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**IMPACT OF AZOLLA PLANT ON DIGESTIBILITY, NUTRITIVE
VALUE AND RUMEN FERMENTATION IN BARKI SHEEP DIETS**

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ABSTRACT: The present study was conducted to evaluate azolla (*Azolla pinnata*) in ruminant feeding by comparing it with clover hay (CH). Twelve adult male Barki sheep, 2 years old, averaging 42.87±2.11 kg of body weight, were used in a palatability and metabolism trial. Animals were separated into two comparable groups according to their live body weight (6 animals/ group). The experimental animals were kept individually in metabolic crates. Results demonstrated that there were no significant differences between Sun-dried Azolla (SDA) and CH in dry matter content, while the content of OM, CP, and EE were significantly higher in SDA (84.16, 26.93 and 3.80%, respectively) than in CH (81.75, 14.48 and 2.70%, respectively). The content of CF % was significantly lower in SDA (13.48%) than in CH (24.82%). The results of the chemical analysis showed that SDA is rich in CP content (26.93%), which exceeds the CP content of CH (14.48%). Digestion coefficients for SDA were significantly ($P<0.05$) higher than CH for DM and CP, but NFE was significantly ($P<0.05$) lower than CH, while, CF did not differ significantly. The nutritive values (DCP and TDN %) for SDA were significantly ($P<0.05$) higher (18.49 and 51.93%) than CH (7.70 and 46.55), the N balance followed similar pattern being significantly ($P<0.05$) higher (19.14 g N/d) than for those fed CH (7.73 g N/d), which was 2.5 times higher. Rumen characteristics (pH, VFA and NH₃-N) started to be significantly ($P<0.05$) higher in animals fed SDA than in those fed CH after six hours of feeding. Generally, *Azolla pinnata* can be used successfully in Barki sheep feeding with beneficial effects on digestibility, nutritive value, nitrogen balance and rumen fermentation.

Key words: *Azolla pinnata*, clover hay, digestibility, rumen fermentation, Barki sheep.

INTRODUCTION

Due to the feed gap in Egypt, intense research efforts must be focused on examining the potential for employing novel, nontraditional sources of animal feeds and enhancing their nutritional value (Ali, *et al.*, 2015, Kewan, *et al.*, 2021, Abo-Donia and Nayel., 2022, Abo-Donia, *et al.*, 2022 and Tadavi *et al.*, 2023).

Azolla is a type of fern that floats freely in the water (Mooventhan *et al.*, 2019; Singh *et al.*, 2020 and Abd El-Ghany 2020). It is associated with fixing cyanobacterium anabaena, which fixes atmospheric nitrogen (Raja *et al.*, 2012), and has the potential to be a sustainable animal feed alternative (Pillai *et al.*, 2002). The Azolla-anabaena symbiosis is outstanding due to its fast biomass production because of its rapid growth

rate, and higher contents of protein, carotene and β carotene (Lakshmanan *et al.*, 2017). Since climate change poses a challenge to livestock production because to its effects on the amount and quality of feed crops, fodder, and forage; this technology has the potential to adapt it and can be helpful for farmers (Kumar and Chander 2017).

Katole *et al.*, (2017) reported that Azolla has a lot of advantages such as Low input costs, ease of growth in the wild and on farms with regulated growing conditions, and quick production of huge amounts of green fodder which is needed in both the fall and summer. It can also fix atmospheric nitrogen and carbon dioxide, converting them into carbohydrates and ammonia, respectively.

Azolla is a good source of high-quality protein. Azolla nutritional content are 21-25.08% CP, 10 -14.39% CF, 3.95% EE, 38.73% NFE, 17.5-27%, total ash and 45-55% essential minerals (Kumar and Chander 2017 and Joysowal *et al.*, 2018). In another report by Mohamed *et al.* (2018) found that the mean concentration of CP, CF, EE, NFE and Ash of *Azolla pinnata* were 31.67%, 11.04%, 5.9%, 36.39% and 15%, respectively. Ghodake *et al.*, (2012) reported that dried Azolla can compose up to 15% of the total concentrate requirement of growing Osmanabadi goats. In this regard Giridhar *et al.* (2012) revealed that Azolla may be given to the animals in a fresh or dried state. It can be fed to cattle, sheep, goats, rabbits, poultry, and pigs either directly or after mixing with concentrates. Also, Chatterjee *et al.* (2013) obtained that Azolla has the potential to be a substitute nutritional supplement that crossbred cattle can use to increase their production in terms of growth, milk, meat, and other aspects. Sihag *et al.* (2018) revealed that concentrate mixture replacement with sun-dried Azolla did not affect N intake, however, fecal nitrogen was significantly higher in 20% Azolla level. While ingested and retained nitrogen g/day and retention of nitrogen percentage were significantly low on the 20% Azolla addition to a concentrate mixture for goats. The results showed that ingestion and retention of nitrogen were not affected due to concentrate mixture replacement with Azolla up to 15% levels.

Hassanein *et al.* (2023) found that Azolla supplementation at 0, 10 and 20% level in Zaraibi dairy goats diets affected on nutrient digestibility of DM, OM, CP, CF, EE and NFE at higher level. In spite, rumen pH in (0 and 3h) were decreased with increasing Azolla levels from (6.69 and 5.53) to (6.6 and 5.42), respectively, the rumen pH in 6h was increased from (6.19 to 6.65). Moreover, ammonia-nitrogen and total volatile fatty acids (TVFA's) in goats' rumen liquor were increased with increasing Azolla level.

The present study was conducted to evaluate using Azolla (*Azolla pinnata*) in ruminant feeding by comparing it with clover hay and investigate its effect on digestibility, nutritive value and rumen fermentation in Barki sheep.

MATERIALS AND METHODS

The current investigation was carried out at Animal Production Department of the Faculty of Agriculture, Menoufia University in compliance with Scientific Research Ethics and Animal Use Committee (SRE & AUC) Faculty of Agriculture, Menoufia University (Reference No. 11-SRE & AUC-MUAGR-02-2024).

Animals and feeds

Twelve adult male Barki sheep, 2 years old, averaging 42.87 ± 2.11 kg of body weight, were used in a palatability and metabolism trials to compare SDA with clover hay. Animals were separated into two comparable groups based on their live body weight (6 animals/group). The experimental animals were kept individually in metabolic crates (1.60m x 0.53m) as stated by Maynard *et al.* (1979) to make separation of urine and feces collection. The first three weeks were regarded as a primary period, the consecutive 5 days were used for samples collection.

Animals in first group received SDA, while the other group received clover hay. Dry matter intake was stabilized for both groups before samples collection at approximately 3.5% of body weight. Feed was offered twice a day at 8.00 am and 4.00 pm. Fresh and clean water was available to all animals.

Fecal samples

Feces were quantitatively collected at 9:00 a.m. before feeding. A quantity of 10% of feces was withdrawn and dried, in a forced air oven, to a constant weight at 70°C for 24 hrs. Dry fecal samples were ground to exceed a 2mm screen and kept in plastic bags for later analysis.

Urine samples

For the determination of nitrogen balance, every day during the collection periods, urine was quantitatively collected at 9:00 a.m. before feeding. Urine was accumulated in plastic containers which contained 50 ml of 0.1N HCl to make pH < 2.00 in order to prevent N loss due to ammonia volatilization and to prevent bacteria from growing in the urine. A quantity of 10% of

the total urine from each ram was withdrawn and kept in glass bottles in deep freezer (-20°C) for later determination of N content.

Complete Chemical analyses

The complete chemical composition of diets and feces was determined according to AOAC (2000).

Rumen fermentation

Samples of rumen liquor collected using a stomach rubber tube inserted into the rumen via the esophagus at 0, 3 and 6h post-feeding to determine the rumen fermentation characters of pH, NH₃-N, and VFA. The liquor was strained through 4 layers of cheese cloth and divided into two parts, then pH immediately determined using the pH meter (Model HI 8424) in the first part. The second part stored in a deep freezer (-20°C) until chemically analyzed using dried glass bottles with 0.5 ml toluene and 1 ml paraffin oil to each sample. Free Ammonia-N in the rumen samples was determined by the Van Slyke method, as described by Ahmed *et al.* (1976). After acidification of rumen liquor samples using concentrated ortho-phosphoric acid and 0.1N hydrochloric acid, the volatile fatty acids (VFA) were determined by the steam distillation methods as described by Eadie *et al.* (1967). Distillation rate was adjusted so that 100-ml distillate was collected. The concentration of VFA was calculated by knowledge of the amount of 0.01 N NaOH required to neutralize the VFA in the distillate.

Statistical analysis

Data were analyzed using Statistical Analytical System (SAS, 2002), Version, 9.3.1, according to the General Linear Model as following:

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

Where:

Y_{ij} = the parameters under analysis.

μ = the overall mean.

T_i = the fixed effect of the treatments.

e_{ij} = the random error component assumed to be normally distributed.

Differences among means were evaluated using Duncan's (1955) Multiple Range test.

RESULTS AND DISCUSSION

Chemical composition of the experimental diets

The proximate analysis of the experimental feeds (SDA and clover hay) is shown in Table 1. There were no significant differences ($P=0.698$) between SDA and Clover hay in both moisture and dry matter content (9.82 and 90.18% vs. 10.00 and 90.00%, respectively). Similar results were obtained by Cruz *et al.* (2023); Jaouedi *et al.* (2022); Sharma *et al.* (2020); Ahmed *et al.*, (2016) who revealed that *Azolla pinnata* contained 89 - 90.6% DM.

The contents of OM, CP, and EE on a DM basis were significantly higher in SDA (84.16, 26.93 and 3.80%, respectively) than in clover hay (81.75, 14.48 and 2.70%, respectively), while the contents of CF and Ash were significantly lower in SDA (13.48 and 15.84%, respectively) than in clover hay (24.82 and 18.25%, respectively). There were no significant differences ($P=0.698$) between SDA and clover hay in nitrogen-free extract. Chemical analysis showed that sun dried azolla is rich in CP content (26.93%), which exceeds the CP content of clover hay (14.48 %). These results were consistent with those by Tadavi *et al.* (2023); Shambhvi *et al.* (2021); El-Deeb *et al.* (2021); Mahanthesh *et al.* (2018); Bhattacharyya *et al.* (2016); Indira and Ravi (2014); Shamna *et al.* (2013) and Gouri *et al.* (2012) who found that CP content of *Azolla* ranged between 25.4 to 28.2%. In the same regard, occasional studies have confirmed that *Azolla* of CP content ranges between 21 to 24% (Cruz *et al.*, 2023; Lal *et al.*, 2022; Boitai *et al.*, 2018; Ara *et al.*, 2015; Acharya *et al.*, 2015; Bhilawade 2015; Paudel *et al.*, 2015; Rawat *et al.*, 2015).

Table 1 : Chemical composition of the experimental ingredients.

Item (%)	Experimental ingredients		P- value
	Azolla	Hay	
Moisture	9.82±0.27	10.00±0.34	0.698
DM	90.18±0.27	90.00±0.34	0.698
On DM% basis			
OM	84.16±0.25	81.75±0.57	0.018
CP	26.93±0.34	14.48±0.36	<0.001
CF	13.48±0.28	24.82±0.37	<0.001
EE	3.80±0.12	2.70±0.11	0.002
NFE	39.95±0.76	39.75±0.58	0.842
Ash	15.84±0.25	18.25±0.61	0.018

DM, dry matter; OM, organic matter; CP, crude protein; CF, crude fiber; EE, ether extract; NFE, nitrogen-free extract.

Digestibility and nutritive values of the experimental diets

The nutrient digestibility and nutritive values of the experimental feeds are shown in Table 2. It is clear that the values of digestibility coefficients for SDA were significantly ($P < 0.05$) higher than clover hay for DM and CP. The corresponding values for SDA were higher than clover hay by 5.49 and 22.17%, respectively. On the other hand, the digestion coefficient of NFE for SDA was significantly ($P < 0.05$) lower than clover hay by 7.43%. There were no significant differences ($P = 0.092$) between SDA and clover hay for CF digestibility. Indira *et al.* (2009) reported that the digestibility coefficients of DM, CP, CF, EE, and NFE of Azolla were significantly higher when fed to cattle. Furthermore, (Bhatt *et al.*, 2021) illustrated that the digestibility of DM, OM, CP, EE, NDF, and ADF improved with added Azolla in calves' and goat's diets. Correspondingly, Wadhvani *et al.* (2010) and Das *et al.* (2018), observed that DM and CP digestibility decreased significantly with the increase of *Azolla filiculoides* in lamb's diet.

Ghodake *et al.* (2012) reported that the digestibility of all nutrients DM, CP, CF, EE, and NFE decreased when azolla levels increased compared to the control group on the Osmanabadi diet. Rao and Reddy *et al.* (2013) studied the development of a feeding system based on *Azolla pinnata* and Sheanut Cake for Nellore sheep reared in different production systems, they found that the digestibility coefficient of all nutrients in the semi-intensive system was higher than in an intensive system. Abou El-Fadel *et al.* (2020) illustrated that digestibility of CF and EE were increased by adding Azolla in crossbred Osimi lambs' diets, while, the digestion coefficient of DM, OM, CP and NFE and feeding value as TDN and DCP were decreased. However, Bhatt *et al.* (2021) reported that digestibility of DM, OM, CP, EE, NDF, ADF and TDN were increased by Azolla level increased in Sahiwal Calves, with reinforcement Hassanein *et al.* (2023) revealed that DM, OM, CP, CF, EE and NFE digestibility were increased by adding Azolla to goat's diet.

Table 2 : Nutrients digestibility and nutritive value of the experimental ingredients.

Item (%)	Experimental ingredients		P- value
	Azolla	Hay	
DM	56.12±0.50	53.04±0.59	0.003
CP	68.65±0.52	53.43±0.50	<0.001
CF	54.66±1.08	51.91±1.01	0.092
EE	60.65±1.68	60.00±1.99	0.806
NFE	51.84±1.12	55.69±0.71	0.016
DCP	18.49±0.24	7.70±0.21	<0.001
TDN	51.93±0.69	46.55±0.43	<0.001

DM, dry matter; CP, crude protein; CF, crude fiber; EE, ether extract; NFE, nitrogen-free extract. DCP, digested crude protein; TDN, total digestible nutrients

The results of the nutritive value followed the same trend of the digestion coefficients (Table 2). The higher values of nutrient digestibility had a reflection on the nutritive value expressed as DCP and TDN%. The values of DCP and TDN% for SDA were significantly ($P < 0.05$) higher than clover hay by 58.36 and 10.36%, respectively. Bhatt *et al.* (2021) reported that nutritive values (TDN) were increased by Azolla level increased in Sahiwal Calves. Likewise, Kumari *et al.* (2021) illustrated that TDN and DCP were increased by the addition of Azolla. However, Abou El-Fadel *et al.* (2020) illustrated that feeding values as TDN and DCP were decreased.

Nitrogen utilization

The results of nitrogen utilization expressed as N intake, fecal N, urinary N and N balance are shown in Table 3. Animals fed SDA showed

significant ($P < 0.05$) increases in N intake, fecal N and urinary N (58.26, 18.26 and 20.86 g N/d, respectively) than in clover hay (31.06, 14.45 and 8.87 g N/d, respectively). However, the N balance in animals fed SDA was significantly ($P < 0.05$) higher (19.14 g N/d) than for those fed Clover hay (7.73 g N/d), which was 2.5 times higher.

Sihag *et al.* (2018) observed that concentrate mixture replacement with sun-dried Azolla did not affect N intake, however, fecal nitrogen was significantly higher in 20% Azolla level. While ingested and retained nitrogen g/day and retention of nitrogen percentage were significantly low on the 20% Azolla addition to a concentrate mixture for goats. The results showed that ingestion and retention of nitrogen were not affected due to concentrate mixture replacement with Azolla up to 15% levels.

Table 3 : Nitrogen balance of the experimental ingredients.

Item	Experimental ingredients		P- value
	Azolla	Hay	
Feed-N	58.26±0.44	31.06±0.65	<0.001
Feces-N	18.26±0.23	14.45±0.27	<0.001
Urine-N	20.86±1.06	8.87±0.28	<0.001
Nitrogen balance	19.14±1.38	7.73±0.64	<0.001

Rumen characteristics

Table 4 shows the rumen characteristics of animals fed the experimental feed stuffs. No differences were reported between Sun dried azolla and clover hay in rumen pH, TVFA and NH₃-N at zero time and three hours post feeding. After six hours of feeding, results illustrated that all studied rumen characteristics were significantly ($P < 0.05$) higher in animals fed sun dried azolla than in those fed clover hay. The values of pH, TVFA and NH₃-N in animals fed dried azolla were 6.52, 18.62 and 11.58,

comparing with animals fed clover hay (6.34, 17.78 and 10.45), respectively. Hassanein *et al.*, (2023) who showed that rumen pH non-significant differences, rumen pH in (0 and 3h) were decreased with increasing Azolla level from (6.69 and 5.53) to (6.6 and 5.42) in 0 and 3h, respectively. However, rumen pH in 6h was increased from (6.19 to 6.65). Ammonia-nitrogen and total volatile fatty acids (TVFA's) in rumen liquor of goats were increased with increasing Azolla level (0, 10 and 20%) in Zaraibi dairy goat diets.

Table 4 : Rumen parameters of the experimental ingredients.

Time	Item	Experimental ingredients		P- value
		Azolla	Hay	
0 h	pH	6.79±0.06	6.70±0.09	0.417
	TVFA, meq/dl	15.73±0.90	14.52±0.65	0.250
	NH ₃ -N, mg/dl	9.94±0.13	9.35±0.16	0.191
3 h	pH	6.48±0.08	6.32±0.11	0.303
	TVFA, meq/dl	18.73±0.50	17.88±0.67	0.334
	NH ₃ -N ,mg/dl	11.64±0.14	10.54±0.18	0.332
6 h	pH	6.52±0.07	6.34±0.11	0.016
	TVFA, meq/dl	18.62±0.49	17.78±0.67	0.001
	NH ₃ -N, mg/dl	11.58±0.15	10.45±0.18	0.001

TVFA, total volatile fatty acids; NH₃-N, ammonia nitrogen.

On the other hand Kumar *et al.* (2015) noted that the average rumen pH in Azolla fed group (6.62) was higher than the control (6.56). Total Volatile Fatty Acid (TVFA) concentration in Azolla fed group (54.42) was lower than the control (55.56). Time of sampling had significant effect on the TVFA concentration. The TVFA concentration was the highest (60.63) at 4 h post-feeding. Ammonia nitrogen (NH₃-N) concentration in Azolla fed group (11.31) was lower than the control (11.59). Time of sampling had significant effect on rumen fermentation pattern of buffalo bulls.

CONCLUSION

Based on the experiment findings, sun dried azolla is rich in CP content which exceeds the CP content of clover hay (13.48%). DM and CP digestibility for sun dried azolla were significantly higher than clover hay, while CF digestibility did not differ significantly. The nutritive values for sun dried azolla significantly improved comparing with clover hay, also N balance followed similar pattern. Rumen characteristics (pH, VFA and NH₃-N) started to be significantly higher in animals fed sun dried azolla than in those fed clover hay after six hours of feeding. Generally sun dried *Azolla pinnata*

can be used successfully in Barki sheep feeding with beneficial effect on digestibility, nutritive value, nitrogen balance and rumen fermentation.

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تأثير نبات الأزولا على معاملات الهضم والقيمة الغذائية وتخمرات الكرش فى علائق أغنام البرقى

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الملخص العربى

أجريت الدراسة الحالية لتقييم استخدام نبات الأزولا فى تغذية المجترات وذلك عن طريق مقارنة الأزولا بدريس البرسيم. تم استخدام ١٢ كيش برقى بالغ عمر حوالى عامان ووزن الجسم 42.87 ± 2.11 كجم. تم تقسيم الأغنام إلى مجموعتين متمثلتين (٦ كباش / مجموعة) تبعاً لوزن الجسم، حيث وضعت الحيوانات فى صناديق هضم منفردة. أظهرت النتائج المتحصل عليها أنه لم تكن هناك فروق معنوية فى محتوى المادة الجافة بين الأزولا الجافة ودريس البرسيم، بينما سجل محتوى المادة العضوية، البروتين الخام، الدهن الخام قيماً أعلى معنوياً ($P \geq 0.05$) فى الأزولا الجافة مقارنة بدريس البرسيم. انخفض محتوى الألياف الخام بشكل معنوياً ($P \geq 0.05$) فى الأزولا الجافة عن دريس البرسيم. أكدت نتائج التحليل الكيماوى على أن الأزولا الجافة غنية فى محتواها من البروتين الخام (٢٦,٩٣%). ارتفع معنوياً معاملات هضم المادة الجافة والبروتين الخام للأزولا الجافة كانت أعلى معنوياً ($P \geq 0.05$) مقارنة بدريس البرسيم، فى المقابل انخفض ($P \geq 0.05$) معامل هضم الكربوهيدرات الذائبة للأزولا مقارنة بدريس البرسيم، ولم يختلف معامل هضم الألياف الخام معنوياً بين العلائق التجريبية. ارتفعت معنوياً القيمة الغذائية سواء فى صورة مجموع المركبات الغذائية المهضومة (TDN) أو بروتين مهضوم (DCP) فى الأزولا الجافة (51.93% و ١٨,٤٩) عن دريس البرسيم (٤٦,٥٥ و ٧,٧٠) على التوالى. سجلت مجموعة الأزولا قيم أعلى ($P \geq 0.05$) لميزان النيتروجين (19.14 g N/d) عن دريس البرسيم بعد ٦ ساعات من التغذية. بشكل عام يمكن استخدام الأزولا بنجاح فى تغذية أغنام البرقى لما لها من تأثير جيد على كلا من معاملات الهضم والقيمة الغذائية وميزان النيتروجين و تخمرات الكرش فى الأغنام البرقى.