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Growth Performance, Carcass Characteristics, Hematobiochemical Parameters, Cecal Bacterial Load and Economic Efficiency of Koekoek Chickens Fed Azolla

Research Article

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ABSTRACT

An experiment was conducted to evaluate the effect of azolla meal (AZM) on growth performance, carcass characteristics, hematobiochemical parameters, cecal bacterial load and economic efficiency of Koekoek chickens. One hundred forty-four chickens were randomly distributed into four treatment groups of 12 chickens in 3 replications in completely randomized design. The first treatment (T1) was control group, in which conventional chicken diet was provided without AZM, whereas T2, T3 and T4 contained 5%, 10% and 15% AZM, respectively. At the end of the experiment, nine chicks per treatment were slaughtered for carcass characteristics assessment. From this slaughtered chickens, cecal content was collected for bacterial load analysis. Before slaughtering, blood samples were collected to analyze their hematobiochemical parameters. The results indicate that the inclusion of up to 10% azolla did not significantly differ ($P \geq 0.05$) from the control group in both growth performance and carcass characteristics. Lymphocytes and packed cell volume (PCV) of T1 were significantly greater ($P<0.05$) than those of T3. Total protein (TP), albumin, globulin and glucose contents of T3 were significantly greater ($P<0.05$) than T4. Cecal bacterial load indicated that *Lactobacillus* was significantly greater ($P<0.001$) in T2 and T3, whereas *E. coli* was significantly lower in T3. The results of the cost–benefit analysis revealed that chickens fed 10% azolla presented significantly greater ($P<0.05$) economic efficiency. Overall, this experiment implies that dietary inclusion of 10% azolla can increase net income without affecting the growth performance, carcass characteristics and health of chickens.

KEY WORDS *Azolla filiculoides*, biochemical attributes, cecal bacteria, economic efficiency, growth performance.

INTRODUCTION

The fast-growing population and rising incomes in developing countries have led to a 57% increase in the global demand for meat between 2005 and 2050 (Alexandratos and Bruinsma, 2012). Poultry is expected to experience one of the greatest rises in demand for animal protein in these parts of the world. Because poultry diets require more protein than those of ruminant animals, the growing demand for poultry intensifies the need for high-protein feed sources. Protein supplements are among the most expensive feed ingredients, and according to Kim *et al.* (2019), soybean meal is the main source of high-quality protein. Feed accounts for up to 70% of total production costs, and therefore, agricultural and industrial byproducts have been evaluated as feed ingredients to reduce those costs (Sayehban *et al.* 2016). Reducing feed costs is therefore essential for poultry industry to maintain competitiveness

and sustainability.

The utilization of crop residues and byproducts in the past as alternatives to soybean meal in feeds was not successful, mainly due to their high fiber content and poor digestibility (Sayehban *et al.* 2016). Thus, studies on the utilization of nonconventional poultry feed ingredients have attracted the attention of scientists worldwide (Bhattacharyya *et al.* 2016). In this regard aquatic plants could offer a sustainable and nutrient-rich alterative for chicken diets, providing essential biological benefits without competing with human food sources.

Azolla, a free-floating aquatic fern that forms a symbiotic relationship with the nitrogen-fixing blue–green alga *Anabaena azollae*, could serve as a sustainable feed substitute for poultry, pigs, and livestock (Mishra *et al.* 2016a). The potential incorporation of azolla meal into future poultry feed formulations holds promise for reducing feed costs while simultaneously ensuring optimal health and immune function in birds (Mishra *et al.* 2016b). Some studies have documented the use of azolla as protein feed supplement and its impact on growth performance, carcass traits and hematobiochemical parameters in chickens (Mishra *et al.* 2016a; Abdelatty *et al.* 2021) in other parts of the world.

Among different species, *Azolla filiculoides* is distributed in the Fogera and Libo Kemkem Plains of Ethiopia (Menale *et al*. 2025). The use of the fern as possible protein poultry feed source is not exploited. Since the chemical composition and the nutritional value of azolla vary with species, location, and growth condition (Brouwer *et al.* 2018), evaluating the effects of its inclusion on various performance parameters of chickens can provide valuable insight to the scientific research and practical applications in poultry nutrition. Besides, different researchers recommend different azolla inclusion rates and focused only on limited parameters (Mishra *et al.* 2016a; Abdelatty *et al.* 2020; Samad *et al.* 2020). According to Hafeez *et al*. (2024), the inclusion levels of azolla are much controversial in the published literatures and little information are available on the effect of azolla on the growth performance, *E. coli*, and other performances. Therefore, it is important to evaluate the impact of *Azolla filiculoides* on growth performance, carcass traits, blood hematological parameters, serum profiles, cecal bacterial counts, and the economic efficiency of Koekoek chickens.

MATERIALS AND METHODS

Ethics approval

The research activity was undertaken after it received approval from Bahir Dar University, College of Agriculture and Environmental Science Research Ethics Review Committee (Ref. No: 004-2025).

Collection and preparation of azolla meal

Azolla filiculoides was collected from Woreta Agricultural, Technical, and Vocational Education Training College farm. The fern was cultivated using cow dung, soil and phosphorus from earthen ponds prepared for this purpose. It was manually harvested every week, washed with pure water and air dried. It was then ground and stored at room temperature in airtight plastic bags until it was used as azolla meal (AZM). AZM samples were chemically analyzed before being used in the broiler diets (Table 1). The experimental feeds were mixed with AZM using horizontal feed mixer.

Table 1 Chemical composition of azolla meal used in the diet

Chemical composition	Amount
Dry matter (%)	90.4
Crude protein (% DM)	29.5
Crude fiber (% DM)	12.8
Ether extract (% DM)	7.1
Ash (% DM)	15.2
Metabolizable energy (kcal/kg DM)	2576.8

Chemical analysis of feed ingredients

Representative samples from each feed ingredient used in the experiment were collected by taking multiple sub samples and analyzed at the animal nutrition laboratory prior to formulating the dietary treatments. The analyses included determination of dry matter (DM), ether extract (EE), crude fiber (CF), and ash content, following the procedures of AOAC (2005). Nitrogen content was measured using the Kjeldahl method, and crude protein (CP) was calculated by multiplying the nitrogen value by 6.25. Metabolizable energy (ME) levels of the feed ingredients were estimated using the following formula (Wiseman, 2013):

ME (kcal/kg DM)= 3951 + 54.4 EE - 88.7 CF - 40.8 Ash

Chickens and management

A total of 144-day-old Koekoek chicks were purchased and brooded together for the first two weeks. After 15 days, twelve chicks per replicate were randomly allocated to each experimental unit. The experimental house was divided into twelve separate pens. The pens were cleaned, disinfected with formalin, left to dry, and bedded with sawdust to a depth of 5 cm before the chicks were introduced. Each pen was heated using a 200 W heat bulb.

During the first week, the brooder temperature was maintained between 35 °C and 32 °C, after which it was gradually lowered by 2–3 °C per week until reaching the optimal temperature. Chicks were exposed to a slowly decreasing lighting period from 24 hours at day-old to 13 hours per day. Fresh and clean water and feeds were provided *ad libitum* throughout the thirteen weeks of experimental period.

Chicks were vaccinated for new castle disease, fowl pox and Gumboro.

Experimental design and preparation of diets

Twelve chicks per replicate, arranged in a completely randomized design (CRD), were assigned to four treatments, each with three replications. Four treatment diets were used at two different stages of growth. In accordance with the recommendation by AFRDO, (2022), starter and finisher diets were prepared in accordance with chicken requirements (Table 2). A starter diet with 22% CP and 2800 kcal ME/kg and a finisher diet with 20% CP and 3000 kcal ME/kg were provided to all the experimental chickens. The treatment diets were T1, T2, T3 and T4, which included 0%, 5%, 10% and 15% AZM, respectively. However, to adjust the nutrient level of the diets, the proportions of maize and soybean meal were changed.

Data collected

Average feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), and mortality percentage were measured to assess differences among the treatment groups. Feed offered and feed refused were weighed and recorded daily throughout the experiment to determine feed intake for each replicate and treatment. Feed intake was calculated by subtracting the refused feed from the amount offered. Individual chick body weights were recorded at the start of the experiment and weekly thereafter until completion. Average daily weight gain was calculated by dividing the total body weight gain by the number of experimental days. The feed conversion ratio was determined as the amount of feed consumed per unit of body weight gained. Daily mortality was recorded for each replicate and treatment, and mortality percentage was calculated by dividing the number of dead chicks by the total number of chicks and multiplying by 100.

Carcass characteristics

At the end of the experiment, 36 chickens (9 per treatment) were slaughtered to assess carcass characteristics. The birds were fasted for twelve hours prior to slaughter. Each chicken was weighed before starvation, slaughtered, and bled for about three minutes. The carcasses were then dipped in hot water and manually defeathered. Following this, the carcasses were eviscerated—removing the head, heart, crop, pancreas, kidneys, lungs, proventriculus, small and large intestines, caeca, urogenital tracts, and lower legs—and hung on the evisceration line to drain for 15 minutes before weighing. The back, thighs, drumsticks, wings, neck, and breast were used to evaluate commercial carcass yield. Dressing percentage was calculated as the carcass weight divided by the slaughter weight, multiplied by 100. The giblets, including the heart, gizzard, and liver, were weighed, and their percentage was calculated relative to the slaughter weight.

Hematobiochemical analyses

At the end of the experiment, three chickens from each replicate were randomly selected for blood sampling. Blood was drawn from the wing vein using a 5 mL syringe fitted with a 22-gauge sterile hypodermic needle. Each sample was divided into two labeled containers for hematological and serum biochemical analysis. Hematological parameters measured included red blood cell (RBC) count, hemoglobin (Hb), lymphocytes (Lym), packed cell volume (PCV), platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), following standard laboratory procedures. Serum biochemical parameters—such as total protein (TP), albumin, cholesterol, triglycerides, low-density lipoprotein (LDL), glucose, alkaline phosphatase (ALP), uric acid, and creatinine—were analyzed using an automated chemistry analyzer (Grindem, 2011). Globulin concentration was calculated by subtracting albumin from total protein.

Cecal bacterial load

At the end of the experiment, the bacterial load was determined by collecting cecal material from each slaughtered chicken. One gram of sample from each sampled chicken was placed into a sterile Petri dish separately after being diluted with 9 mL of 1% peptone broth and homogenized. The contents were subsequently placed in a sterile tube and mixed via a single-tube mixer, resulting in a single pooled sample for each group. Total bacterial counts were determined using plate count agar. Viable counts of *Lactobacillus* and *E. coli* were obtained by plating serial 10-fold dilutions (prepared in 1% peptone solution) onto lactobacilli medium III agar and MacConkey agar plates, respectively. The lactobacilli medium III agar plates were incubated aerobically at 39 °C for 24 hours, while the MacConkey agar plates were incubated aerobically at 37 °C for 24 hours. *Salmonella* spp. were enumerated using a colorimetric reaction on xylose lysine deoxycholate (XLD) agar plates incubated at 37 °C for 48 hours.

Economic efficiency analysis

The cost–benefit analysis of the use of AZM in the diet of chickens was computed considering the total variable cost (TVC) and net income. The TVC was calculated by adding the cost of feeds, the cost of chicks and miscellaneous costs (labor, medicines, heaters and litter). The net income was computed by subtracting the total cost from the sale of chickens (total return).

Table 2 Feed ingredients and calculated chemical compositions of the experimental diets[1]

Feed Ingredients (%)	Starter				Finisher			
	T1	T2	T3	T4	T1	T2	T3	T4
Maize	48.0	46.4	43.7	41.2	59.0	57.8	55.8	53.6
Soybean meal	32.0	28.6	26.3	23.8	26.0	22.2	19.2	16.4
Azolla meal	0.0	5.0	10.0	15.0	0.0	5.0	10.0	15.0
Noug cake	6.0	6.0	6.0	6.0	5.0	5.0	5.0	5.0
Wheat bran	6.0	6.0	6.0	6.0	5.0	5.0	5.0	5.0
Bone meal	2.0	2.0	2.0	2.0	1.5	1.5	1.5	1.5
Salt	1.0	1.0	1.0	1.0	0.5	0.5	0.5	0.5
Vitamin premix	1.2	1.2	1.2	1.2	1.0	1.0	1.0	1.0
L-Lysine	1.0	1.0	1.0	1.0	0.6	0.6	0.6	0.6
DL-Methionine	0.8	0.8	0.8	0.8	0.4	0.4	0.4	0.4
Dicalcium phosphate	2.0	2.0	2.0	2.0	1.0	1.0	1.0	1.0
Total	100	100	100	100	100	100	100	100
Calculated composition								
Crude protein (%)	21.89	21.98	22.03	21.93	20.05	20.10	19.95	20.03
Crude fiber (%)	3.53	3.71	3.81	3.62	3.45	3.40	3.51	3.39
Metabolizable energy (kcal/kg)	2810.5	2813.5	2808.5	2812.5	3008.5	3015.5	3012.0	3019.5
Calcium (%)	1.14	1.12	1.15	1.12	1.00	1.02	0.98	1.02
Total phosphorus (%)	0.81	0.83	0.78	0.79	0.73	0.74	0.71	0.73
Available phosphorus (%)								

[1] T1: Control (0% air dried azolla); T2: 5% air dried azolla; T3: 10% air dried azolla and T4: 15% air dried azolla.

The economic efficiency was calculated as follows: Economic efficiency= net revenue / feed cost (Hassan and Awad, 2017).

Statistical analysis

All data collected during the feeding trial were analyzed using analysis of variance (ANOVA) with the general linear model (GLM) procedure in SAS software version 9.3 (SAS, 2004). When significant treatment effects were detected ($P<0.05$), mean comparisons were conducted using Tukey's HSD test. The statistical model applied for data analysis was:

$$Y_{ij}= \mu + T_i + e_{ij}$$

Where:
Y_{ij}: response variable.
μ: overall mean.
T_i: effect of the i^{th} treatment (i=1- 4).
e_{ij}: random error term.

RESULTS AND DISCUSSION

The effect of different levels of dietary AZM on growth performance parameters is presented in Table 3. There was a significant difference in final body weight (FBW) and BWG between the treatment groups. Accordingly, T4 had significantly lower ($P>0.05$) FBW and BWG values than the other treatment groups. This effect is likely attributed to reduced palatability and increased bulkiness caused by the higher fiber content of the increased azolla inclusion level (Hafeez *et al.* 2024).

Another reason could be due to its anti-nutritional factor content. High levels of azolla reduce weight by increasing both the metabolic rate and energy consumption, thus reducing the digestibility of ingredients (Abd and Taha, 2021). Azolla contains up to 50 g kg-1 (poly)phenolic compounds (Brouwer *et al.* 2018) of which condensed tannins in particular may decrease the digestibility of azolla feed. Similarly, *A. filiculoides* contains 1.6% alkaloid (Menale *et al.* 2025), which could be a limiting factor for its digestibility and affect growth performance. Similar results were reported by Abdelatty *et al.* (2020), who reported that body weights did not differ significantly between groups with up to 10% azolla feeding for broiler chickens.

On the other hand, these results disagreed with those of Samad *et al.* (2020), who reported that chickens fed 15% azolla had significantly higher FBW and BWG. Similarly, increasing the level of azolla in the diet of broiler chickens to 12% significantly increased the final body weight and daily body weight gain (Kamel and Hamed, 2021). These variations could be attributed to the differences in the species of azolla and the stage of maturity of the plant species used (Samad *et al.* 2020). Similarly, Acharya *et al.* (2015) also reported that White Pekin broiler ducks supplemented with 10% azolla presented the lowest body weight gain. Despite these discrepancies, 10% AZM in the current study did not cause reduced growth performance which supports that AZM can be included at least at 10%.

In this study, there was no significant difference in the feed conversion ratio (FCR) between the treatment groups. This result is in agreement with the findings of Ara *et al.* (2018) in layers and Samad *et al.* (2020) in broilers.

Table 3 Effect of Azolla meal on the growth performance of Koekoek chickens

Parameter	Treatments[1]				SEM	P-value
	T1	T2	T3	T4		
IBW (g/chick)	83.6	84.3	84.1	83.8	2.1	0.999
FBW (g/chick)	1598.6[a]	1594.4[a]	1608.0[a]	1498.6[b]	16.8	0.037
BWG (g/chick/day)	19.8[a]	19.6[a]	19.7[a]	18.4[b]	0.2	0.015
FI (g/chick/day)	59.2	59.6	58.5	57.7	0.6	0.723
FCR	2.97	3.03	2.97	3.13	0.04	0.485
Mortality (%)	0.00	2.78	2.78	5.56	1.4	0.487

T1: Control (0% air dried azolla); T2: 5% air dried azolla; T3: 10% air dried azolla and T4: 15% air dried azolla.
IBW: initial body weight; FBW: final body weight; BWG: body weight gain; FI: feed intake and FCR: feed conversion ratio.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

On the other hand, the FCR was found to be improved linearly with the addition of azolla meal up to 12% (Kamel and Hamed, 2021). The variation might be due to the azolla strain and poultry species used. The results indicated that the inclusion of 5% to 10% *Azolla filiculoides* in the diet had effects similar to those of the commercial diet (no azolla) on the growth performance of Koekoek chickens. The result of this research implies that there could be maximum safe level of inclusion for poultry which needs to be investigated.

The effects of AZM on the carcass characteristics of Koekoek chickens are shown in Table 4. The dressing percentage and major cut-up parts, including the breast, thigh, drumstick, and wings were among the traits that differed significantly among the treatments. The dressing percentage of chickens in T1, T2 and T3 were similar and significantly higher (P<0.05) than those in T4. This can be explained by the factors influencing growth performance mentioned previously. Besides, the antioxidant properties of carotenoids and biopolymers as noted by (Acharya *et al.* 2015), likely contributed to the observed benefits. In line with this study, Mishra *et al.* (2016a) also reported that the dressing percentage of Chabro chicken supplemented with up to 10% AZM was not significantly different from that of the control group. However, these results disagreed with the finding of Punyatong *et al.* (2024), who reported that chickens provided with different levels of azolla had nonsignificant differences.

Consistent with the current findings, Mishra *et al.* (2016a) reported that thigh and drumstick properties were not significantly different when chickens were fed 0, 5%, 7.5% or 10% AZM in their diets. The inconsistencies in carcass trait reports are primarily related to the growth performance of the experimental animals. The results of the dressing and major cut-up percentages in the current study are therefore parallel to the significant difference in the final body weight of the chickens. In line with this study, Punyatong *et al.* (2024) noted that 15% fresh azolla supplementation resulted in a lower wing percentage.

In contrast, Bhattacharyya *et al.* (2016) reported nonsignificant differences. These discrepancies suggest that the effects of azolla supplementation may vary depending on the levels of supplementation, age of the broilers, and the type of feed used (Khan *et al.* 2024).

As shown in Table 5, there was no significant difference in hematological indices between the treatment groups except for lymphocytes and packed cell volume (PCV). The similarity of the RBC and Hb between the treatment groups suggests that oxygen-carrying capacity is unaffected showing no indication of anemia.

Kamel and Hamed (2021) reported similar RBC among the treatments in broilers. Mishra *et al.* (2016b) reported that the Hb concentrations of broilers fed 0%, 5%, 7.5% and 10% AZM did not significantly differ. Similarly, Verma *et al.* (2024) reported similar Hb levels among the treatment groups of quail fed 0%, 5% or 10% azolla. The MCV, MCH and MCHC in the present study were not significantly different. The difference could be attributed to species and its processing method, nutrient composition, and anti-nutritional factors of azolla.

In the present study, T3 had significantly lower (P>0.05) lymphocytes than the control group, while there was no difference between the azolla-fed groups. This could be because of subtle changes in immune modulation or availability of micronutrients. However, on the basis of the findings of Kamel and Hamed (2021), lymphocytes were significantly greater in broilers fed 4% azolla, followed by those fed 12%, and the least significant was the control group. In another study, Chabro chickens fed 5% and 7.5% azolla had significantly more lymphocytes than the control chickens. In contrast, Kumar *et al.* (2018) reported nonsignificant differences between treatments.

The normal range of PCV in chickens is 22%-35% (Bounous and Stedman, 2000). The PCV of T1 and T2 were significantly greater (P<0.05) than that of T3, which is in line with the finding of Verma *et al.* (2024), who reported 5% azolla-fed quails presented significantly greater PCV.

Table 4 Carcass characteristics of Koekoek chickens fed azolla meal

Carcass traits	Treatments				SEM	P-value
	T1	T2	T3	T4		
Slaughter weight (g)	1621.6^{a}	1572.0^{ab}	1624.0^{a}	1503.0^{b}	18.1	0.024
Dressed weight (g)	936.8^{a}	897.0^{a}	939.0^{a}	823.0^{b}	15.1	0.003
Dressing (%)	58.0^{a}	57.0^{a}	58.0^{a}	54.7^{b}	0.5	0.013
Breast (%)	17.3	17.3	17.5	17.0	0.1	0.524
Thigh (%)	9.8^{a}	9.4^{b}	9.7^{a}	9.2^{b}	0.2	0.005
Drumstick (%)	9.9^{a}	9.6^{a}	10.0^{a}	8.2^{b}	0.2	0.002
Wing (%)	4.3^{a}	4.1^{a}	3.9^{a}	3.1^{b}	0.1	<0.001
Back (g)	120.7	118.6	119.4	118.0	0.7	0.634
Neck (g)	52.2	51.0	53.6	50.0	0.6	0.143
Skin (g)	88.0	86.4	87.5	86.3	0.6	0.802
Heart (g)	9.5	9.3	10.8	9.0	0.3	0.256
Liver (g)	37.0	36.3	39.0	35.7	1.0	0.794
Gizzard (g)	49.3	47.3	50.0	45.4	0.7	0.059
Giblet (%)	6.0	6.0	6.0	5.7	0.1	0.441

T1: Control (0% air dried azolla); T2: 5% air dried azolla; T3: 10% air dried azolla and T4: 15% air dried azolla.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Hematological parameters of Koekoek chickens fed azolla meal

Hematological parameters	Treatments				SEM	P-value
	T1	T2	T3	T4		
RBC (x10^{6}/µL)	2.30	2.21	2.14	2.15	0.03	0.160
Hb (g/dL)	15.90	17.30	16.85	17.20	0.27	0.241
Lym. (x10^{3}/µL)	62.52^{a}	55.72^{ab}	43.21^{b}	51.84^{ab}	2.56	0.024
PCV (%)	27.80^{a}	27.50^{ab}	25.50^{c}	26.30^{bc}	0.32	0.003
PLT (x10^{3}/µL)	7.70	8.60	12.60	10.60	1.12	0.557
MCV (fL)	120.30	124.10	125.80	122.30	0.69	0.068
MCH (pg/cell)	69.40	78.20	83.30	79.70	2.48	0.243
MCHC (g/dL)	57.40	63.00	66.10	65.20	1.77	0.349

T1: Control (0% air dried azolla); T2: 5% air dried azolla; T3: 10% air dried azolla and T4: 15% air dried azolla.
RBC: red blood cells; Hb: hemoglobin; Lym: lymphocytes; PCV: packed cell volume; PLT: platelets; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin and MCHC: mean corpuscular hemoglobin concentration.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Serum biochemical indices of Koekoek chickens fed azolla meal

Biochemical indices	Treatments				SEM	P-value
	T1	T2	T3	T4		
TP (g/dL)	3.9^{ab}	3.8^{ab}	4.5^{a}	3.1^{b}	0.1	0.004
Albumin (g/dL)	2.6^{a}	2.4^{ab}	2.6^{a}	2.1^{b}	0.1	0.013
Globulin (g/dL)	1.3^{ab}	1.2^{ab}	1.6^{a}	1.1^{b}	0.1	0.024
Cholesterol (mg/dL)	133.6	132.1	133	137.3	1.7	0.786
Triglycerides (mg/dL)	43.4	43.4	45.2	42.6	0.9	0.851
LDL (mg/dL)	28.0	20.0	27.3	30.0	1.5	0.069
Glucose (mg/dL)	287.0^{a}	210.0^{b}	256.0^{a}	208.7^{b}	10.4	<0.001
ALP (nm/L)	1063.0	1092.5	917.3	821.0	46.7	0.114
Uric acid (mg/dL)	3.7^{b}	3.3^{b}	3.6^{b}	8.5^{a}	0.7	<0.001
Creatinine (mg/dL)	0.37	0.35	0.48	0.28	0.03	0.176

T1: Control (0% air dried azolla); T2: 5% air dried azolla; T3: 10% air dried azolla and T4: 15% air dried azolla.
TP: total protein; LDL: low-density lipoprotein and ALP: alkaline phosphatase.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

This may indicate mild hemodilution or lower plasma protein concentration. In contrast, a nonsignificant effect has been reported in other studies (Mishra *et al.* 2016b; Kamel and Hamed, 2021). The reported values in this finding were within the normal range reported for chickens.

In general, the hematological profile in this study suggested that AZM didn't cause physiological stress or toxicity. This might be due to the combined effect of balanced nutrient and antioxidant properties of the fern that help maintain the synthesis of normal blood cells and organ fun-

ction. The minor reduction in lymphocytes and PCV of T3 is more likely due to an adaptive physiological adjustment.

Total plasma protein is a common parameter utilized to estimate avian body condition. The normal ranges of TP, albumin and globulin for chickens are 3.0-4.9 g/dL, 1.17-2.74 g/dL and 1.83-2.1 g/dL, respectively (Meluzzi *et al.* 1992). In this regard, there was a significant difference (P<0.05) in total protein, albumin and globulin content between the treatments. T4 was significantly lower than T3, while there was no difference between chickens fed 0, 5% and 10% AZM (Table 6). This may be due to reduced protein digestibility, the possibility of essential amino acid imbalance, lower feed intake, and anti-nutritional factors. Similarly, Mishra *et al.* (2016b) reported that 0%, 5% and 10% azolla had no significant effect on the total protein or albumin content of Chabro chickens. The high protein content in the blood is reflected by protein deposition in the meat (Adriani *et al.* 2021). Therefore, the high body weight and dressing percentage reported in this study could be related to the plasma levels of protein, which might have contributed to tissue growth.

On the other hand, the total protein and albumin contents of 5% AZM-fed quails were significantly greater than those of control quails and 10% azolla-fed quails (Verma *et al.* 2024).

AL-Rekabi *et al.* (2020) and Kamel and Hamed (2021) reported that azolla-fed broilers had significantly higher total protein and globulin levels than control broilers. Further research to investigate digestibility, amino acid profile and additional liver function is suggested to confirm the mechanism.

The glucose and uric acid contents exhibited highly significant (P<0.001) differences between the treatment groups. T1 and T3 had significantly higher (P<0.05) glucose levels than the other treatment groups. This might be due to altered carbohydrate availability or non-linear effects of the bioactive compounds of azolla. Sherif *et al.* (2022) also reported significantly greater glucose content in control groups than in azolla-treated quails. On the other hand, Mishra *et al.* (2016b) reported no significant difference among the experimental groups. This difference could be as a result of differences in azolla inclusion level, composition of feed or strain of birds used.

The normal range of uric acid content in chickens is 1.9-12.5 mg/dL (Clinical Diagnostic Division, 1990). The uric acid content under T4 was significantly greater than that under the other treatments. This could be due to the relatively high azolla percentage in the diet of the treatment group, which could have resulted in high uric acid concentrations. High protein intake influences the concentration of uric acid in the blood, as it is produced as a result of protein metabolism (Chen *et al.* 2025).

In the present study, 10% AZM improved the abundance of the useful bacteria *Lactobacillus* and decreased the abundance of the pathogenic bacteria *E. coli* (Table 7). Azolla supplementation increased the proportion of propionate in animals, indicating a change in the microbiota (Abdelatty *et al.* 2020). This might be due to the fact that azolla contains important substrates that could act as prebiotics which might reach optimal level to stimulate *Lactobacillus* growth. An increase in *Lactobacillus* spp. decreases the gut pH, delaying the growth and colonization of pathogenic bacteria (Park and Kim, 2018). In another study by Arram *et al.* (2023), the use of 5% azolla improved beneficial microflora and reduced the number of pathogens in the cecal. In contrast, no significant difference was found in Japanese quail fecal excretion of *E. coli* (Hafeez *et al.* 2024). Abdelatty *et al.* (2021), on the other hand, reported that azolla meal included at the 5% and 10% concentrations had no effect on *Lactobacilli* in broilers. In this study, treatments with relatively high *Lactobacillus* abundances resulted in relatively high slaughter weights, possibly because, as reported by Borda-Molina *et al*. (2021), bacteria are positively correlated with bird body weight.

The results of the cost–benefit analysis of Koekoek chickens fed different proportions of AZM are presented in Table 8. The feed cost, sale of chicken, net income and economic efficiency were significantly (P<0.05) differed among the treatment groups. The feed cost of T1 was significantly greater (P<0.05) than those of T3 and T4. This is attributed to the lower cost of AZM preparation than that of SBM and maize on the market under Ethiopian context. A similar result was also reported by Mishra *et al.* (2016a), who revealed that the feed cost of the control group was significantly greater than that of the 10% azolla-fed group. Owing to the lower FBW, significantly lower (P<0.05) chicken sales were recorded in the treatment group fed 15% AZM (T4) than in the other groups.

The net income of chickens fed 10% AZM (T3) was significantly greater (P<0.05) than that of the control and T4 groups. This is due to the combined effect of higher FBW and lower feed cost. Similarly, Kamel and Hamed (2021) reported that broilers fed 12% AZM presented greater net returns. Islam and Nishibori (2017) also reported that the use of azolla in the diet of broilers resulted in improved net profit compared with that of the control groups. The economic efficiency of T3 was significantly greater (P<0.001) than that of the other experimental groups. This result is in agreement with the report of El-Ghany (2020), who reported that the inclusion of azolla at the 10% level provided the maximum economic benefit in poultry production. Similarly, Kamel and Hamed (2021) reported that broilers fed a 12% azolla diet presented significantly greater profitability and economic efficiency than the control group.

Table 7 Cecal bacterial load (log cfu/g) of Koekoek chickens fed azolla meal

Cecal bacterial load CBL	Treatments				SEM	P-value
	T1	T2	T3	T4		
Total bacteria	4.02	3.96	4.04	3.99	0.02	0.297
Lactobacillus	3.83^{b}	3.99^{a}	4.07^{a}	3.78^{b}	0.03	<0.001
E. coli	3.84^{a}	3.77^{a}	3.42^{b}	3.84^{a}	0.06	0.008
Salmonella	3.88	3.92	3.80	3.89	0.02	0.094

T1: Control (0% air dried azolla); T2: 5% air dried azolla; T3: 10% air dried azolla and T4: 15% air dried azolla.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 8 Effects of Azolla meal on the economic efficiency of Koekoek chickens

Items	Treatments				SEM	P-value
(Birr/chicken)	T1	T2	T3	T4		
Chicks purchase	60	60	60	60	-	-
Feed cost	277.6^{a}	256.2^{ab}	245.6^{b}	234.9^{b}	5.45	0.005
Other costs[1]	65.9^{b}	81.2^{ab}	85.5^{ab}	90.9^{a}	3.22	0.017
Total variable cost	403.5^{a}	397.2^{a}	391.3^{b}	385.7^{b}	2.65	0.044
Sell of chicken	553.6^{a}	553.3^{a}	560.3^{a}	530.7^{b}	3.52	<0.001
Net income	149.8^{b}	156.1^{ab}	169.1^{a}	145.1^{b}	3.12	0.008
Economic efficiency	0.54^{c}	0.62^{b}	0.70^{a}	0.62^{b}	0.02	<0.001

[1] Labor, medicine, heater and litter costs.
T1: Control (0% air dried azolla); T2: 5% air dried azolla; T3: 10% air dried azolla and T4: 15% air dried azolla.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

In spite of the important findings of this research, lack of nutrient digestibility and amino acid utilization measurement might limit comprehensive understanding of the biochemical and physiological reasons behind the observed results.

CONCLUSION

AZM inclusion in the diet of Koekoek chickens resulted in variable effect on growth performance, carcass characteristics, hematological and serum biochemical, cecal microbiota, and economic efficiency depending on the level of inclusion. The addition of 10% AZM as a protein source showed improved overall performance, with significantly greater net profit associated with high *Lactobacillus* and low *E. coli* count. Furthermore, total protein, albumin, globulin and glucose at 10% AZM were within the normal ranges, indicating efficient nutrient utilization and metabolic function. On the contrary, inclusion of AZM at 15% reduced growth performance, serum protein, and blood glucose, signifying that extreme inclusion could impair energy metabolism and protein digestibility. In conclusion, the use of 10% AZM in the diet is the optimal inclusion to maintain metabolism, healthy microbiota and improve profitability.

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