantly at T₂ 10% AZM level.

Key words: *Azolla*, Broiler chickens, Carcass characteristics, Chemical composition, Growth performance, Nutrient evaluation.

INTRODUCTION

The intensive poultry production system, mainly comprising broilers and layers, is dependent on the adequate supply of feed and water, protected housing and other infrastructural facilities for its rapid growth. Broilers need adequate amounts of all essential nutrients to be productive and healthy. Requirements for these nutrients vary in quantity depending on species, age, productive state, environmental condition and health status (Anonymous, 2011). For the sustainability of the venture, it is necessary that resources are available locally and competition with humans is minimal. Thus, the availability of quality feed at a reasonable cost is key to successful poultry operations (Basak *et al.*, 2002; Toor and Goel, 2024).

The major concern of any livestock industry particularly in developing countries is the attainment of sustainable production. This has proved difficult in most of these countries because of the dependency and excessive demand for conventional feed ingredients that are either imported or expensive where they locally exist. Given that feed is the largest single expense related to chicken production, nutrient management is a top priority for today's contemporary poultry business (Srilatha *et al.*, 2018). Thus, for poultry to contribute effectively to poverty alleviation and improve food security (Toor and Goel, 2024), it is essential to increase their productivity by improving strategies of feeding through the utilization of unconventional local feed resources (Semenye, 1990). The high protein requirement of broilers coupled with the limited ability to satisfy these needs from non-protein nitrogen sources necessitates the search for locally available protein-rich feed resources that

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are not directly consumed by man such as the species of the hydrophytes *Azolla*.

Azolla is a small-leaved floating aquatic fern that grows naturally on stagnant water in drains, canals, ponds, ditches, rivers and water bodies including marshy lands and wetlands. It is native to the tropics, subtropics and warm temperate regions of Africa, Asia, North and South America and parts of Australia. *Azolla* is a genus of seven species (*A. pinnata*, *A. filiculoides*, *A. mexicana*, *A. nilotica*, *A. caroliniana*, *A. japonica* and *A. microphylla*) of aquatic ferns. Of these species, the water fern *Azolla pinnata*, is perhaps the most promising from the point of view of ease of cultivation, minimal water for propagation, growth in unexploited niches, high productivity and good nutritive value (Prabina and Kumar, 2010; Chandrababu and Parvathy, 2022; Nimbalkar and Patil, 2024).

The present study was therefore undertaken to evaluate the nutritive value of *Azolla pinnata* meal, the

growth performance, meat composition and carcass and internal organs characteristics of broiler chickens fed *Azolla* meal-based diets.

MATERIALS AND METHODS

The experiment was conducted at the Poultry Unit of Teaching and Research Farm, Bayero University, Kano, which falls within latitude 11° 58' 47" North and longitude 8° 25' 52" East of the equator in the Sudan savanna zone bordering the guinea savanna vegetation in the south. The research was carried out under the ethical guidelines for non-regulated procedures of the University. *Azolla pinnata* was sourced from a rice paddy field in Barwa town, Kano, Nigeria. Contaminants (weeds, straws, stones, snails etc.) and other foreign particles adhered to the leaves and roots were removed after collection. It was dried under shade on a clean concrete floor for 7 days, packaged in sacks, stored at room temperature and used for the formulation of experimental rations. Graded levels of *Azolla* were incorporated into the diets prior to mixing and subsequent feeding.

The experiment was laid in a Completely Randomized Design (CRD) comprising a total of one hundred and sixty (160) day-old strain of Cobb 500 broiler chicks randomly assigned into 4 dietary treatment groups with 4 replicates, containing 10 birds per replicate. Four

iso-nitrogenous and iso-caloric experimental broiler starter and finisher diets were formulated with AZM inclusion at different levels. The experimental diets were tested at 0, 10, 20 and 30% levels of AZM inclusion designated as T_1 , T_2 , T_3 and T_4 respectively. Birds were fed starter mash at 1 - 4 weeks and finisher mash at 4 - 6 weeks both containing AZM including a control diet with no AZM as presented in Tables 1 and 2. Data were collected to determine the growth performance, feed intake and feed conversion ratio of broiler chickens fed with the experimental diets.

At the end of the feeding trial, birds were fasted for feed for 12 hours and one bird per replicate was randomly selected for carcass evaluation. The dry matter, crude protein, crude fibre, ether extract, total ash and nitrogen free extract chemical composition of AZM and samples of broiler breast meat were determined by the methods of the Association of Official Analytical Chemists (AOAC, 2005) at the animal science laboratory of the Department of Animal Science, Faculty of Agriculture, Bayero University. Data generated were statistically assessed by analysis of variance (ANOVA) using the general linear model procedures of Statistical Analysis Software (SAS) package version 9.1 (2003). Least Significant Difference (LSD) was used to separate the significantly different means at a probability level of 5%.

Table 1: Ingredient Composition of Experimental Diets at Broiler Starter Phase (0-4 weeks).

Feed ingredient (kg)	Treatments (% AZM)			
	T_1	T_2	T_3	T_4
<i>Azolla</i> meal (AZM)	0	10	20	30
Maize grain (MZ)	61	55	51	47
Soya bean meal (SBM)	35	31	25	19
Fish meal	1.6	1.6	1.6	1.6
Bone meal (BM)	1.5	1.5	1.5	1.5
Premix*	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Methionine	0.2	0.2	0.2	0.2
Lysine	0.2	0.2	0.2	0.2
Total	100	100	100	100
Calculated composition				
ME (Kcal/kg)	2956	2974	2981	2991
Crude protein (%)	23.21	23.32	23.41	23.62
Crude fibre (%)	4.1	4.8	5.4	6
Ether extract (%)	4.1	4.2	4.2	4.2
Lysine (%)	2.7	3.1	3.4	3.8
Methionine (%)	0.7	0.8	1	1.1
Calcium (%)	0.8	1.1	1.3	1.6
Phosphorus (%)	0.9	0.9	0.9	0.8

*Composition of premix (Vitamin-mineral mixture): Vitamin A: 2 400 000 IU; Vitamin D: 1 000 000 IU; Vitamin E: 16 000 IU; Vitamin K: 800 mg; Vitamin B1: 600 mg; Vitamin B2: 1 600 mg; Vitamin B6: 1 000 mg; Vitamin B12: 6 mg; Niacin: 8 000 mg; Folic acid: 400 mg; Pantothenic acid: 3 000 mg; Biotin: 40 mg; Antioxidant: 3000 mg; Cobalt: 80 mg; Copper: 2000 mg; Iodine: 400; Iron: 1200 mg; Manganese: 18 000 mg; Selenium: 60 mg; Zinc: 14 000 mg.

RESULTS AND DISCUSSION

Proximate composition of *Azolla pinnata* meal

The proximate analysis of AZM was carried out to determine dry matter, crude protein, crude fibre, ether extract, total ash and nitrogen free extract (Table 3). All the values obtained fall within the range for nutrient content in cereals as reported by Olomu (2011). The results revealed that AZM contained (%DM) 22.50 crude protein, 7.37 crude fibre, 3.59 ether extract, 19.46 ash and 47.08 nitrogen free extract. A higher value of 24.26% was reported by Cherryl *et al.* (2014) which was higher than the value obtained in the present study. Alalade and Iyayi (2006) reported crude protein values within the range of 19-31% which is consistent with the current study. The values obtained for crude fibre, ether extract and total ash in the present study were found to be similar to values reported by Basak *et al.* (2002) who reported that the crude fibre content of AZM varied from 7.27 - 15.71%, ether extract and ash varied from 3.5 - 3.7% and 15.6 - 24.2% respectively. Prasanna *et al.* (2011) reported a total ash content value of 16.21% whereas Anitha *et al.* (2016) reported 10.50 - 15.82% of total ash content which were lower than the value obtained in the present study. The possible reason for variability in the chemical composition could be the response of *Azolla* to environmental conditions like temperature, light intensity, water availability and soil nutrients which affect its chemical composition.

Growth performance of broiler chickens fed diets containing graded levels of AZM

There were significant differences ($P < 0.05$) amongst treatments for feed intake (FI), daily weight gain (DWG)

and feed conversion ratio (FCR) (Table 4). The highest feed consumption and the highest weight gain in AZM fed diets were recorded in diet containing 10% AZM. This result is contrary to the findings of Basak *et al.* (2002) and that of Bhuyan *et al.* (1998) who reported that the supplementation of broiler feed with AZM had no significant effects on feed consumption. However, the findings of previous studies such as that of Bacerra *et al.* (1995) were in line with the results of the present study which studied the influence of AZM on the performance of growing birds and reported a significant difference in feed consumption between treatments. The increase in feed intake in broiler chickens fed diets containing 20 and 30% levels of AZM could be attributed to its palatability. The significantly high feed intake recorded in this study was similar to the result reported by Dhumal *et al.* (2009) which demonstrated a significant increase in feed intake with an increase in AZM level up to 30%. However, Bacerra *et al.* (1995) indicated a benefit from AZM supplementation at a low level of inclusion which is consistent with the current study. Conversely, Ara *et al.* (2015) found a linear reduction in feed intake with increasing AZM levels in the diets of broiler chickens. The decrease in the feed consumed may be due to reduced palatability (Bested and Morento, 1985) and increased bulkiness of AZM (Bacerra *et al.*, 1995) which reduced its utilization.

Alalade and Iyayi (2006) attributed the lower growth rate of broiler chicken fed high levels of AZM to the lower feed intake. The inclusion of 10% AZM in broiler diets significantly increased weight gain than other AZM-fed dietary treatments which is consistent with the findings of

Table 2: Ingredient composition of experimental diets at broiler finisher phase (4-6 weeks).

Feed ingredient (kg)	Treatments (% AZM)			
	T ₁	T ₂	T ₃	T ₄
<i>Azolla</i> meal (AZM)	0	10	20	30
Maize grain (MZ)	63	60	59	56
Soya bean meal (SBM)	33	26	17	10
Fish meal	1.6	1.6	1.6	1.6
Bone meal	1.5	1.5	1.5	1.5
Premix*	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Methionine	0.2	0.2	0.2	0.2
Lysine	0.2	0.2	0.2	0.2
Total	100	100	100	100
Calculated nutrient composition				
ME (Kcal/kg)	3120	3146	3149	3156
Crude protein (%)	20.41	20.46	20.47	20.49
Crude fibre (%)	4.1	4.6	5.1	5.7
Ether extract (%)	4.1	4.1	4.2	4.2
Lysine (%)	2.7	3	3.4	3.8
Methionine (%)	0.7	0.8	0.9	1
Calcium (%)	0.8	1.1	1.3	1.6
Phosphorus (%)	0.9	0.9	0.8	0.8

*Vitamin-mineral mixture.

Basak *et al.* (2002). The findings of the present study are also in line with those of Samad *et al.* (2020) who reported that the addition of AZM up to 15% level enhances the growth performance traits without negative effects on nutrient digestibility of broiler chickens. They concluded that AZM can be added to the broiler diet at a 10% level without adverse effects on birds' performance. Improved DWG was recorded in birds fed 10% AZM. The significant difference in DWG is in line with the findings of Anitha *et al.* (2016) who studied the influence of AZM usage on the production performance of broiler chickens and reported a significant difference in body weight gain. Seth *et al.* (2013) detected an increase in weight gain in chickens fed 5% and 10% AZM-based diets over control whose result is contrary to the findings of this study. The large amount of crude fiber in diets containing high levels of AZM (20-30% inclusion level) led to a decrease in body weight by reducing the digestion, absorption and availability of nutrients. These findings are in agreement with that of Saikia *et al.* (2014) who demonstrated that the lowest body weight gain was in the group supplemented with a 15% level of AZM and they explained that the increase in the fiber content in high concentrations of AZM supplemented feed negatively affects the appetite of the birds and consequently reduces the growth rate. Treatment 4 (30% AZM) presented the best FCR amongst the treatment groups. Studies from Namra *et al.* (2010) indicated an improved FCR in AZM supplemented broiler feed which is in agreement with the present study.

Survivability during the experimental period was non-significant which indicates that AZM had no deleterious effect on broiler chicken's performance. This result is similar with that of Castillo *et al.* (1981) who also found no toxic effect of dietary AZM in broilers. The difference in the

results obtained from different studies may be dependent on the environment, management and quality of the AZM.

Carcass characteristics of broiler chickens fed diets containing graded levels of AZM

There were no significant ($P>0.05$) differences in all the parameters measured of carcass and internal organs characteristics except for empty gizzard weight. The value for empty gizzard weight ranged between 54(g) and 44(g) with the lowest weight recorded in T_2 and the highest in T_3 . Birds fed 20% AZM presented higher empty gizzard weight as illustrated in Fig 1. The non-significant difference observed in this study follows the findings of Pinkihan (2013) who reported no significant effect of AZM in terms of dressing percentage and Dhumal *et al.* (2009) who reported non-significant differences amongst the means of various traits such as carcass yield percentage and abdominal fat pad.

Results exhibited corroborated the findings of Mateos *et al.* (2012) that the change in the quantity and quality of crude fibre content affects the development and the function of the digestive organs including the size of the empty gizzard and gastrointestinal tract. Similarly, the results are per the finding of Pinkihan (2013) who reported that the weight of giblets from birds fed 20% AZM was numerically heavier. There were no significant ($P>0.05$) differences observed for non-edible parts in all the parameters measured across the treatments and the reason for this was not clearly understood.

Composition of broiler chickens meat fed *Azolla pinnata* meal-based diets

The result for the proximate composition of broiler chickens' breast meat is presented in Table 5. The inclusion level of AZM significantly ($P<0.05$) affected the content of some components of broiler breast meat. The values for dry matter were significantly higher ($P<0.05$) in the control group and lower in 10% AZM group. Similarly, the values for protein content were significantly ($P<0.05$) higher in 10% AZM and lower in 30% AZM inclusion. The values ranged from 29.87 to 32.37%. The incorporation of AZM had a beneficial impact on the chemical composition of broiler chicken meat, elevating the protein concentration in breast meat, as Szczurek (2008) previously demonstrated an increase in the

Table 3: Proximate composition of *Azolla pinnata* meal.

Parameters (%)	Composition
Dry matter	90.30
Crude protein	22.50
Crude fibre	7.37
Ether extract	3.59
Total ash	19.46
Nitrogen free extract	47.08

Table 4: Growth performance of broiler chickens fed *Azolla pinnata* Meal-based diets.

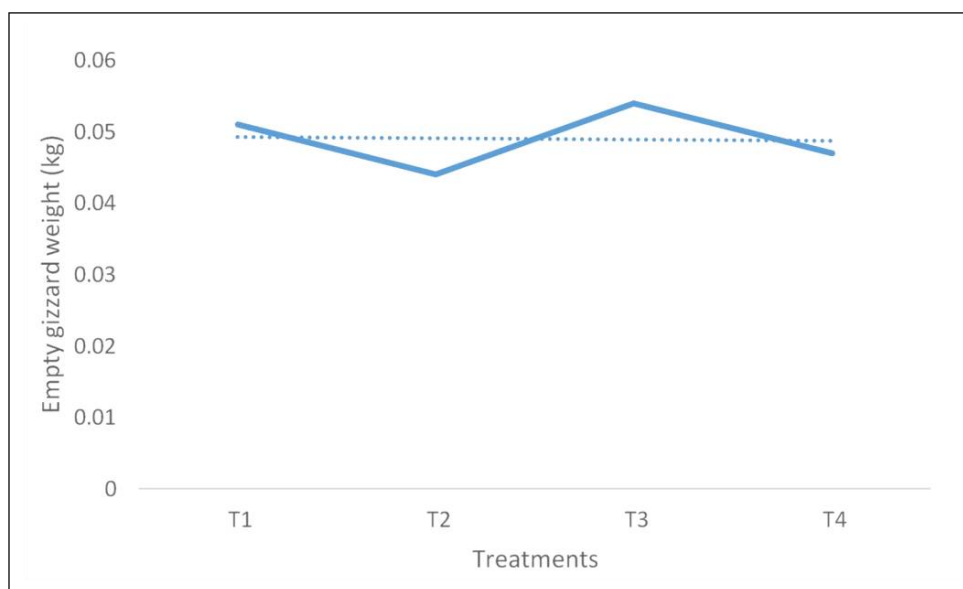
Parameters	Treatments (% AZM)				SEM
	T_1 (0%)	T_2 (10%)	T_3 (20%)	T_4 (30%)	
Initial weight (g)	0.450	0.45	0.45	0.45	.003
Final weight (g)	2.19	2.13	1.91	1.93	0.05
Total weight Gain (g)	1.74	1.68	1.46	1.48	0.05
Daily weight Gain (g)	0.052 ^a	0.051 ^{ab}	0.045 ^b	0.046 ^{ab}	0.001
Total feed intake (g)	2.91 ^a	2.20 ^b	2.08 ^c	1.80 ^d	0.005
Feed conversion ratio	1.68 ^a	1.34 ^b	1.44 ^{ab}	1.23 ^b	0.04
Mortality (%)	0.5	0	0.5	0	0.19

^{a,b,c} Values with common superscripts in a row did not differ statistically ($p>0.05$), SEM = Standard error of means.

Table 5: Composition of Broiler Chickens Meat Fed *Azolla pinnata* Meal-based diets.

Parameters (%)	Treatments (% AZM)				SEM
	T ₁ (0%)	T ₂ (10%)	T ₃ (20%)	T ₄ (30%)	
Dry matter	97.25 ^a	96.80 ^b	96.90 ^{ab}	97.08 ^{ab}	0.03
Protein	29.92 ^b	32.37 ^a	30.88 ^{ab}	29.87 ^b	0.18
Ether extract	8.33 ^b	18.33 ^a	17.62 ^a	6.09 ^c	0.12
Total ash	6.78	6.78	6.8	8.63	0.2

^{a,b,c} Values with common superscripts in a row did not differ statistically ($p>0.05$), SEM =Standard error of means.

**Fig 1:** Heavier empty gizzard weight of broiler chickens fed 20% *Azolla pinnata* meal-based diet.

crude protein content and reduced proportion of fat in broiler chickens fed barley-based diets. Significant reduction ($P<0.05$) of fat content in the experimental broiler meat was recorded in the control and 30% AZM groups which is quite similar to the findings of Szczurek (2008). No significant differences were observed for ash content amongst the treatment groups. This result contradicts the findings of Sharma (2014) who reported that no significant differences between treatments of broiler breast meat were observed at different levels of AZM inclusion in the diets of broiler chickens.

CONCLUSION

The present study concluded that AZM can be incorporated at 10% level in broiler chickens' diet for better growth and improved meat protein content. Its inclusion in broiler chickens' diets poses no health risk to both broiler chickens and consumers. Further studies should investigate other performance indices for enhanced production that is cost effective.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the University's Committee of Experimental Animal Care and Handling Techniques.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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