

Effects of Graded Levels of Azolla (Azolla Pinnata) Meal on Egg Production and Quality Traits in Bovans Brown Laying Hens

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Abstract

An experiment was conducted to evaluate the effect of inclusion of Azolla (*Azolla pinnata*) meal on feed intake, production performance, and egg quality traits of laying hens. Four experimental diets were formulated by incorporating Azolla (*Azolla pinnata*) meal at 0 (T₁), 5% (T₂), 10% (T₃) and 15% (T₄) inclusion levels and in which an attempts was made to maintain a constant crude protein. A total of 120 Bovans Brown laying hens (23-week-old) were randomly assigned to twelve experimental pens, with four dietary treatments and three replicate pens per treatment, each containing ten hens. The experiment lasts for 84 days, during which daily feed intake, egg production, and egg weight were recorded. Initial and final body weights were measured at the beginning and end of the trial, respectively. Egg quality traits were evaluated in four sampling rounds, using six freshly laid eggs per replicate. Dietary inclusion of Azolla meal had no significant effect on daily feed intake ($P > 0.05$). However, hen-day egg production (89.48 to 97.09g), egg weight (59.23 to 60.57g), and egg mass (0.53 to 59kg/hen) increased significantly with increasing levels of Azolla meal ($P < 0.05$). The maximum value of egg weight was recorded on the Azolla meal dietary inclusion particularly T₃ and T₄. Egg-shell weight, shell thickness, yolk weight, yolk height, Haugh unit, and yolk color score were also significantly improved as the levels of Azolla meal increased ($P < 0.05$). Feed utilization efficiency, total return, net return and marginal rate of return (MRR) improved, while total feed cost statistically decreased with increasing dietary Azolla mea inclusion. In conclusion, dietary inclusions of Azolla (*Azolla pinnata*) meal up to 15% improve egg production performance, enhance egg quality, and increase economic efficiency by reducing feed costs in Bovans Brown laying hens.

Introduction

The rapid changes of lifestyle and the dietary habits have contributed to increasing the prevalence of non-communicable human diseases like coronary heart diseases, obesity, and diabetes (Popkin 2006). At the same time, growing global food insecurity and the adverse effects of climate change have intensified the search for sustainable feed resources for food and nutritional supplementation. Currently, over 820 million people worldwide suffer from hunger (FAO 2020), while global food demand continues to rise as the world population projected to exceed 9 billion by 2050 (UN 2015). The poultry industry, one of the fastest-growing agricultural sectors, plays a vital role in enhancing food and nutrition security by providing affordable, high-quality protein within a short production cycle (Kamel and Hamed, 2021; Alem 2024). However, the rapid expansion of poultry production is constrained by the rising cost limited availability of conventional feed ingredients, which accounts for 70–80% of total production costs (Kamel and Hamed, 2021; AL-Shwilly 2022).

Ethiopia possesses one of the largest livestock populations, in the world contributing about 16.5% to the national gross domestic product (GDP) and 45% to the agricultural GDP, while supporting the livelihoods of more than one-third the population (FAO, 2019). According to the CSA 2020/2021 survey, the country is home to approximately 57 million chickens, predominantly local breeds reared under traditional production system in rural areas (CSA 2021). Despite this large population, poultry productivity remains

low due to traditional management practices and chronic feed scarcity, which are further aggregated by seasonal fluctuations and poor feed quality. With the increasing demand for animal-source foods, the identification and utilization of affordable and sustainable feed resources are essential to improve poultry productivity and strengthen food security. These rising cost and limited availability of conventional feed ingredients have therefore increased interest in nutrient-dense non-conventional feed resources as alternative to reduce grain considerable attention as a promising feed ingredient for poultry. Among these, *Azolla* is one of such kinds of feed which have gained attention as a promising alternative for poultry feed due to its richness by available nutrients and essential mineral profiles. It is a small free floating aquatic fern native to Asia, Africa, and America (Pillai et al. 2002). *Azolla pinnata* in particular is widely adopted in the tropical areas as in Ethiopia. It grows naturally in stagnant water bodies with a pH of 4–7 (Kumar and Chander 2017; Iqra et al., 2019; Sonu et al. 2023).

There are multiple reasons for considering *Azolla* (*Azolla pinnata*) meal as a potential feed resource for sustainable, viable and profitable small scale poultry production. In terms of production benefits, it is rapidly growing, self-perpetuating, high N₂ fixer, high biomass per unit area, easy for cultivated, takes up much less space and water for propagation than the traditional and conventional feed resources (Kumar and Chander 2017). On a dry matter (DM) basis evidence existed that, *Azolla* is rich sources of nutrients containing 25–35% protein, 10–15% essential minerals including calcium, phosphorous, magnesium, potassium, manganese and iron (Kumar et al. 2018), and 7–10% amino acids like lysine, arginine, methionine, isoleucine, leucine, phenylalanine, glycine and valine, making it a viable option for feed shortages (Kathirvelan et al. 2015; Brouwer et al. 2018; Fadzlin et al. 2023), along with appreciable quantities of vitamin A and vitamin B-12 (Pillai et al. 2002) and carotene contents ranged from 206–619 mg per kg (Lejeunea et al. 2000). Despite the fact that on feed trials, several studies have reported that inclusion of *Azolla* in poultry diets improves production performance, body weight gain and feed conversion ratio with inclusion ranging of 2.5 to 10% without any negative health effects (Khatun et al. 2008; Brouwer et al. 2018; Kumar et al. 2018; Wasihun et al. 2019; Samad et al. 2020; Seid 2023). Even though, effects of feeding *Azolla* meal on both layers and broilers diet on feed intake, production performance, and egg quality characteristics, there is a limited research data targeting on the use of *Azolla* meal in commercial layer diets, along with it different researchers test a few inclusion level of *Azolla* in other parts of the world including Africa; but to our knowledge, and under Ethiopian conditions studies on layer chickens have been very limited when compared with broiler chickens. Therefore, the main objective of present study was to evaluate the effects of of sun-dried *Azolla* meal at different inclusion levels on egg production performance and egg quality traits of Bovans Brown laying hens.

Material and methods

Ethical consideration

The study was conducted in compliance with the Act for the Protection of Animals Used for Scientific Purposes of the Federal Democratic Republic of Ethiopia, which is aligned with European Union legislation governing the use of animals in research. All animal welfare procedures were reviewed and

approved by the Ethical Review Board of the Faculty of Veterinary Medicine and Agriculture, Addis Ababa University (Certificate Ref. No. VM/ERC/05/84/17/2025).

Description of the study Area

The research was carried out at the poultry farm of College of Veterinary Medicine and Agriculture, Addis Ababa University located in Bishoftu, approximately 47 km southeast of Addis Ababa.

Management of birds and experimental setup

At 23 weeks of age, a total of 120 Bovans Brown layer hens, with an initial body weight of 1.567 ± 0.008 kg (mean $\pm$ SD), were randomly allocated to four dietary treatments using a Completely Randomized Design (CRD), with three replications per treatment. Twelve experimental pens were uniformly partitioned, with concrete floors covered with disinfected teff straw litter. Prior to the commencement of the experiment, all pens, feeders, drinkers, and laying nests were thoroughly cleaned, disinfected, and sprayed to control external parasites. Each treatment pen housed ten randomly assigned birds. All hens were vaccinated and routine health and sanitation measures were maintained throughout the experimental period. Birds were offered experimental diets at a rate of 125g/bird/day and clean drinking water was provided *ad libitum*. Fluorescent lamps were installed to ensure uniform lighting. Birds were allowed a one-week adaptation period to the experimental diets before the commencement of data collection.

Cultivation of Azolla and meal production

Azolla culture was obtained from the fodder production unit of Sebeta Fishery and Aquatic Life Research Center, Sebeta. Cultivation was carried out on polyethylene sheets placed in twelve artificially constructed earthen ponds, each measuring 3 m $\times$ 1.5 m with a depth of 0.2 m following the method described by Kumar and Chander (2017). Ponds were overlaid with polyethylene sheet to prevent water seepage and root penetration. A layer of 40 kg fertile soil was spread evenly over the polyethylene sheet, followed by the addition of 15 kg of fresh cow dung. The initial water depth was maintained at approximately 15 cm and later increased to three-fourth of the pond depth and then, 1 kg of *Azolla* culture was inoculated. Biomass harvesting was conducted using perforated plastic trays. Harvested *Azolla* was washed with clean water and air-dried for 3 days using framed nets in a well-ventilated room, with the biomass turned 3 to 4 times daily to ensure uniform dehydration while retaining its green color. *Azolla* meal was finally prepared and stored in a dry place for feeding birds in concentrate mix.

Preparation and formulation of experimental diets

Four experimental diets containing graded levels of Azolla meal were formulated to be nearly isocaloric and isonitrogenous providing 2750 kcal/kg DM ME and 16.5% CP. The diet formulation principles were based on nutritional requirements for laying hens as outlined by NRC (1994). The diets were formulated to contain a maximum of 8% CF and EE, and at a minimum of 3.9% Calcium and 0.8% of Phosphorous, using Microsoft Excel Solver. The experimental diets were designated as T₁ (0% AM), T₂ (5% AM), T₃ (10% AM) and T₄ (15% AM) as presented in Table 1.

Table 1
Ingredient composition of experimental diets (%).

Ingredient Item	T1	T2	T3	T4
White maize	60	60	59	56
White middling	6.8	4	3	4
Nougseed cack	5	4	3	3
Soybean meal	13	12	12	10
Meat and bone meal	5.9	5.3	3	2
Azolla meal	0	5	10	15
Salt	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.6	1	1.5	1.7
Lime stone	8	8	7.6	7.5
L-lysine	0.1	0.1	0.2	0.2
DL- methionine	0.1	0.1	0.2	0.1
Total	100.00	100.00	100.00	100.00
T1: 0% inclusion of <i>Azolla pinnata</i> meal; T2: 5% inclusion of <i>Azolla pinnata</i> meal; T3: 10% inclusion of <i>Azolla pinnata</i> meal; T4: 15% inclusion of <i>Azolla pinnata</i> meal.				

Data collection, measurements and calculations

Following a one-week acclimatization period to the experimental diets, the amount of feed offered, and refused was recorded daily. Initial BW of the hens was recorded at the beginning of the experiment by weighing birds per pen and computing the average. At the end of the feeding trial, final BW measured using a digital balance with an accuracy of ± 1 g, and the BW change was calculated as the difference between final and initial BW. BW gain was calculated to assess relative growth performance during the experimental period using the following formula:

$$\text{Body weight gain (\%)} = (\text{Body weight change} \div \text{Initial body weight}) \times 100 \text{ (1)}$$

Eggs were collected three times daily and the number of eggs produced per day, and weight of each egg was recorded on the day of collection to determine the mean egg weight. Hens in each replicate was monitored and recorded daily. Egg production was calculated on a hen-day basis. Hen-day egg production (HDEP) was computed using the following equation (Ahmed et al. 2013):

$$\text{HDEP (\%)} = (\text{Total eggs produced per day} \div \text{Number of hens present on that day}) \times 100 \quad (2)$$

The egg mass was calculated by multiplying the mean egg weight by the corresponding HDEP value. Feed conversion ratio (FCR) was then determined by dividing the feed intake by egg mass (Araujo et al., 2015).

Egg quality parameters were evaluated four times at regular intervals throughout the 84-day experimental period. During each sampling round, six freshly laid eggs were randomly collected from each replicate; the data obtained from the four sampling intervals were averaged to determine a single mean value for each treatment, which was then used for statistical analysis. The individual egg weights were measured using a digital balance with a precision of ± 0.01 g. Egg length and egg width were measured using Vernier caliper with a least count of 0.01 mm. Egg shape index was calculated from egg length and width as described by Duman et al. (2016). Eggs were broken onto a flat, transparent glass surface using a spatula to facilitate the measurement of internal quality traits. Shell thickness was measured using a Vernier caliper after removing the shell membrane and expressed as the average thickness of the broad end, equator, and narrow end of the shell. Albumen width was measured at both the narrowest and widest points of the thick albumen surrounding the yolk, and the average value was used for analysis. Tripod micrometer was employed to measure the height of the yolk and thick albumen, while their respective weights were measured using a precision balance. Albumen and yolk percentage were calculated as the proportion of their individual weights relative to total egg weight. Yolk-to-albumen ratio was computed as the ratio of yolk weight to albumen weight. Yolk index (YI) was calculated using the following formula (Kul and Seker 2004):

$$\text{YI} = \text{Yolk height} \div \text{Average yolk diameter} \times 100 \quad (3).$$

Haugh Unit (HU) was calculated from albumen height and egg weight using the formula proposed by (Haugh 1937):

$$\text{HU} = 100 \times \log_{10} (H - 1.7W^{0.37} + 7.6) \quad (4)$$

Where H represents albumen height and W is egg weight. Yolk color was assessed using the Roche Yolk Color Fan, which ranges from 1 (pale yellow) to 15 (deep orange) (Beardsworth and Hernandez 2004). Shell weight was obtained by subtracting the combined weights of albumen and yolk from the total egg weight, eggshell percentage was calculated as the proportion of shell weight relative to total egg weight.

Chemical analysis

The DM, CP, CF, ash and EE content were determined procedures outlined by AOAC (2019). The metabolizable energy (ME) content of the feed ingredients was estimated using the equation of (Wiseman 1987):

$$\text{ME (kcal/kg DM)} = 53 + 38 \times \text{CP} + 85.4 \times \text{EE} + 37 \times \text{NFE} - 46.2 \times \text{CF}. \quad (5)$$

Calcium (Ca) concentration was determined using an atomic absorption spectrophotometer, while Phosphorous (P) was quantified at 630 nm following the method described by APHA (1980). Chemical analyses of *Azolla* meal were performed at the Animal Nutrition and Soil Laboratory of Hawassa University. In contrast, proximate analyses of the experimental diets were conducted at the Ethiopian Agricultural Authority, Animal Products and Input Quality Testing Center in Addis Ababa. All analysis was carried out in triplicates.

Partial budget analysis

The economic feasibility of *Azolla* meal into the diet of experimental hens was evaluated by integrating production performance data with the associated costs of feed formulation, following the procedures described by Kumar et al. (2018). The economic efficiency assessment considered both variable and fixed costs and analyze following the Knott et al. (2003) procedure.

Statistical analysis

Statistical analyses were performed using one-way ANOVA procedures of SAS package program (SAS 2013; Version 9.4). Treatment means were computed with their corresponding standard error of the mean (SEM). Differences among treatment means were compared using Duncan's Multiple Range Test (DMRT) (Duncan 1955), due to its higher statistical power to detect true differences between treatment means and statistical significance was declared at $p < 0.05$. The statistical model used for data analysis was as follows:

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

Where Y_{ij} = Observation of the response variable for the j^{th} replicate of the i^{th} treatment; μ = Overall mean; T_i = Fixed effect of the dietary treatment and e_{ij} = random error associated with each observation.

Results and discussion

Nutritional composition of *Azolla* meal and experimental diets

The chemical composition of the major feed ingredients and experimental layer diets were presented in Table 2.. Chemical analysis showed that *Azolla* meal contained 90% DM, 23.4% CP, 14.4% CF, 3.2% EE

and 20.4% total ash, 38.6% NFE, and a ME value of 1987.4 kcal/kg. The DM content of *Azolla* meal obtained in the present study is comparable with values reported by Shinde et al. (2017; 90%) and Mangesh et al. (2018; 91.8%), but slightly lower than those reported by Kumar et al. (2012) and Shukla et al. (2018). The CP content of *Azolla* meal in this study was consistent with the findings of Alalade and Iyayi (2006) and Iqra et al. (2019), whose reported CP values ranging from 20% to 22.79%. However, higher CP contents of 28.24% and 28.59% are reported by Indira et al. (2009) and Ahirwar and Leela (2012), respectively. In contrast, Pinkihan, (2013) reported a lower CP content of 10.6%. The variation in CP may be attributed to differences in nutrient inputs, environmental conditions, and processing methods. For instance, Ncube et al. (2015) reported that shade drying retained significantly higher CP levels compared with sun-drying and oven drying methods.

Table 2
Chemical composition of *Azolla Pinnata* meal and experimental diets (%).

Ingredient Item	<i>Azolla</i> meal	T1	T2	T3	T4
Dry Matter	90.0	89.6	890.4	89.2	89.0
Crude Protein	23.4	15.51	15.96	16.43	16.74
Crude Fiber	14.4	3.48	3.77	4.21	4.82
Ether Extract	3.2	4.01	3.92	3.74	3.69
Total Ash	20.4	13.09	13.55	13.94	13.91
Calcium (Ca)	1.9	3.69	3.69	3.76	3.86
Phosphorous (P)	0.5	0.76	0.78	0.82	0.83
Metabolizable energy (kcal/kg)	1987.4	2820	2785	2743	2691
T1: 0% <i>Azolla pinnata</i> meal; T2: 5% <i>Azolla pinnata</i> meal; T3: 10% <i>Azolla pinnata</i> meal; T4: 15% <i>Azolla pinnata</i> meal					

Consistent with the present finding, Boitai et al. (2018) reported an EE content of 3.52% in *Azolla* meal. However, the EE content of *Azolla* meal observed in the current study was higher than the values and reported by Ali and Lesson (1995; 1.6%) and Sonam et al. (2020; 1.64%). In contrast, a comparatively higher EE value (3.74%) is reported by Wasihun et al. (2020). The CF content of *Azolla* meal in the present study was comparable with the values reported by Wasihun et al. (2020; 14.21%) and Seid (2023; 14.23%). Conversely, lower CF values ranging from 7.4% to 13.1% are reported by Singh and Subudhi (1978), Joysowal et al. (2018) and Sonam et al. (2020). However, the present CF value was lower than those reported by Kumar et al. (2012) and Iqra et al. (2019). The ash content of *Azolla* meal recorded in this study was higher than the values reported by Khatun et al. (2008; 16.29%) and Boitai et al. (2018; 18.01%), but slightly lower than the value reported by Kumar et al. (2012; 36.1%). The Ca and P contents of *Azolla* meal in the present study were 1.9% and 0.5%, respectively, which are in agreement the findings

of Iqra et al. (2019). Calculated value of ME was 1987.4 kcal/kg DM, which is lower than the value of 2431 kcal/kg reported by Khatun et al. (2008) but higher than 1614.07 kcal/kg Seid (2023).

Productive performance of Bovans Brown layers

The production performances of hens feed *Azolla* meal was presented in Table 3. Mean daily feed intake increased significantly ($P < 0.05$) from 111.27 g/hen/day in the control group (T1) to 113.61 g/hen/day in the highest inclusion (T4). Similar reports of unaffected feed intake are reported by Wasihun et al. (2020) and Seid, (2023). The feed conversion ratio (FCR) is presented as follows: T1 (2.10), T2 (2.00), T3 (1.96) and T4 (1.93). Although the statistical analysis showed no significance differences among treatments ($P > 0.05$), the progressive reduction in FCR from T1 to T4 suggests a positive numerical response to increasing levels of *Azolla* meal. The decreasing trend in FCR implies that hens fed diets supplemented with *Azolla* meal required less feed to produce the same amount of egg mass, indicating improved biological efficiency. The best numerical performance recorded in T4 (1.93). However, the lack of statistical significance indicates that the observed differences could be due to normal biological variation among experimental units rather than a strong treatment effect. Contrary to the present findings, Khatun et al. (2008) reported no variation in feeding efficiency of laying hens fed diets containing up to 10% *Azolla* meal.

Table 3
Effects of dietary inclusion of *Azolla Pinnata* meal on egg production performance of layers

Parameters	T1	T2	T3	T4	SEM	P-Value
Feed intake (g/hen/day)	111.27 ^d	112.23 ^c	113.45 ^b	113.61 ^a	0.05	< .001
Feed conversion ratio	2.10	2.00	1.96	1.93	0.39	0.490
Initial body weight (g)	1557.33	1553.74	1560.44	1558.78	1.06	0.123
Final body weight (g)	1681.42 ^b	1723.69 ^a	1661.88 ^c	1609.66 ^d	12.3098	< .001
Body weight gain (g)	124.09 ^b	169.95 ^a	101.44 ^c	50.86 ^d	12.93	< .001
Hen day egg production	89.48 ^c	93.29 ^b	96.56 ^a	97.09 ^a	0.56	< .001
Egg weight (g)	59.23 ^d	59.70 ^c	60.21 ^b	60.57 ^a	0.09	< .001
Egg mass (Kg/hen)	0.53 ^c	0.56 ^b	0.58 ^a	0.59 ^a	0.00	< .001
Mortality ratio (%)	0.00	0.00	0.00	0.00	0.00	< .001
T1: 0% <i>Azolla pinnata</i> meal; T2: 5% <i>Azolla pinnata</i> meal; T3: 10% <i>Azolla pinnata</i> meal; T4: 15% <i>Azolla pinnata</i> meal; SEM: standard error of the mean; FI: feed intake; ^{abcd} different superscript letters following the mean values in the same row are statistically different ($p < 0.05$).						

Initial BW was comparable across treatments, while significant ($P < 0.05$) differences were observed in final BW or BW gain among hens fed *Azolla* meal inclusion particularly T3 and T4 compared to the control group (T1) and the lowest inclusion level (T2). Reduction in BW gain observed at higher inclusion levels may be attributed to the fact that BW gain in laying hens was not directly proportional to egg production, and excessive BW gain does not necessarily enhance laying performance. These findings suggest that *Azolla* meal inclusion at 10% and 15% may promote preferential nutrient partitioning toward egg production rather than body tissue accretion. Similar observations are reported by Sushree et al. (2017) and Boitai et al. (2018) who found that dietary inclusion of *Azolla* up to 10% reduced BW of laying hens. However, the present results are in contrasts with the findings of Wasihun et al. (2019) who reported that the final body weight of Koekoek layer chicken increased when the inclusion level of *Azolla* meal grow up to 15% of substitution of soyabean meal.

As presented in Table 3, increasing levels of *Azolla* meal markedly improved hen-day egg production (HDEP). The average egg mass and egg weight were increased significantly ($P < 0.05$) as *Azolla* meal inclusion increased particularly higher in T3 and T4 relative to the other treatment groups. These results indicate that *Azolla* meal enhanced egg output. Consistent with the present findings, previous studies have reported improvements in egg production and egg weight following *Azolla* meal supplementation in layer diets. Kumar and Chander (2017) reported a 10–15% increase in egg laying capacity with *Azolla* meal inclusion, while Lakshmanan et al. (2017) showed that a 6.64% improvement in egg production and 6.62% increase in egg weight in hens fed *Azolla*-based diets. The present study further demonstrates that inclusion of *Azolla* meal up to 15% in layer diets had a beneficial effect on egg size. Similar findings are reported by Khatun et al. (1999), who observed improved egg size when *Azolla* was included at levels up to 15%. These improvements are attributed to the rich nutritional profile of *Azolla*, which supports reproductive performance and physiological efficiency in laying hens (Dhumal et al. 2009; Khatun 2015). In addition, all treatment groups recorded zero mortality throughout the experimental period, indicating that *Azolla* meal inclusion up to 15% was safe and did not adversely affect bird health. Similar observations have been reported in earlier studies, which found no adverse effects of *Azolla* on birds' liability (Boitai et al. 2018).

Effects of *Azolla* meal on egg quality

The effects of *Azolla* meal inclusion on egg quality traits were presented in Tables 4 and 5. Egg weight, shell weight and shell thickness increased significant ($P < 0.05$); at the highest level of *Azolla* meal inclusion when compared with the control group (T1). Shell thickness is the most important parameter of laying hens, which is required to form the structure and handling of eggs, importantly it is crucial in upholding the hygiene of table eggs, as any damage or cracks will make the eggs more susceptible to bacterial contamination. Egg length and egg width showed a steady increasing trend from 57.33 mm to 59.23 mm, and 44.20 mm to 45.03 mm, respectively, however, these differences were not statistically significant ($P > 0.05$). Similarly, egg shape index and egg volume are ranged from 74.93% (T1) to 77.07% (T4), and 54.43 cm³ (T1) to 62.10 cm³ (T4), respectively, indicating that eggs produced across all treatments had acceptable oval shape and volume, with no significant differences among treatments.

Shell percentage also showed a non-significant increasing trend ($P > 0.05$) with increasing levels of *Azolla* meal. The egg shape index recorded over the 12-weeks experimental period indicates that eggs were within the normal range. According to Duman et al. (2016), eggs are classified as sharp, normal, and round when shape index values are < 72 , between 72, and 76 and > 76 , respectively. This classification confirms that inclusion of *Azolla* meal did not adversely affect egg size or shape. The present findings further revealed that eggshell weight and eggshell thickness were significantly improved ($P < 0.05$) at 15% inclusion. These results agree with the findings of Seid (2023). However, they contrast with the reports of Alalade et al. (2007) and Khatun et al. (2008), who observed no significant effect on eggshell thickness with dietary inclusion of *Azolla* meal up to 15% and 20%, respectively. As evident from the Table 4, most of the external egg quality parameters were not significantly differs from the controlled group (T1) when dietary inclusion levels *Azolla* meal increased. These findings are inconsistent with Seid (2023), who found a significant external quality measurement.

Table 4
External egg quality traits of hens fed different levels of *Azolla pinnata* meal.

Parameters	T1	T2	T3	T4	SEM	P-Value
Egg weight (g)	57.33 ^c	58.33 ^b	59.90 ^{ab}	60.33 ^a	1.57	0.030
Egg length (mm)	57.33	57.47	58.90	59.23	2.16	0.890
Egg width (mm)	44.20	43.87	44.10	45.03	1.64	0.960
Egg shape index (%)	77.07	76.33	74.93	76.07	1.44	0.770
Egg volume (cm ³)	54.43	52.73	57.97	62.10	11.07	0.930
Shell weight (g)	5.30 ^c	5.57 ^{bc}	5.87 ^{ab}	6.00 ^a	0.09	$< .001$
Shell thickness (mm)	0.35 ^c	0.37 ^b	0.39 ^{ab}	0.41 ^a	0.01	$< .001$
Egg shell (%)	9.27	9.57	9.83	9.97	0.33	0.490
T1: 0% <i>Azolla pinnata</i> meal; T2: 5% <i>Azolla pinnata</i> meal; T3: 10% <i>Azolla pinnata</i> meal; T4: 15% <i>Azolla pinnata</i> meal; SEM: standard error of the mean; ^{a,b,c} Different superscript letters following the mean values in the same row are statistically different ($p < 0.05$).						

Table 5
Internal egg quality traits of hens fed different levels of *Azolla Pinnata* meal.

Parameters	T1	T2	T3	T4	¹ SEM	P-Value
Albumen weight (g)	35.80	35.80	36.43	36.40	1.69	0.990
Albumen height (mm)	7.13 ^c	7.83 ^{bc}	8.60 ^{ab}	8.97 ^a	0.22	< .001
Albumen length (mm)	85.60 ^b	86.00 ^b	93.67 ^a	94.97 ^a	1.02	< .001
Albumen width (mm)	75.40	77.80	79.97	81.57	3.08	0.550
Albumen index (%)	8.83 ^b	9.57 ^{ab}	9.90 ^{ab}	10.17 ^a	0.27	0.030
Albumen (%)	62.40	61.33	60.73	60.43	1.31	0.730
Yolk weight (g)	16.23 ^b	16.97 ^{ab}	17.60 ^{ab}	17.83 ^a	0.34	0.040
Yolk height (mm)	16.97 ^b	17.13 ^b	18.17 ^a	18.87 ^a	0.16	< .001
Yolk length (mm)	40.97	42.80	42.80	45.00	4.23	0.940
Yolk width (mm)	37.80	38.73	39.73	39.60	1.05	0.570
Yolk index (%)	44.97	44.43	45.73	47.67	1.28	0.360
Yolk color (Roche scale)	6.33 ^c	8.67 ^{bc}	11.00 ^{ab}	12.33 ^a	0.71	< .001
Haugh Unit (HU)	79.90 ^c	84.17 ^{bc}	88.27 ^{ab}	90.23 ^a	1.27	< .001
Yolk (%)	28.3	29.10	29.44	29.60	2.05	0.580
T1 = 0% Azolla meal, T2 = 5% Azolla meal, T3 = 10% Azolla meal, T4 = 15% Azolla meal, ¹ SEM = standard error of the mean; ^{a,b,c} Different letters following the mean values in the same row are statistically different (p < 0.05).						

As shown in Table 5, most internal egg quality traits improved with increasing levels, especially at 10% and 15% showing significant ($P < 0.05$) differences among treatments. Albumen weight, albumen width, yolk length, yolk width and yolk index were not significantly ($P > 0.05$) affected by the inclusion of *Azolla* meal. This finding contradicts earlier reports by Seid, (2023) who observed significant effects of *Azolla* on these parameters. However, other internal egg quality traits, like albumen height, albumen length, albumen index, yolk weight, yolk height, yolk color, and HU were significantly improved ($P < 0.05$). In the current study, the highest HU values were recorded from hens fed diets containing the highest level of inclusion. Similar improvements are reported by Wasihun et al. (2020) and Sied (2023) in Koekoek and Bovans Brown layers fed diets containing *Azolla* meal, respectively. Yolk color score increased progressively with increasing levels of *Azolla* meal. The deep orange yolk color observed is likely due to the high carotenoid content of *Azolla* (Pillai et al. 2004). These results were consistent with the findings of Sujatha et al. (2013), who reported increased albumen heights and albumen index in layers

supplemented with *Azolla* meal up to 15%. Likewise, Boitai et al. (2018) and Wasihun et al. (2020) documented significant improvements in yolk color with increasing dietary *Azolla* meal.

Economic efficiency of *Azolla* meal inclusion in the layers diet

The partial budget analysis illustrated in Table 6 demonstrated that inclusion of *Azolla* meal in the diets of Bovans Brown laying hens improved the economic performance of layer production system through reduced feed costs and increased egg revenue. The total feed intake was similar across the dietary treatments which were about 302.4kg, indicating that dietary inclusion of *Azolla* meal did not materially affect the overall experimental feed intake. However, the amount of *Azolla* meal consumed increased progressively with dietary inclusion level, from 15.12kg in T2 to 45.36 kg in T4. In terms of productive performance, egg production showed a favorable numerical trend with increasing *Azolla* meal inclusion, which was rising from 2,299 eggs in T1 to 2,454 eggs in T4. Correspondingly, the total return (TR) increased from 43,681 ETB in the control group (T1) to 46,626 ETB at the highest inclusion level (T4). These results showed that inclusion of *Azolla* meal up to 15%, increased profitability through the combined effect of lower feed cost and improved the egg output. The net return (NR) also followed a similar trend, which raised 26,669.5 ETB in T1 to a maximum of 29,213.1 ETB in T4. These findings are consistent with the previous reports that *Azolla* meal can reduce dependence on expensive conventional feed costs, enhanced economic sustainability and improve farm profitability in poultry production systems (Lakshmanan et al. 2017; Omnia et al. 2025). Similarly, Seid (2023) reported that improved cost efficiency in laying hens supplemented with *Azolla* meal due to favorable egg production relative to feed investment.

Table 6
Partial budget analysis of laying hens fed graded levels of *Azolla Pinnata* meal.

Parameters	T1	T2	T3	T4
Total feed consumed (kg)	302.10	302.10	4302.4	302.4
Total <i>Azolla</i> meal (kg) (optional)	0.00	15.12	30.24	45.36
Total variable cost (ETB)	17,011.5	17,229.10	17,740.30	17,412.90
Total number of eggs produced	2,299	2,376	2,441	2,454
Total return (ETB)	43,681	45,144	46,379	46,626
Net return (ETB)	26,669.50	27,914.90	28,638.70	29,213.10
Marginal rate of return	0.00	5.72	2.70	6.34
T1: 0% <i>Azolla pinnata</i> meal; T2: 5% <i>Azolla pinnata</i> meal; T3: 10% <i>Azolla pinnata</i> meal; T4: 15% <i>Azolla pinnata</i> meal; ETB; Ethiopian birr.				

The marginal rate of return (MMR), which measures the additional net benefit obtained per unit of extra cost, was positive in all *Azolla* meal supplemented treatments. The highest MMR was recorded in T4

(6.34), followed by T2 (5.72), and T3 (2.70). This suggests that every additional ETB invested in the 15% of *Azolla* diet generated approximately 6.34 ETB in extra net benefit. According to the partial budget evaluation guidelines, a MRR greater than 1 (100%) is generally considered economically acceptable for technology adoption in livestock production (Byerlee et al. 1988). In this study, all supplemented treatments were exceeded this threshold. The lower MRR value recorded in T3 compared with T2 and T4 suggests that economic response to *Azolla* meal inclusion may not always be linear. Similar non-linear trends have been reported in broiler trials, where excessive inclusion levels without balanced formulation may lead to reduce nutrient digestibility and impair nutrient absorption due to the presence of anti-nutritional factors (Alem and Bekele 2024). Such kind of variation can arise from interactions among feed cost, egg production response, and experimental diet formulation efficiency. Nevertheless, the recovery of MRR at the highest dietary inclusion level indicates that Bovans Brown layers can efficiently utilize relatively higher levels of *Azolla* meal when diets are properly balanced dietary formulations.

Conclusion and recommendation

Azolla (*Azolla pinnata*) meal contains valuable protein, minerals and pigment that are suitable for feeding Bovans Brown laying hens. The results of this study demonstrate that *Azolla* meal plays a significant role in improving daily feed intake efficiency, egg laying performances and without any adverse effect on feed conversion ratio. Economically important egg quality parameters including eggshell weight, shell thickness, Haugh unit, and yolk color score were notably improved when hens offered diets containing graded level of sun-dried *Azolla* meal. Importantly, all experimental layers recorded zero mortality throughout the trial period. The economic analysis result further showed that the feed costs of the dietary treatments significantly reduced and improved the total return with increasing inclusion levels of *Azolla* meal in the diet. The biological and economic effect observed in the current study indicates that *Azolla* meal can enhance profitability and sustainability of small-scale poultry production. It is therefore recommended that *Azolla* meal be incorporated in to layer diets at the inclusion levels of 5% to 15% provides the best balance between egg production and economic efficiency. To reduce dependence on expensive conventional protein sources, farmers are encouraged to adopt cultivation due to its low production cost and high nutrient density. Further research is recommended to assess the long-term effects of *Azolla* meal feeding across the entire laying cycle and large-scale farm economics to refine the inclusion thresholds and confirm commercial applicability. In additional, extension services and farmer training programs on *Azolla* cultivation and meal preparation are essential to ensure consistent quality and safe integration in to layer diets.

Declarations

Data availability statement

The data presented in this study are available on request from the corresponding author.

Author contributions

Conceptualization: Berhan M.T., Etalem B.T. Methodology, validation and data curation: Berhan M.T., Etalem B.T. Investigation and formal analysis: Esubalew T.M. Writing original draft preparation and visualization: Esubalew T.M. Writing-review and editing: Berhan M.T., Etalem B.T. Project administration: Berhan M.T. All authors have read and agreed the published version of the manuscript.

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Conflict of interest

There is no conflict of interest with any individual regarding the materials discussed in this manuscript.

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