Availability, biomass production, growth performance and chemical composition of Azolla *filiculoides*: A potential feed for Chicken in Ethiopia

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Abstract

This research was conducted to assess the utilization, availability and chemical composition of *A. filiculoides* in the water logged areas of South Gondar Zone and evaluate its chemical composition and growth performance under different fertilizer application. 304 farmers from three rural kebeles were interviewed to assess the availability and utilization. To evaluate the growth performance and chemical composition, twelve earthen ponds of four treatments with three replications were used. Based on the survey, *Azolla* was found to be the most dominant and one of chicken feed aquatic plant. Based on the experiment, the biomass, Relative Growth Rate (RGR) and Doubling Time (DT) showed highly significant difference (P<0.001) among the treatment groups and T2 and T4 exhibited significantly better performance than other treatments. The proximate analysis revealed that the Crude Protein (CP), Crude Fiber (CF), ash and Metabolizable Energy (ME) showed significant difference among treatments. The CP content of T3 (32.26%) and T4 (32.07%) were significantly higher (P<0.01) than the control and T2. The secondary chemical analysis revealed that alkaloids were present in higher percentage than the recommended proportion. Some proportion of *A. filiculoides* can be considered to be used as feed source due to chickens' preference to feed in the villages, its high biomass yield, growth performance and CP content.

Key words: *A. filiculoides;* biomass; relative growth rate; chemical composition

Introduction

To address the problem of high feed cost of chicken and reduce competition with human food, search for alternative source are of prime importance (Churyumova et al., 2021). A promising solution is the continuous search on the utilization of the different non- conventional feed sources with high nutritive values as a complement or replacement for standard feed (Bhatt et al., 2020). Among the non-conventional feed sources, aquatic plants are receiving a lot of attention in nutrition due to their broad range of uses in animal and human food (El-Ghany. 2020). Some researchers explored the possibilities of using aquatic plants (water hyacinth, *Azolla* and duck weed) as non-conventional feed source for livestock and chicken. *Azolla* is an aquatic fern that floats over rice fields, small ponds, stagnant water of ponds, drains, rivers, canals, marshy fields and wetland paddy in tropical and subtropical countries (Kumari et al., 2017). *Azolla* is promising in terms of ease of cultivation, minimal water requirement for propagation, rapid biomass production, the growth in unexploited niches, productivity and nutritive value and above all cost of production is very low (El-Ghany, 2020; Kathirvelan et al., 2015).

In northwestern Ethiopian particularly in Fogera and Libokemkem plains, *Azolla* is distributed after its introduction in 2004 to be used as an input for rice production and feed for chicken and fish. But the availability, utilization, biomass production and chemical composition as a possible chicken feed were not evaluated and documented. Since the relevant output of a farming system is the product of quantity and quality, simultaneous analysis of biomass productivity and chemical composition is needed to determine the potential for growing *Azolla* for protein feed (Brouwer et al., 2018). Quite a lot of research activities reported a high but wider range of protein content and some variations in DM yield and other nutrients. These could be due to species, location and management/input applied. Therefore, the objective of this investigation was to assess the availability and utilization of *A. filiculoides*, as well as to evaluate its biomass production, growth performance and chemical composition in order to explore its potential as a chicken feed resource.

Materials and methods

Study area, population and sample size determination of the survey

The study was conducted in Fogera and Libokemkem districts in south Gondar Zone of Ethiopia. Fogera is situated 11° 58′ latitude North and 37° 41′ longitude East (Taddesse *et al*, 2016). Libo Kemkem is another district in the zone which is $11^0 57'$ latitude North and $37^0 34'$ longitude East. The two districts were selected purposively based on their potential of growing rice crop and *Azolla* as most part of the land is water logged in main rainy season. Two kebeles from Fogera namely Wagetera and Quhar Michael and Shinna from Libo Kemkem districts were selected. To conduct the survey on village rural farmers, the sample size was determined based on the following formula by Yamane (1967):

$$n = \frac{N}{1+N(e2)}$$

Where n = sample size, N =the total households in the study area and e = the level of precision.

Treatment and experimental design

The experimental *Azolla* fern species is *A. filiculoides,* which were obtained from Bahir Dar fisheries and other aquatic life research center. The experiment took place at Woreta Agricultural TVET College research site between February and April of 2023. The experimental design was randomized complete design (CRD) of four treatments with three replications. Twelve pits with length, width and depth of 1.5m, 1.5m and 0.3m respectively were prepared for the treatment. The pits receive treatments randomly in lottery method. The treatments were:

T1 = Soil only
T2 = Soil + Cow dung + 25g of super phosphate (Bhatt et al., 2020)
T3 = Soil + Poultry litter + 25g of super phosphate (Utomo et al., 2019)
T4 = Soil + Cow dung + Poultry litter + 25g of super phosphate

Management of experimental plots

The pits were prepared, covered with plastic sheet and at the top end stones were placed to make the sheet in place. About 6 kg of sieved fertile soil were uniformly spread over the sheet of each pit. Slurry made of 2kg cow dung (1-2 days old) and 25g of Super Phosphate in 10 liters water was poured in to the pits receiving T2. For T3, 112.5g dried poultry litter and 25g of Super Phosphate in 10 liters water were used. For the last treatment 1kg of cow dung, 60g of poultry litter and 25g of phosphate was mixed and added to the treatment pits. More water was poured to make the water level reach about 20 cm in each treatment pit. After two days bout 200gm of fresh and pure culture of *Azolla* was inoculated in each pit. To maintain a faster growth, cow slurry (1 kg) and phosphate of about 20gm were poured at every 10th day gap for treatment 2. For treatment 3, 60g of poultry litter and phosphate of about 20gm were poured at every 10th day gap. For treatment 4, cow slurry (0.5 kg), 50g of poultry litter and phosphate of about 20g were poured at every 10th day gap. Every two weeks, 20 % of the water was replaced with fresh water to prevent nitrogen build up in the pit.

Data collection

Formal survey was used to identify the availability and utilization of *Azolla* and other aquatic plants. In order to determine the chemical composition, *Azolla* samples were taken using a wooden square of 0.5m by 0.5m quadrat. The collected samples were dried in an open air in shades and weighed using sensitive weighing balance. For the experiment, once the pit was covered by the growing *Azolla* and form a full mat, two third of the fern was harvested using sieve based on Bhatt et al. (2020) methodology. The harvested *Azolla* was washed to remove the extraneous materials from it using pure water. Days of first harvest and frequency of harvesting were recorded. After each harvest the fresh *Azolla* was washed, weighed and recorded to estimate relative growth rate (RGR), doubling time (DT) and biomass yield.

Chemical composition of *Azolla*

Representative samples were taken to Hawassa University, animal nutrition laboratory for chemical analysis. Samples were dried in an oven at 65ºC for 72 hours, ground and passed through a one millimeter sieve for partial dry matter determination. All samples were analyzed for dry matter (DM), ether extract (EE), crude fiber (CF) and ash contents (AOAC, 2005). Nitrogen was determined by Kjeldhal procedure and crude protein (CP) was calculated by multiplying N content by 6.25. The calcium content was determined by atomic absorption spectrometer after dry ashing. The metabolizable energy (ME) levels of feed ingredients were calculated using the formula:

ME (kcal/kg DM) = 3951 + 54.4 EE - 88.7 CF - 40.8 ash (Wiseman, 2013).

After the research was completed, a representative sample from T2 (selected due to its performance) was used for the analysis of secondary chemicals. Sample extraction for determination of total phenolic, condensed tannin, flavonoids and alkaloid contents were done by using standard methods at Jimma University.

Statistical Analysis

The collected data such as availability and utilization of aquatic plants were filled, coded and analyzed using Statistical Package for Social Sciences (SPSS) version 25 software and summarized, and analyzed for descriptive statistics and frequencies. Chi-square (χ^2) for association values was used to determine the relationships between the categorical variables.

Relative growth rate (RGR) and Doubling time (DT) were calculated as in the following equations:

$RGR = \frac{(logW2 - logW1)}{(t2 - t1)}$ (Pabby et al., 2001) $DT = \frac{(t2 - t1) \times log2}{\log(W2/W1)}$ (Kannaiyan and Kumar 2005)

In both equations, t1= time initial (0 day); t2 = time of harvest (days); W1 = fresh *Azolla* biomass at initiation of experiment (grams); W2 = fresh *Azolla* biomass at harvest time (grams).

The collected data from the experimental units was subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure by SAS software Version 9.3. When treatment effects were found to be significant ($P<0.05$), mean separation were undertaken using Tukey HSD test. The following statistical model was used for data analysis.

$Yij = \mu + Ti + eij$

Where,

Yij is all dependent variables (dry matter yield, frequency of harvest and chemical composition)

μ is the overall mean

Ti is the fixed effect of ith treatment group (i = 1, 2, 3, 4)

eij is the random error

Result and Discussion

Farmers' response on availability and utilization of aquatic plants

The availability and utilization of aquatic plants in water logged areas of South Gondar Zone is presented in Table 3. From the interviewed farmers, majority (77.6%) of the respondents said that they encounter large area coverage of aquatic plants in their surroundings. The availability and distribution was supported by Haroon (2020) who identified different aquatic macrophytes around Nile river in Egypt. The result was also confirmed by Tewabe & Asmare (2020) as they reported the distribution of water hyacinth and *A. filiculoides* in water logged areas around Lake Tana. *Azolla* was found to be the dominant (with 59.3% of respondents) aquatic plant followed by water hyacinth. The members in the focus group discussion also agree on the broader area distribution of *Azolla* than water hyacinth. Similarly, (Ibrahim, 2020) reported that *Azolla* and water hyacinth were among the dominant aquatic plants distributed in Ajiwa dam of Nigeria. These aquatic plants could be significant sources of proteins and minerals suitable for incorporation into the animal diet.

According to the current finding, 126 (92.6%) confirmed chicken were noticed feeding on water hyacinth and *Azolla*. The result is supported by Yosefe et al. (2015) who reported that green grass, weeds leaf, different cereals leaf, different fruit leafs, enset leaf, cabbage were among the feed resources of village chickens in Ethiopia. Poel et al. (2013) reported that aquatic protein sources are very interesting poultry feed because of high protein content. This is mostly due to the scavenging nature of chicken under the rural village poultry production system. It implies that aquatic plants in the study area should be considered to be evaluated and tested as chicken feed resources.

Growth performance

The growth performance of *A. filiculoides* under different fertilizer application is presented in Table 3. The biomass yield, Relative Growth Rate (RGR) and Doubling Time (DT) exhibited highly significant difference between the treatment groups. T1 has significantly lower ($P<0.01$) fresh and air dried *Azolla* biomass while T2 and T4 have the highest yield. Similarly Hossain et al. (2021), observed that maximum growth of *A. pinnata* when there was higher P content than no P in the water. It is also in line with the finding of Utomo et al. (2019) who reported that weight of *Azolla* was significantly higher in fertilized earthen pond than unfertilized ones. This could be due to insufficient concentration of phosphorus inhibit growth rate of *Azolla* species.

Significantly lower and higher ($P<0.01$) Relative Growth Rate (RGR) of *A. filiculoides* in this study was 0.0371 (T1) and 0.0666 (T2) respectively. In agreement with this, Hossain et al. (2021) reported that the RGR of *A. pinnata* was significantly lower in treatments which receive no P content. Temmink et al. (2018) in a laboratory experiment also observed significant increase in RGR in *A. filiculoides* when culture medium was supplied with 0.3 ppm P. The result was lower than the finding of Vroom et al. (2024) who reported 0.08–0.15 g^{-1} $d^{-1.}$

Table 1. Farmers' response on availability and utilization of aquatic plants by animals

Variables	Kebeles	Category	N	%	χ 2	P-Value
Have you ever seen aquatic plants?	Kuhar Michael	Yes	58	58		
		No	42	42		
	Wagetera	Yes	104	100		
		No	0	0		
	Shina	Yes	74	74		
		No	26	26		
	Total	Yes	236	77.6	26.46	<0.001
		No	68	22.4		
Which aquatic plant/s is/are the dominant ones?	Kuhar Michael	Water hyacinth	18	31		
		Azolla	0	0		
		Water lettuce	40	69		
	Wagetera	Water hyacinth	38	36.5		
		Azolla	66	63.5		
		Water lettuce	0	0		
	Shina	Water hyacinth	0	0		
		Azolla	74	100		
		Water lettuce	0	0		
	Total	Water hyacinth	56	23.7		
		Azolla	140	59.3		
		Water lettuce	40	17		
What type of aquatic plants you see chicken are eating?		Both water hyacinth and *Azolla*	72	57.1		
		Only water hyacinth	24	19		
		Only *Azolla*	30	23.9		

Table 2. Growth performance of *A. filiculoides* under different fertilizer application

Parameters	Treatments				SEM	P value
	T1	T2	T3	T4		
Fresh biomass yield (g)	2141.2^{c}	5376.7^{a}	3556.5^{b}	5379.4^{a}	411.93	0.0001
Air dried biomass yield (g)	219.16^{c}	541.32^{a}	363.98^{b}	557.51^{a}	42.43	0.0001
Relative Growth Rate (g/g/day)	0.0371^{c}	0.0666^{a}	0.0539^{b}	0.0596^{ab}	0.004	0.0001
Doubling Time (days)	8.2^{a}	4.5^{b}	5.6^{b}	5.1^{b}	0.39	0.0001
Frequency of harvest	4.33^{c}	9^{a}	6.67^{b}	9^{a}	0.59	0.0001

T1: Control (only soil), T2: cow dung and phosphorus, T3: poultry litter and phosphorus, T4: cow dung, poultry litter and phosphorus; SEM: standard error of the mean; a, b, c means with different superscripts in each row are significantly different at P < 0.05.

Table 3. Chemical composition of *A. filiculoides* under different fertilizer application and from the villages

Parameters	Treatments				SEM	P-value	Village samples
	T1	T2	T3	T4			
Dry Matter (%)	90.59	90.35	90.09	90.04	0.13	0.5	90.67
Crude Protein (% DM)	27.43^{b}	29.52^{b}	32.26^{a}	32.07^{a}	0.67	0.004	21.45
Crude Fiber (% DM)	18.88^{a}	12.86^{b}	18.92^{a}	14.23^{b}	1.00	0.025	27.15
Ether Extract (% DM)	4.95	7.13	6.09	5.3	0.38	0.18	3.68
Ash (% DM)	19.32^{a}	15.23^{b}	16.09^{b}	14.36^{b}	0.61	0.001	28.64
Ca (ppm)	29.07^{b}	58.15^{a}	32.4^{b}	48.45^{ab}	4.52	0.04	33.96
P (ppm)	1173.7	1511.2	1398.5	1652.2	84.72	0.25	1360.4
ME (Kcal/Kg DM)	1756.9^{b}	2576.8^{a}	1947.1^{b}	2391.1^{a}	111.31	0.004	574.5

T1: Control (only soil), T2: cow dung and phosphorus, T3: poultry litter and phosphorus, T4: cow dung, poultry litter and phosphorus; SEM: standard error of the mean; a, b, c means with different superscripts in each row are significantly different at P < 0.05.

In this finding, significantly longer (P<0.01) DT of 8.2 days was recorded in control group while the shorter DT of 4.5 days was observed in T2. The result was supported by Azab & Soror (2020), who reported that the doubling time of *Azolla* sp. by using different fertilizers range from an average of 7 days to 9.2 days for organic and inorganic treatments, respectively. Similarly, *A. fliculoides* is able to double its biomass in one week in an N-free nutrient solution growing in a P-rich environment (Temmink et al., 2018). The DT of T2 was 4.5 days which is in the range of Hossain et al. (2021) and Kathirvelan et al. (2015) who found that *Azolla* doubling time differ in a range from 2 to 5 days. For *A. filiculoides* under this study, the highest value of the maximum RGR reached 0.0666 g/g/d corresponded to 4.5 days of DT was observed in T2. So, using cow dung and P as fertilizer could help to get the better growth performance from the fern.

Chemical composition of *Azolla*

The chemical composition of *Azolla filiculoides* from the study area is presented in table 4. There was a significant difference (P<0.05) in most of the parameters under study except the DM, EE and P. The percent crude protein content of the feed ingredient is among the most important factor for researchers to be used for livestock diet. In this regard, the CP content of the *Azolla* from the village (21.45%) is similar with the finding of Manjula et al. (2022) who reported 21.8%. The value was higher than the values of 17.59% and lower than 26.5% as reported by Kathirvelan et al. (2015) and Bhatt et al. (2020) respectively.

The CP content of T3 (32.26%) and T4 (32.07%) are significantly (P<0.01) higher than the other two treatment groups. The result is supported by Azab & Soror (2020) who obtained the highest protein content (30.25%) grown under poultry manure. Similar result was obtained by Utomo et al. (2019) who reported that the CP content of *Azolla* fertilized with chicken excreta (25.34%) were significantly higher than those fertilized with goat fecal compost (22.96%). In another study, CP content of *Azolla* fertilized with swine slurry was less (22%) (Leterme et al., 2010). This could be due to the availability of more nitrogen and phosphorus in poultry litter than cow dung which influences nutrient content of *Azolla*. The CP content of *A. filiculoides* in this study confirms that it is in the range for protein feed types for chicken.

The ash content of *Azolla* collected from the village is higher than that of the treatment groups (Table 4). The contents from treatment groups range in between 14.36% (T4) and 19.32% (T1). The finding is in agreement with the review by Swain et al. (2022) who reported 12.3%-19.9% total ash content in *Azolla*. However, the *Azolla* in T1 showed significantly higher (P<0.01) ash content, 19.32% than the others. This value is lower than the findings by Manjula et al. (2022) and Abou El-Fadel et al. (2020) who reported 22.81% and 27.14% ash for *Azolla* meal.

According to the current study, the ME of samples from the village is 574.5 Kcal/KgDM, which is very low due to higher amount of CF and ash content. On the contarary, the values range in between 1756.9 Kcal/KgDM (T1) and 2576.8 Kcal/KgDM (T2) from the treatment groups. This result was supported by Namra et al. (2010) who revealed the presence of high levels of energy in *Azolla* which is important for both digestion and availability of nutrients. The higher ME obtained in this research is comparable with the finding of Khatun et al. (2008) who reported 2431 Kcal/KgDM for *A. pinnata.* Besides the CP content, the ME of *A. filiculoides* should be taken in to consideration when the fern is going to be used as chicken feed.

Secondary chemicals

The alkaloid percentage of *A. filiculoides* (1.6) in this study was higher than the maximum limit for chicken feed (Table 5). For chickens, alkaloid toxicity can be dangerous, with concentrations exceeding 0.05% in feed causing signs of toxicosis, and 0.2% causing decreased weight gain, and 0.3% potentially leading to death (Carmen, 2024). Others including total phenols, tannins and flavonoids with proportion of 0.691 mg/g, 4.43 mg/g and 0.067 mg/g respectively were also available in the fern. The availability of these chemicals in different *Azolla* types at different concentrations has been reported (Mithraja et al., 2011; Tran et al., 2020). According to Brouwer et al. (2018) *A. filiculoides* contain fewer (poly) phenols than *A. pinnata* when grown at ambient CO2, making it a more suitable protein feed. This indicates that some proportion of *A. filiculoides* under this study can be used in the diet of chickens.

Conclusion

Based on this research, *A. filiculoides* is the most abundant aquatic plant in which chickens were seen feeding on it. The growth performances and chemical compositions suggested that it could be ample and good source of crude protein chicken feed. So, some proportion of *A. filiculoides* could be considered as non-conventional feed but there should be further research to evaluate the effect of feeding the fern on chicken performances.

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