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Research Article
Impact of Replacing Concentrated Ingredients by Azolla Plant on Dairy Cattle Performance

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Abstract

Background and Objective: Azolla is a small aquatic fern that has gained attention as a potential feed source for dairy cattle. So, the present study aimed to explore the effect of partially substituting dry Azolla to replace part of the concentrated feed mixture (CFM) in Holstein dairy cows' diets. **Materials and Methods:** About 12 Holstein dairy cows (450±40 kg live body weight, 2nd milk season, 20±3 kg daily milk yield), divided into three groups (4 animals per each group) to examine the effects of the three treatment groups based on swing over method technique in dairy cattle trials. First treatment (control) fed CFM without any addition, treatment (R1) 1.2 kg of dry Azolla replaced 1.5 kg of CFM and treatment (R2) 2.4 kg of Azolla replaced 3 kg of CFM. The trial lasted for 12 weeks; thus, milk production was recorded daily and milk samples were taken every 21 days. **Results:** There was a significant difference ($p>0.05$) in nutrients digestibility between the experimental diets in every nutrient, except for digestible crude protein, dry matter, organic matter, crude fiber and nitrogen-free extract all had digestion coefficient values that were higher with control than with R1 or R2. The data shows a significant increase in milk yield with 2.4 kg of Azolla and a 4.92 and 3.10% increase in fat-corrected milk when 1.2 kg of Azolla replaces concentrated feeds. Blood parameters were in the normal range for all animals inside the experimental groups. **Conclusion:** Including Azolla in dairy cattle rations could positively affect milk yield without altering milk composition, blood parameters and digestion coefficients.

Key words: Azolla plant, concentrate feed mixture, nutrient digestibility, fat corrected milk, milk yield, Holstein cows

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Livestock production is an essential part of the agricultural sector, with ruminants playing a critical role in providing food and livelihood security for rural families. The demand for animal products is increasing due to changes in consumer markets, resulting in two significant obstacles to meeting this demand. Firstly, competition for land in urban areas is increasing, which occupies the land used for animals. Secondly, climate change has a negative impact on water and animal availability, as well as the availability of fodder in current production areas. Therefore, it is essential to identify new and sustainable feed materials for animal production[1,2]. One such alternative is *Azolla pinnata*, a floating aquatic fern that can double its biomass every three days and can be produced in various locations, such as pits, stagnant water and water channels. Azolla, a floating fern, is gaining attention as an alternative feed for dairy cattle. This aquatic plant has been used in Asia and other parts of the world for centuries to increase animal productivity. Azolla can improve milk production up to 15% when fed alongside traditional feeds such as hay or silage. In addition, it also increases the quality of milk produced due to its high content of essential fatty acids and amino acids which are key components in producing healthy milk products.

Azolla pinnata is a potential source of livestock feed that contains a wide range of nutrients. It has a protein content of 25-35%, a mineral content of 10-15% and a combination of amino acids, bio-active substances and biopolymers on a dry matter basis[3]. *Azolla pinnata* is one of the best species among the eight species of Azolla worldwide. It works as a food supplement for many species, particularly dairy cows. However, it should be avoided near factory water and wastewater due to its ability to absorb heavy elements[4]. The benefits of feeding Azolla extend beyond just improved performance from dairy cows. It can also help reduce costs associated with purchasing expensive commercial feeds while providing additional nutrients not found in regular diets. Additionally, this aquatic plant requires minimal land area compared to conventional crops used for livestock feed making it a more efficient use of resources than traditional options like soybeans or corn-based grains which require large lands.

Azolla, a floating plant with small, overlapping leaves and roots that hang in the water, is abundant in iron, copper, manganese, vitamin A, chlorophyll and carotenes, ranging from 1000-8600 ppm on a dry weight basis for iron, 3-210 ppm for copper and 120-2700 ppm for manganese. It contains 4.8-6.7% dry-weight crude fat, including 6.1-7.7% omega-3 and 12.8-26.4% omega-6 polyunsaturated fatty acids. As it does not accumulate plant secondary compounds, it is an ideal protein source for monogastric animals. Azolla nutrients are highly digestible due to its high protein and low lignin contents, making it an economic and efficient feed substitute for livestock. One hectare of Azolla can produce 540-720 kg of protein per month. Studies show that feeding Azolla in the diet of dairy cows resulted in a 15-20% increase in milk yield[5].

Azolla is a type of plant that can be found in ponds, ditches and wetlands in tropical and warm temperate regions worldwide. It requires water or wet mud to grow and cannot survive under dry conditions for more than a few hours. Azolla can tolerate water with a pH range of 3.5 to 10, but it grows best in water with a pH between 4.5 and 7. The ideal temperature for Azolla growth is between 18 and 28°C. As the salinity of the water increases, the growth rate of Azolla gradually decreases. Azolla prefers partial shade with around 50% sunlight and its growth declines rapidly under heavy shade. The plant propagates through vegetative means and nursery ponds are typically used to supply sufficient volume to ensure quick coverage in a wetland field. Previous studies have noted the potential of Azolla as a source of livestock feed for dairy cows[6-8]. Taking these findings into consideration, the present study was conducted to assess the impact of Azolla inclusion in lactating Holstein cow rations on digestibility, blood parameters, milk yield and composition under Egyptian field conditions.

MATERIALS AND METHODS

The current study was carried out at the Animal Production Station, General Association for Agricultural Reform, Quesna, Menoufia. This experiment lasted for 12 weeks, started October 2018 up to the end of December 2018 to assess the effect of using Azolla on milk production and its composition.

Azolla plant source: The experiment began 45 days after purchasing 20 kg of Azolla inoculants from farms associated with the Faculty of Agriculture at the University of Alexandria. The four concrete ponds, with a total area of 60 m^2, used cultivated Azolla inoculants. To prepare the ponds, 30 kg of cow dung was transformed into slurry using 10 L of water and it was spread evenly over the entire area. The water level was raised to 12 cm and the ponds were left for 20 days with daily stirring. Fresh green Azolla was then inoculated and 15 kg of dung was transformed into a slurry with 2 L of water and added to the ponds every 10 days as needed. Azolla was harvested using plastic sieves, washed with clean water and

then exposed to the sun for 3 days, with regular flipping during the day.

Animals: During a 12-week trial period, a group of 12 second-season Holstein milking cows were chosen based on their average body weight (450±40) and average milk yield (20±3) kg/day. These cows were provided with periodic vaccinations in accordance with standard protocols and given unrestricted access to feed and water[9]. Throughout the trial, the cows were divided into three different treatment groups based on a swing over method technique in dairy cattle trials[10].

Experimental rations: Three treatments (Control-R1-R2-Control) were used. Twelve dairy cows 4 animals per group used as follows: 7 control group at the beginning and the end of the feed trial on the concentrated feed mixture (CFM) and rice straw, group 2 (R1) feed on control ration with replacing 1.2 kg of Azolla instead of 1.5 kg of CFM and group 3 (R2) fed on control ration with 2.4 kg instead of 3 kg of CFM. The structure of concentrate feed mixture (CFM formula was as follows: 45% yellow corn grains, 13.30% corn gluten feed, 10% sunflower meal (28% crude protein), 10% dried distiller's grains with soluble (DDGs), 9.50% soybean meal (48% crude protein), 5% sugar cane molasses, 2.4% mono calcium phosphate, 2% wheat bran, 1.5% calcium carbonate, 1% sodium chloride and 0.3% vitamins and minerals premix.

Feeding procedures: The concentrated feed and Azolla were served 3 times a day, the first at 7:30 in the morning, the second at 3:30 in the evening and the third at 11:30 in the evening.

Sampling: About 72 feces samples (12 cows, 3 days, 2 samples each day) were taken during the collection period from all animals at the end of each treatment from the end of the rectal opening, then dried at a temperature of 70°C for a period of 24 hrs and then kept for chemical analysis (dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash).

About 48 blood samples were taken from 4 animals at the end of each treatment. Samples were taken 4 hrs after morning feeding from the abdominal vein and placed in glass tubes with anticoagulant and then analyzed.

About 252 milk samples were taken three times a day at 7:30 am, 3:30 pm and 11:30 pm, during the 3rd week in each period during 6 successive days of each treatment from all the animals. The sample was taken during each milking time then the three samples were mixed for each day.

Samples analysis: Chemical analysis of feedstuffs and feces samples was carried out to determine the percentage of content according to the methods of AOAC[11]. The nitrogen-free extract (NFE) was calculated by difference.

Milk samples: Milk composition analysis was conducted four times throughout the experiment, with samples taken every 10 days. Infrared spectrophotometer (MilkoScan™ Mars, FOSS, Nils Foss Allé 1, DK-3400 Hillerod, Denmark) was utilized to evaluate the milk samples for various components such as total solids, fat, total protein and lactose. To determine the fat corrected milk (FCM), the Gaines's[10] equation mentioned in Ghoneem and Mahmoud[9], was applied. The equation is as follows:

$$\text{FCM (4\%)} = 0.4\ (\text{milk yield (g)}) + 15\ (\text{fat yield (g)})$$

Blood samples: Blood samples were collected from each animal's jugular vein 5 hrs after morning feeding. The samples were placed in glass tubes containing EDTA as an anticoagulant. After centrifugation for 15 min at 5,000 rpm, the blood plasma was separated and stored at -20°C until further analysis. Plasma total protein[12] albumin[13], Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) levels were measured using a commercial kit from Biodiagnostic Co., a provider of diagnostic and research reagents located in Dokki, Giza, Egypt. The measurements were conducted using a T80 UV, VIS Spectrometer from PG Instruments Ltd., United Kingdom.

Statistical analysis: Data analyses were carried out using one way ANOVA procedure of SAS[14] according to the following model:

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

where, Y_{ij} is the parameter under the analysis of the ij cows of lactation trails, μ is the overall mean, T_i is the effect of treatment, e_{ij} is the experimental error for ij on the observation. The variations between means were separated using Duncan's New Multiple Range test[15].

RESULTS AND DISCUSSION

Chemical composition of the experimental feeds and rations: Results of chemical composition of Azolla in Table 1 shown that the crude protein content 29.44% and ether extract 9.86% these values reflected on the composition of the experimental rations. These results were nearest from data

obtained by Basak *et al.*[4], so Azolla meal has the following air-dry composition: 25.78% crude protein, 15.71% crude fiber, 3.47% ether extract, 15.76% ash and 30.08% nitrogen free extract. At the same context, Kumar *et al.*[8] and Yee *et al.*[16] found that the Azolla chemical analysis as follow 8.7% DM, 16-28% CP, 20-30% CF, 3.1% EE, 35.4% NDF and 23.97% ADF, 8.5-11.7% cellulose and low content of carbohydrates being 34.9%. Also, Parashuramulu *et al.*[17] noticed that Azolla is rich in trace minerals, has a 21.37% CP, 1759 kcal ME kg^{-1} energy content and a significant amount of metabolizable energy. Sharma *et al.*[18] recorded the following values for chemical analyses of sun-dried Azolla as follow; 20.40% ash, 40.60% NFE, 0.47% phosphorus, 1.66% calcium, 90.20% DM, 79.60% OM, 23.45% CP, 12.45% CF and 3.10% EE. On the other hand, there were some researchers found the lower values of chemical composition as follow; Al-Suwaiegh[19], Azolla meal has a dry matter (DM) content of 22.93%, crude protein (CP) content of 11.63%, ether extract (EE) content of 2.8%, ash content of 15.59%, nitrogen-free extract (NFE) content of 47.03. Additionally, Kumar *et al.*[20] reported the following data: DM content of 4.7%, organic matter (OM) content of 82.66%, CP content of 22.48%, crude fiber (CF) content of 14.7%, EE content of 4.5%, ash content of 17.34%, NFE content of 40.98.

Digestion coefficients and nutritive values of the experimental rations: Results of digestion coefficients and nutritive values were shown in Table 2 cleared that, there was a significant difference ($p>0.05$) among the experimental rations in all nutrients. Except digestible crude protein. The values of digestion coefficients for dry matter, organic matter, crude fiber and nitrogen-free extract were higher with control followed by R1 then R2 respectively, being 82.37, 79.73 and 77.26 % for DM, 83.45, 81.03 and 78.67% for OM, 61.91, 56.85 and 51.85% for CF and 62.83, 58.9.

About 9 and 55.35% for NFE, respectively. Results of the crude protein digestion coefficient did not appear significantly different between R1 and control (79.73 and 80.86%) followed by R2 78.56 as a significantly lower value. Ether extract digestibility recorded a higher value with R2 (84.30%) followed by R1 (83.18%) then (82.04%). According to Parashuramulu *et al.*[17], *in vitro* DM and OM digestibility are 7.36 MJ kg^{-1} DM, 79.55 and 63.8%, respectively.

The nutritive values in Table 2 cleared that the replacing process of CFM by Azolla with level 1.2 kg (R1) did not influence total digestible nutrients followed by R2 (50.55%) on a significant level 0.01. Results of digestible crude protein cleared that there were insignificant differences among treatments (10.57, 10.99 and 11.30% for control, R1 and R2, respectively). Numerically, the animals fed on 3 kg Azolla instead of the same quantity of concentrate recorded higher values in digestible crude protein by 6.9%. These results agreed with the results confirmed by the following researcher in the same context. According to Peters[21], feeding buffaloes a diet consisting of rice straw and 45% Azolla accelerated their growth rate compared to a diet consisting only of rice straw. According to Samanta and Tamang[22], Azolla can be included up to 20% of the goat concentrate mixture without causing any harm. Additionally, adding Azolla meal to the diet of Nellore sheep or buffalo instead of groundnut protein increased DM digestibility and feed efficiency[23]. Also, Toradmal *et al.*[24] recorded that Azolla meal can replace up to 15% of the concentrate mixture in the diet of Osman badi goats due to its high fiber content. When sun-dried Azolla was used to replace 25% of the protein in a concentrated mixture in buffalo, there were no discernible variations in the nutrients' digestibility[25].

Effect of the experimental rations on blood parameters: The effect of the experimental rations on blood parameters were insignificant ($p>0.05$) among all the groups as showed in Table 3.

The results of the average plasma total protein (g dL^{-1}) in the control, R1 and R2 groups were 7.23, 7.17 and 7.13 g dL^{-1}, respectively. The plasma albumin levels (g dL^{-1}) in control, R1 and R2 groups were 4.15, 4.21 and 4.32 g dL^{-1}, respectively. The plasma globulin levels (g dL^{-1}) in Control, R1 and R2 groups were 3.85, 3.82 and 3.81, respectively. The results of the average of plasma AST (65.57, 660.5 and 66.5 g dL^{-1}) and ALT (25.25, 25.43 and 25.75 g dL^{-1}) in control, R1 and R2 groups respectively. The average plasma protein level (g dL^{-1}) was non-significant and similar outcomes were obtained in crossbreed calves with Azolla feeding[6]. Since the results are within the normal range, it has no detrimental impact on the kidney. As a result, incorporating *Azolla pinnata* into a diet is safe for kidney health and has no negative consequences.

Effect of the experimental rations on milk yield and milk composition: Table 4 presents the findings regarding milk production, which demonstrate the impact of adding Azolla to the diets of dairy cattle. The results indicated a significant increase in milk yield, with a 10.93 and 7.69% rise in actual production when 3 kg of Azolla was included. Furthermore, when 1.5 kg of Azolla replaced concentrated feed in R2 and R1, respectively, there was a corresponding increase of 4.92 and 3.10% in fat corrected milk. The findings were agreed with findings in review of literature by El Naggar and El-Mesery[26] discovered that substituting 15-20% of commercial feed with dried Azolla did not affect milk production but resulted in a 20-25% reduction in the use of commercial feed. According to

Table 1: Chemical composition of the experimental feedstuffs and the experimental rations

	Experimental feeds			Experimental rations		
Item	CFM	Azolla	Rice straw	Control	R1	R2
Dry matter (DM)	87.90	92.09	89.88	88.33	88.64	88.94
Organic matter (OM)	90.32	85.57	80.82	88.25	88.12	87.34
Ash	9.68	14.43	19.18	11.75	12.25	12.66
Crude protein (CP)	16.16	29.44	01.05	13.08	13.79	14.39
Crude fiber (CF)	15.92	11.92	67.43	21.48	21.69	21.73
Ether extract (EE)	02.27	09.86	02.22	02.26	2.76	3.25
Nitrogen free extract (NFE)	43.87	26.44	10.12	41.96	40.84	39.43

CFM: Concentrate feed mixture, Control: CFM+Rice straw, R1: CFM+Rice straw+1.2 kg Azolla and R2: CFM+Rice straw+2.4 kg Azolla

Table 2: Effect of the experimental rations on digestion coefficient and nutritive values

	Experimental rations				
Item	Control	R1	R2	±SEM	p-value
Digestibility					
Dry matter (DM)	82.37^{a}	79.73^{b}	77.26^{c}	0.790	0.002
Organic matter (OM)	83.45^{a}	81.03^{b}	78.67^{c}	0.747	0.003
Crude protein (CP)	80.86^{a}	79.73^{ab}	78.56^{c}	0.439	0.080
Crude fiber (CF)	61.91^{a}	56.85^{b}	51.85^{c}	1.480	0.000
Ether extract (EE)	82.04^{b}	83.18^{ab}	84.30^{a}	0.435	0.085
Nitrogen free extract (NFE)	62.83^{a}	58.99^{b}	55.35^{c}	1.111	0.000
Nutritive values					
Total digestible nutrients (TDN)	54.39^{a}	52.57^{a}	50.55^{b}	0.625	0.010
Digestible crude protein (DCP)	10.57	10.99	11.30	0.307	0.685

Arithmetic means differ ($p<0.05$) in the same row with different letters superscripts and SEM: Standard error of the mean, Control: CFM+Rice straw, R1: CFM+Rice straw+1.2 kg Azolla and R2: CFM+Rice straw+2.4 kg Azolla

Table 3: Effect of the experimental rations on blood parameters

	Experimental rations				
Item	Control	R1	R2	±SEM	p-value
Total protein (g dL^{-1})	7.23	7.17	7.13	0.116	0.948
Albumin (g dL^{-1})	4.15	4.21	4.32	0.083	0.729
Globulin (g dL^{-1})	3.85	3.82	3.81	0.131	0.992
AST (U L^{-1})	65.57	66.05	66.50	1.444	0.966
ALT (U L^{-1})	25.25	25.43	25.75	0.853	0.971

Control: CFM+Rice straw, R1: CFM+Rice straw+1.2 kg Azolla, R2: CFM+Rice straw+2.4 kg Azolla, AST: Aspartate aminotransferase and ALT: Alanine transaminase

Table 4: Effect of the experimental rations on milk production and composition

	Experimental rations				
Item	Control	R1	R2	±SEM	p-value
Milk yield					
Actual milk	21.13^{b}	22.17^{ab}	23.44^{a}	0.900	0.078
FCM, (4% fat)	19.63	20.24	21.14	0.529	0.526
Milk analysis					
Fat	3.57	3.47	3.39	0.158	0.904
Protein	3.48	3.46	3.42	0.034	0.737
Lactose	5.34	5.28	5.18	0.042	0.326
Ash	0.83	0.83	0.83	0.012	1.000
Solid not fat (SNF)	9.65	9.56	9.42	0.065	0.380
Total solids (TS)	13.22	13.03	12.81	0.118	0.414

Arithmetic means differ ($p<0.05$) in the same row with different letters superscripts, SEM: Standard error of the mean, Control: CFM+Rice straw, R1: CFM+Rice straw+1.2 kg Azolla, R2: CFM+Rice straw+2.4 kg Azolla and FCM: Fat corrected milk

review by El Naggar and El-Mesery[26], incorporating 1.5-2 kg of fresh Azolla into the daily diet of dairy cattle led to a 15% increase in milk yield and saved 20-25% on commercial feed. Similarly, when dairy cows replaced 15-20% of their commercial feed with Azolla, there was a corresponding 15-20% rise in milk production. This could potentially be

attributed to Azolla's high protein content and low levels of lignin[26]. El Naggar and El-Mesery[26] clarified that including 1.5-2 kg of fresh Azolla in the daily diet of dairy cattle increased milk yield by 15% and saved 20-25% on commercial feed. In the case of dairy cows, replacing 15-20% of commercial feed with Azolla resulted in a 15-20% increase in milk production, possibly due to Azolla's high protein content and low lignin levels[26]. Additionally, supplementing crossbred bovine animals' diets with 2 kg of Azolla per day increased milk yield and FCM by 11.2 and 12.5%, respectively, while also improving feed conversion efficiency[6]. Also, Kumar *et al.*[8] noticed that including 1.5-2 kg of fresh Azolla in cow diets resulted in a 20.96% increase in milk yield, while buffalo milk yield increased by 16.9%. In another study, Meena *et al.*[27] observed that supplementing buffalo diets with 1.5 kg fresh Azolla per day, along with cottonseed cake, increased milk production by 16.25% and improved animal health. At the same context, Nidhi *et al.*[28] reported a significant 11.85% increase in milk yield when crossbred cows' diets were supplemented with Azolla at a ratio of 1 part Azolla to 1 part concentrate. Furthermore, Kumar *et al.*[29] found that fresh Azolla can be effectively used as a feed supplement for dairy cows, with potential benefits observed at daily amounts of up to 1000 g in milk production ranging from 7 to 13%.

Data of milk analysis in Table 4 cleared that the addition of Azolla as a replacement of concentrated feeds to dairy cattle feeding did not influence the percentages of different milk constituents. In the same context, Gowda *et al.*[30] recorded that adding 2 kg of green Azolla to dairy cattle's diet did not increase milk fat %. On the other hand, Lavania *et al.*[31] observed an 18% improvement in milk fat yield in their three-month feeding trial where they supplied an additional 1.5 to 2 kg green Azolla to the treatment group of mid lactation Kankrej cows. The milk protein % of crossbred dairy calves did not improve after the addition of 2 kg of fresh Azolla to their diet[6]. In addition, Ravindra *et al.*[32] reported that the substitution of dry Azolla for concentrate combination in the Barbari doe's diet had no effect on milk lactose. Numerically, there was a decline in milk contents with an increase of Azolla from 1.2 kg in ration one and 3 kg in ration two compared with control ration. These results were relevant with increasing milk production, thus the relationship between milk production and milk analysis was an inverse relationship. So, solids not fat values were 9.42, 9.56 and 9.95% with R2, R1 and control respectively. Total solids values were 12.81, 13.03 and 13.22% with R2, R1 and control ration respectively. These values agreed with findings obtained by Khare *et al.*[6] and Lavania *et al.*[31].

According to results obtained in the current study and previous studies, the Azolla plant could be a suitable feedstuff for livestock in general and dairy cattle in particular. Results of milk production approved that the Azolla plant has a positive effect on milk yield by 11% without an insignificant impact on milk composition. At the same time, blood constituents were in the normal range without adverse effects on digestibility. So, Azolla plants need more studies on their higher contribution to dairy cattle as concentrated feed replacers. Also, to increase the usage of the Azolla plant commercially, it needs increased awareness among livestock project owners with precise scientific data. Specialists and researchers should be aware of the limitations of Azolla usage in animal feeding, such as seeds, equipment and complete data for appropriate cultivation methods.

CONCLUSION

Summing up, the results of replacing dried Azolla with 1.2 kg (8%) and 2.4 kg (16%) of dietary concentrate feeds for dairy cattle rations positively affect milk yield. At the same time, the replacement concentrated feeds by the Azolla plant did not negatively affect milk analysis, digestion coefficients, or nutritive values. Also, blood parameters cleared that Azolla had no adverse effects on dairy cattle's rations on their health or performance.

SIGNIFICANCE STATEMENT

This study cleared that using Azolla in dairy cattle feeding lies in its ability to provide a rich source of protein, essential amino acids, vitamins, minerals and bioactive compounds. Incorporating Azolla into the diet of dairy cattle can potentially improve milk quality and quantity, support animal health and productivity and offer environmental benefits. This study will help researchers to investigate more inclusion rate of using Azolla in dairy cattle rations. Also, this study will encourage the concept of producing Azolla as a commercial feed material.

REFERENCES

1. Schor, J.B., 2005. The new consumers: The influence of affluence on the environment. Ecol. Econ., 55: 444-445.
2. Hopkins, D.L., D.F. Stanley, E.S. Toohey, G.E. Gardner, D.W. Pethick and R. van de Ven, 2007. Sire and growth path effects on sheep meat production. 2 Meat and eating quality. Aust. J. Exp. Agric., 47: 1219-1228.
3. Pillai, K., 2021. Azolla-A sustainable feed substitute for livestock. Small Enterprises Dev. Manage. Ext. J., 28: 51-58.
4. Basak, B., M.A.H. Pramanik, M.S. Rahman, S.U. Tarafdar and B.C. Roy, 2002. Azolla (*Azolla pinnata*) as a feed ingredient in broiler ration. Int. J. Poult. Sci., 1: 29-34.

5. Broster, W.H., V.J. Broster and A.J. Clements, 1993. Feed utilization by the dairy cow over multiple lactations: A review. Livest. Prod. Sci., 34: 1-21.
6. Khare, A., A. Chatterjee, M. Mondal, M. Karunakaran, M.K. Ghosh and T.K. Dutta, 2016. Effect of supplementing *Azolla microphylla* on feed intake and blood parameters in growing crossbred female calves. Ind. J. Anim. Nutr., 33: 224-227.
7. Singh, B., G.S. Meena, K.C. Meena, R.K. Meena, B. Singh and D. Indoria, 2017. Effect of a wonder herb Azolla on buffaloes milk yield. Int. J. Curr. Microbiol. Appl. Sci., 6: 1059-1066.
8. Kumar, A.S., S. Murugesan and P. Balamurugan, 2020. Feeding of Azolla as a green fodder feed supplement on productive performance and milk composition of crossbred dairy cows in Theni District of Tamil Nadu, India. Int. J. Curr. Microbiol. Appl. Sci., 9: 1382-1388.
9. Ghoneem, W.M.A. and A.E.M. Mahmoud, 2022. Impact of incorporating *Thymus vulgaris* as leaves or essential oil in Damascus goats ration on lactation performance. Int. J. Dairy Sci., 17: 1-12.
10. Gaines, W.L., 1941. A supplemental note on the correlation between fat percentage and yield of milk, milk fat, milk energy. J. Dairy Sci., 24: 159-164.
11. Cunniff, P. and AOAC, 1995. Official Methods of Analysis of AOAC International. 16th Edn., Association of Official Agricultural Chemists International, Rockville, Maryland, ISBN: 9780935584547.
12. Gornall, A.G., C.J. Bardawill and M.M. David, 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 177: 751-766.
13. Doumas, B.T., W.A. Watson and H.G. Biggs, 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chim. Acta, 31: 87-96.
14. SAS., 2004. SAS/ETS 9.1 User's Guide. SAS Institute Inc., Cary, North Carolina, USA, ISBN: 1-59047-244-6, Pages: 2416.
15. Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.
16. Yee, T.J., N.A. Kamaruddin and S.S.S. Mohamad, 2022. Nutritional evaluation of *Azolla pinnata* and *Azolla microphylla* as feed supplements for dairy ruminants. J. Agrobiotechnol., 13: 17-23.
17. Parashuramulu, S., P.S. Swain and D. Nagalakshmi, 2013. Protein fractionation and *in vitro* digestibility of Azolla in ruminants. Online J. Anim. Feed Res., 3: 129-132.
18. Sharma, N.K., S. Chaudhary, H. Purohit and U. Ram, 2022. A study on nutritive value and production of green Azolla (*Azolla pinnata*). Pharma Innovation J., 11: 2687-2689.
19. Al-Suwaiegh, S., 2023. Effects of feeding *Azolla pinnata* on growth performance, nutrient digestibility and blood metabolites of growing Ardi goats in subtropics. Adv. Anim. Vet. Sci., 11: 331-337.
20. Kumar, M., R.K. Dhuria, D. Jain, R. Nehra, T. Sharma, U.K. Prajapat *et al*., 2018. Effect of inclusion of sun dried Azolla (*Azolla pinnata*) at different levels on the growth and performance of broiler chicks. J. Anim. Res., 8: 629-632.
21. Peters, G.A., 1984. Azolla-Anabaena Symbioses: Basic Biology, Use, and Prospects for the Future. In: Practical Application of Azolla for Rice Production, Silver, W.S. and E.C. Schröder (Eds.), Springer, Dordrecht, Netherlands, ISBN: 978-94-009-6203-3, pp: 1-14.
22. Samanta, G. and Y. Tamang, 1995. Feeding value of Azolla (*Azolla pinnata*) in goats. Ann. Zootech., 44: 62-62.
23. Renu, K., R.K. Dhuria, N.V. Patil and R.K. Sawal, 2018. Effect of different levels of azolla (*Azolla pinnata*) incorporation in pelleted complete feed on growth performance of camel calves. J. Camel Pract. Res., 25: 307-309.
24. Toradmal, S.D., R.R. Shelke and N.R. Sonone, 2020. Effect of azolla on growth performance of osmanabadi goat kids. Int. J. Fauna Biol. Stud., 7: 45-48.
25. Bhatt, N., N. Tyagi, R. Chandra, D.C. Meena and C.K. Prasad, 2020. Growth performance and nutrient digestibility of *Azolla pinnata* feeding in Sahiwal calves (*Bos indicus*) by replacing protein content of concentrate with *Azolla pinnata* during winter season. Indian J. Anim. Res., 55: 663-668.
26. El Naggar, S. and H.S. El-Mesery, 2022. *Azolla pinnata* as unconventional feeds for ruminant feeding. Bull. Natl. Res. Cent., Vol. 46. 10.1186/s42269-022-00752-w.
27. Meena, G.S., B.L. Dhaka, B. Singh, R.K. Meena and K.C. Meena, 2017. Effect of Azolla as feed supplement on milk yield in buffaloes. Int. J. Curr. Microbiol. Appl. Sci., 6: 3490-3494.
28. Nidhi R., K. Kumari, F. Singh and V.R. Gilhare, 2015. Effect of *Azolla*-supplemented feeding on milk production of cattle and production performance of broilers. Appl. Biol. Res., 17: 214-218.
29. Kumar, U., M.L. Reager and B.S. Mitharwal, 2020. Cultivation of Azolla (*Azolla pinnata*) and its use as cattle feed supplement. Int. J. Curr. Microbiol. Appl. Sci., 9: 1174-1178.
30. Gowda, N.K.S., A. Manegar, S. Verma, N.C. Valleesha, G. Maya, D.T. Pal and K.P. Suresh, 2015. Azolla (*Azolla pinnata*) as a green feed supplement for dairy cattle-An on farm study. Anim. Nutr. Feed Technol., 15: 283-287.
31. Lavania, P., S.C. Jingar, H.L. Bugalia, S.M. Meena and A. Kumar, 2019. Effect of *Azolla* feeding as a supplement on milk and reproduction performance on zebu cattle under field condition. Int. J. Chem. Stud., 7: 3167-3168.
32. Ravindra, K., P. Tripathi, U.B. Chaudhary, R.B. Sharma and M.K. Tripathi, 2016. Replacement of concentrate mixture with dried Azolla on milk yield and quality in Barbari does. Anim. Nutr. Feed Tech., 16: 317-324.