

Nutrients Digestibility, Nitrogen Balance and Rumen Fermentation Parameters of Farafra Sheep Fed on *Sesbania sesban* and Reed Plants

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Summary

This study aimed to determine the effects of the dietary feeding of sesban (*Sesbania sesban* (L.) Merr.) and reed forage (*Arundo donax* L.) on nutrient digestibility, nitrogen retention, ruminal protozoa count, and rumen fermentation in sheep. Twelve Farafra rams (50 ± 0.25 kg, 2 ± 0.2 years) were allocated to three treatments. The control group was fed a diet containing 700 g concentrate mixture with fresh alfalfa *ad libitum*. In the second and third treatments, alfalfa was replaced with fresh sesban or reed forage *ad libitum*, respectively. Forage intake of sesban and alfalfa was higher ($P < 0.05$) than reed forage. Fiber digestibility was higher ($P < 0.05$) with reed compared to other treatments. Digestible crude protein of control and sesban treatments was higher ($P < 0.05$) than reed. Nitrogen intake, digestion and retention of the control and sesban treatments were higher ($P < 0.05$) than reed treatment. No differences were found among the treatments for ruminal pH, ammonia-N, total volatile fatty acids, and total protozoal count. In conclusion, the results of nutrient digestibility, rumen fermentation and nitrogen from sesban and reed forage showed that alfalfa can be replaced with sesban or reed forage in the diet of sheep. Moreover, sesban and reed forage can be considered as sources of protein and energy for rams during the summer season.

Key words

Sesbania sesban (L.) Merr., reed forage, sheep, nutrient digestibility, rumen fermentation

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Introduction

Insufficient feed, both in quantity and quality, is the main barrier to livestock production in tropical and subtropical regions (Gouda et al., 2022; Kholif et al., 2022). In Egypt and many other countries, alfalfa crops have been reduced to allow more area for wheat according to government policy (Nikiel and Eltahir, 2021). A possible alternative is *Sesbania sesban* (L.) Merr. and reed forages (*Arundo donax*, L), which naturally grow over huge land areas.

Multipurpose tree legumes grow well in tropical environments without requiring a lot of agronomic inputs, indicating their use as supplementary feeds for medium- and small-scale livestock farmers in the tropics (Gebreyowhans and Zegeye, 2019). The New Valley is Egypt's largest governorate, which occupies half of the western desert and accounts for 48% of the country's total surface area. Some areas contain much salt, thus making the growth of crops difficult (Abdel-Shafy and Kamel, 2016). Cultivation of *Sesbania sesban* and reed plants is considered one of the solutions to overcome the problem of high levels of salinity in New Valley governorate, in addition to using it as a high-quality green fodder for animals, especially small ruminants.

Reed plants can grow fast and tolerate soil salinity and survive in high water salinity compared with other plants (Müller et al., 2022). Reed forage is more palatable and richer in nutritive value while young compared to older one (Ahmed et al., 2009). Previous studies showed that reed exerted antibacterial, antifungal, anthelmintic, anticancer and other pharmacological effects (Al-Snafi, 2015). Its high nitrogen (121 g kg^{-1}), potassium (10.9 g kg^{-1}), and manganese (2.65 g kg^{-1}) content makes it a particularly good fodder plant for ruminants (Baran et al., 2002). The young stems attain the highest total carbohydrate and crude fiber concentrations (Al-Sodany et al., 2013). It also enhances milk production in dairy cows (Nour et al., 1995) and performance of fattening sheep (Ahmed et al., 2011) which is attributed to the presence of components reported to be galactogogues (Al-Snafi, 2015), and has no hazardous or anti-nutritional effect when fed to animals (Ahmed et al., 2011).

Sesbania sesban (called sesban) is one of the fodder trees that is used to alleviate feed deficiencies used fresh or as hay for animals because of its high protein content (23.8%) (Mohsin and Al-Hamdani, 2012). Supplementing *Sesbania sesban* increases feed intake, digestibility, nitrogen retention, and performance of lactating cows (Khalili and Varvikko, 1992) and lambs (Mekoya et al., 2009; Zaki et al., 2015). El-Mogazy et al. (2017) observed that feeding adult Zaraibi bucks on diets containing sesban at 40, 50 and 80% increased daily water consumption without affecting ruminal pH or ammonia-N ($\text{NH}_3\text{-N}$) or nutrient digestibility, while increasing ruminal total volatile fatty acids (VFA) (in the diet with 40% sesban). Recently, Fargaly et al. (2022a) observed that feeding fresh *Sesbania sesban* and reed grass to growing Farafra lambs to replace alfalfa (represents 40% of total diet) did not affect body weight, average daily gain, feed conversion rate, hot carcass, dressing percentage and carcass components.

We hypothesized that the nutrient and secondary metabolite (e.g., tannins and saponins) concentrations in sesban and reed plants candidated them to replace alfalfa in diets of rams without negative effects on digestion or ruminal fermentation. Therefore, the present study aimed to assess the effect of feeding alternative

forages, including sesban and reed plants on dry matter (DM) intake (DMI), nutrient digestibility, nitrogen retention and ruminal fermentation. Additionally, the study compared the chemical composition and feeding value of sesban and reed plants versus alfalfa, which is a traditional green fodder in Egypt.

Material and Methods

Study Location

This experiment was carried out at the experimental farm of the Department of Animal Production, Faculty of Agriculture of New Valley, Al Kharga city ($25^{\circ}26'N$ and $30^{\circ}32'E$). Animals were managed and cared for in accordance with the guide of Agricultural Research and Teaching of Federation of Animal Science Societies (Fass, 2010). The protocol of the experiment was revised and approved by the Institutional Animal Care and Use Committee of the Faculty of Agriculture, New Valley University, New Valley, Egypt.

Animals, Management and Feeding

Twelve castrated Farafra rams with almost similar weights ($50 \pm 0.25 \text{ kg}$) and ages (2 ± 0.2 years old) were randomly assigned to three treatments ($n = 4$ rams per treatment) and kept in stainless metabolic cages ($168 \text{ cm long} \times 76 \text{ cm wide} \times 107 \text{ cm high}$) equipped with a system to facilitate complete urine and feces. Cages were designed for separate collection of feces and urine, and equipped with feed, drinking, urine and fecal containers. The trial lasted 22 days, including a 15-day adaption period followed by a 7-day fecal collection period. Samples of feces and urine were collected twice daily at 06:00 and 18:00 h. The cages were kept in semi-opened concrete floor pens. The rams in the first treatment were fed a control diet that consisted of 700 g of concentrate mixture with fresh alfalfa *ad libitum* at 07:00 and 16:00 h. The second (Sesban treatment) and third (Reed treatment) groups were fed 700 g of concentrates with fresh sesban or reed forage *ad libitum*, respectively. The rams were fed 60% of their daily requirements as a concentrated mixture based on the National Research Council was recommended for adult rams with 50 kg weight (NRC, 2007), with the remaining coming from green fodders. The rams were offered the concentrate feed mixture, followed by the fodder. Adjustments were made to the quantity of the diet offered to ensure the collection of 10% orts. The rams were fed twice daily at 08:00 and 16:00 h. The compositions of the concentrate mixture were as follows: 11% of soybean meal, 21% of wheat bran, 65% of yellow corn, 2% of limestone, and 1% of salt.

Moreover, the concentrate and forages (alfalfa, sesban, and reed) were chemically analyzed for the nutrient contents according to the Association of Official Analytical Chemists (AOAC, 2019). The chemical composition of these forage sources is shown in Table 1. The required daily amount of alfalfa was cut in the evening and distributed on the ground to be fed on the next day, to avoid bloating incidences. The whole plants of sesban and reed plants (both leaves and branches of each one) were daily cut before feeding in the same day into small pieces (2 to 3 cm) before offering to the animals.

Table 1. Chemical composition (%) of alfalfa, sesban, reed plants, and concentrate feed mixture (DM basis)

Item	CFM1	Reed	Sesban	Alfalfa
Dry matter	94.8	33.1	37.2	31.3
Organic matter	85.8	88.4	93.8	89.9
Crude protein	14.6	12.3	16.2	15.7
Crude fiber	8.8	22.8	18.7	21.6
Ether extract	5.3	3.3	3.9	3.6
Nitrogen free extract	57.1	50.1	55.0	49.0
Neutral detergent fiber	14.7	57.3	24.7	32.7
Acid detergent fiber	2.7	29.9	22.1	19.6
Cellulose	1.3	22.0	11.6	9.5
Hemicellulose	12.0	27.4	2.6	13.1
Gross energy (MJ/Kg DM)	17.3	17.4	18.6	17.8

Note: Concentrate mixture (CFM) contained (%): 11% of soybean meal, 21% of wheat bran, 65% of yellow corn, 2% of limestone, and 1% of salt.

Nutrient Digestibility Coefficient and Nitrogen Balance Measurements

Daily feed intake of concentrate mixture and forages (alfalfa, sesban, and reed) was estimated by subtracting residual feed from that offered during the fecal collection period. The feed remains were obtained, dried, combined, and crushed through a 1-mm screen before being stored for chemical analysis. Urine was collected via the metabolism cages using steel buckets underneath the cage to prevent fecal contamination. After each sample (triplicate) was taken, the total volume of urine was recorded and buckets were cleaned and replaced to prevent any contamination or dilution between samples. Total fecal output was mixed and weighed daily. A representative sample (10%) of daily fecal and urine samples was obtained from each ram, promptly weighed and mixed and preserved at -20 °C. The fecal or urine samples of each animal (14 samples produced from each animal twice daily for 7 days) were pooled and mixed, and 10% of the total amount was taken for chemical analysis at the end of the collection period. The feces were dried for 24 h at 60–70 °C, then milled through a 1-mm mill screen and kept until chemical analysis. Nitrogen balance was calculated as: $N \text{ retained (g day}^{-1}) = N \text{ intake} - N \text{ excretion in feces} - N \text{ excretion in urine}$.

The chemical analysis of feed residue and feces (triplicate samples) was conducted using protocols developed by AOAC (2019). The Van Soest (1991) techniques were used in determining the neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). The difference between NDF and ADF was used to calculate hemicellulose, whereas the difference between ADF and ADL was used to calculate cellulose. Nutrient content changes between consumed feed and feces as a proportion of their intake were used to determine nutrient digestibility. The feeding values in terms of total digestible nutrient (TDN)

and digestible crude protein (CP) (DCP) were estimated using chemical analyses of ingredients and the apparent digestibility of various nutrients in the consumed rations (McDonald et al., 2010). Gross energy (GE) was calculated according to the British Ministry of Agriculture, Fisheries and Food (MAFF) report (MAFF, 1975).

Rumen Liquor Parameters

During the last successive two days of the experiment, individual samples of rumen fluid (100 mL) were obtained from the ventral, caudal and dorsal sacs using a stomach tube for determination of pH and concentration of fermentation end-products. To prevent contamination with saliva, the first 50 mL ruminal fluid were discarded. Samples were obtained right before feeding time (07:00 h), as well as 4 h (11:00 h) and 8 h (15:00 h) afterward. The pH of ruminal fluid was measured immediately using a pH meter (HI98127 pHep®4 pH/Temperature Tester, Hanna® instrument, Italy). The rumen fluid samples were separated into two parts, of which the first was filtered through one layer of cheesecloth before being counted for protozoa. The total protozoa count was performed according to Dehority (2018). Nonetheless, the second half was filtered through four layers of cheesecloth and the filtrate was then utilized to immediately measure the pH with a digital pH meter (Beckman, model 45, USA), and NH_3 -N concentration was determined according to Chibnall et al. (1943). Strained rumen liquid samples were acidified with 0.1 N of hydrochloric acid and concentrated orthophosphoric acid to inhibit microbial activity before storage for analysis and then stored frozen at -20 °C for VFA. Using the Markham micro distillation equipment, the total VFA concentration was measured by titration, after steam distillation of a 4 mL sample (Warner, 1964).

Statistical Analysis

The Shapiro-Wilk test was used to test the normal distribution of variables. For ruminal protozoal count that showed significance for the Shapiro-Wilk test, data transformation (i.e., natural log) was applied before reanalysing the normality of the residuals. The data were statistically analyzed using the general linear model (GLM) procedure of the Statistical Analysis System SAS (SAS Institute, Cary, NC, USA). The one-way analysis of variance was used to compare the digestibility, nitrogen retention, and nutritive values of the different groups. The Duncan Multiple Range Test was used to determine significant variations in treatment means. The data are presented as mean \pm standard error. P -values of <0.05 were deemed significant. The following model was used:

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

The rumen liquid parameters data were analyzed according to the following statistical model:

$$Y_{ijk} = \mu + T_i + M_j + (TM)_j + E_{ijk}$$

where, Y_{ij} = experimental observation, μ = general mean, T_i = the effect of treatment, i = control, Sesban and Reed treatments, e_{ij} = the errors related to individual observation, M_j = the effect of time sampling after feeding, j = zero, four hours and eight hours, $(TM)_j$ = interactions between time and treatment.

Results

Dry Matter Intake, Nutrients Digestibility and Nutritive Value

Table 2 shows the daily DMI of rams that were fed with different forages. The daily roughages intake of rams that were fed with alfalfa and sesban was higher ($P < 0.05$) by approximately 24.05% and 30.62% than that of reed plants, respectively. However, the roughages and total DMI of the rams that were fed with alfalfa and sesban did not differ.

Digestibility of different nutrients in all groups that were fed on different forages was almost similar (Table 2). However, the NDF and ADF digestibility in the reed group was higher ($P < 0.05$) than that of the sesban group. Additionally, the digestibility value of cellulose and hemicellulose of the reed group was 32% and 17.95% higher than that of the sesban group, respectively.

The nutritive value in terms of the total digestible nutrients (TDN) was not significantly affected among the groups. However, the digestible crude protein (DCP) of the control and sesban groups was higher ($P < 0.05$) than that of the reed group (Table 2).

Table 2. Effect of feeding sesban and reed plants on intake, nutrient digestibility, and nutritive values of experimental diets fed to Farafra sheep (n = 4).

	Diet ¹			SEM	P value
	Control	Sesban	Reed forage		
DM intake (g/sheep/d)					
Concentrate	664	664	664	0	1.000
Roughage	923 ^a	1011 ^a	701 ^b	58.7	0.026
Total DM intake	1587 ^a	1674 ^a	1365 ^b	58.7	0.026
Nutrient digestibility (%)					
Dry matter	82.9	82.51	80.49	2.17	0.744
Organic matter	84.7	83.6	81.6	2.22	0.682
Crude protein	82.1	80.98	79.93	2.03	0.793
Ether extract	83.9	79.33	83.15	1.68	0.300
Nitrogen free extract	87.6	85.51	82.27	2.03	0.350
Neutral detergent fiber	70.4 ^a	53.62 ^b	78.96 ^a	4.76	0.024
Acid detergent fiber	60.3 ^{ab}	46.50 ^b	72.12 ^a	5.00	0.032
Cellulose	58.5	52.71	77.56	7.49	0.160
Hemicellulose	80.4	69.49	84.69	6.22	0.302
Nutritive value (%)					
Total digestible nutrient ²	79.2	80.2	75.6	2.07	0.381
Digestible crude protein	12.5a	12.6 ^a	10.7 ^b	0.28	0.017

Note: Means within the same row bearing different superscripts significantly differ ($P < 0.05$).

¹ Diet: Sheep were fed a diet containing 700 g concentrate mixture and *ad libitum* fresh alfalfa (Control treatment), or fresh sesban (Sesban treatment) and reed forage (Reed treatment) replacing alfalfa.

² Calculated according to NRC equation (NRC, 2001).

Nitrogen Retention

With no differences between the control and sesban treatments, Reed treatment lowered N intake, N digested and N retained (Table 3). However, there was no difference in the percentage of N-retained to N-intake or N-digested among the groups.

NH₃-N concentrations at 0 h postfeeding, while Reed treatment lowered it at 4 h, with no effects due to treatments at 8 h post feeding. Both Sesban and Reed treatments decreased ruminal VFA concentration, while Reed treatment decreased their concentrations at 4 h without affecting it at 8 h post feeding.

Rumen Fermentation Activities

Treatments did not affect ruminal pH at all sampling hours (Table 4). Sesban and reed treatments lowered ($P < 0.05$) ruminal

Sesban and Reed treatment increased ruminal protozoal count at 0 h post feeding, while decreased it at 8 h post feeding (Table 5). However, Sesban treatment lowered the count at 4 h post feeding.

Table 3. Nitrogen balance of Farafra rams fed on the experimental diets (n = 4)

DM intake (g)	Diet ¹			SEM	P value
	Control	Sesban	Reed forage		
N-intake (g)	38.6 ^a	41.7 ^a	29.1 ^b	1.67	0.002
Fecal-N (g)	7.39	8.43	6.15	0.793	0.259
N-digested (g)	31.2 ^a	33.3 ^a	23.0 ^b	1.17	0.003
Urinary-N (g)	4.38	4.26	3.08	0.39	0.131
N-Retained	26.8 ^a	29.0 ^a	19.9 ^b	1.15	0.006
N-Retained (g/N-intake) (g)	69.8	69.5	68.7	3.12	0.933
N-Retained/N-digested (%)	86.0	87.1	86.4	1.623	0.900

Note: Means within the same row bearing different superscripts significantly differ ($P < 0.05$).

¹ Diet: Sheep were fed a diet containing 700 g concentrate mixture and *ad libitum* fresh alfalfa (Control treatment), or fresh sesban (Sesban treatment) and reed forage (Reed treatment) replacing alfalfa.

Table 4. Effect of feeding sesban and reed plants on ruminal fermentation of experimental diets fed to Farafra sheep (n = 4)

	Time	Diet ¹			SEM	P value
		Control	Sesban	Reed forage		
pH	0 h	7.34	7.3	7.29	0.061	0.845
	4 h	6.15	6.45	6.39	0.160	0.682
	8 h	7.29	7.45	7.46	0.189	0.661
	Mean	6.93	7.07	7.05	0.172	0.655
Ammonia-N mg/100 ml	0 h	6.08 ^a	5.48 ^b	5.93 ^b	1.568	0.032
	4 h	17.19 ^a	17.94 ^a	11.27 ^b	5.734	0.029
	8 h	6.82	6.37	6.67	1.447	0.083
	Mean	10.03 ^a	9.93 ^a	7.96 ^b	1.355	0.034
Volatile fatty acids meq/100 ml	0 h	8.49 ^a	8.48 ^a	6.91 ^b	0.472	0.022
	4 h	19.96 ^a	16.03 ^b	17.13 ^b	2.240	0.017
	8 h	10.84 ^b	13.2 ^a	9.12 ^c	0.922	0.026
	Mean	13.10 ^a	12.57 ^a	11.05 ^b	1.111	0.024

Note: Means within the same row bearing different superscripts significantly differ ($P < 0.05$).

¹ Diet: Sheep were fed a diet containing 700 g concentrate mixture and *ad libitum* fresh alfalfa (Control treatment), or fresh sesban (Sesban treatment) and reed forage (Reed treatment) replacing alfalfa.

Table 5. Effect of feeding sesban and reed plants on ruminal protozoal count ($\times 10^6$ mL⁻¹) (n = 4).

Time	Diet ¹			SEM	P value
	Control	Sesban	Reed forage		
0 h	1.98 ^c	2.77 ^a	2.19 ^b	0.393	0.011
4 h	3.17 ^a	1.57 ^b	3.29 ^a	0.133	0.033
8 h	2.43 ^a	1.74 ^c	2.09 ^b	0.277	0.024
Mean	2.53 ^a	2.03 ^b	2.52 ^a	0.222	0.013

Note: Means within the same row bearing different superscripts significantly differ ($P < 0.05$).

¹ Diet: Sheep were fed a diet containing 700 g concentrate mixture and *ad libitum* fresh alfalfa (Control treatment), or fresh sesban (Sesban treatment) and reed forage (Reed treatment) replacing alfalfa.

Discussion

Chemical Composition of Sesban and Reed Forages

The chemical composition differed between sesban, alfalfa, and reed plants. The variability observed between plants could be due to differences in genetics, climatic conditions, soil types, soil fertility, agronomical management, fertility status, parts of the plant (leaves, twigs, whole forage and green pods) and other environmental factors (Ammar et al., 2022; Metoui et al., 2019). The sesban tree has high nitrogen content in its foliage and is a great protein supplement for roughage that is lacking in protein (Manaye et al., 2009; Sabra et al., 2010). This tree's leaves and branches provide significant protein levels (ranging from 20% to 25%) that are easily digested by ruminants (Gomase et al., 2012), it is within the acceptable range reported for different foliage plants (Lee, 2018) and is above the minimum threshold of 80 g kg⁻¹ DM required for rumen microbial growth and activity (Soliva et al., 2015). Sesban contains a high CP level, indicating that it might be used to supplement protein in ruminant diets that are low or lacking in CP (Gebreyowhans and Zegeye, 2019). Reed grass has a moderate CP level (12.25%), which is similar to that found (12.25%) by De la Cruz (1983). The higher percentage of fiber fractions (NDF and ADF) found in the reed forage than in alfalfa and sesban may be due to its low CP content. Mahmoud et al. (2017) used some grass and legume and other plants and found that the crude fiber and ether extract contents were high in the species that contained less protein and low in species with high protein content.

The chemical composition of sesban in our study was similar to that reported by El-Mogazy et al. (2017) who observed that sesban contained 15% CP, 58.5% NDF and 37.4% ADF. The chemical composition of sesban and reed forage in the present study was close to alfalfa as both sesban and reed had fair contents of protein and energy which could be used as feed for rams in Mediterranean regions, especially during the drought season (Soltan et al., 2017).

Dry Matter Intake, Nutrient Digestibility and Nutritive Value

The lower intake in the reed plant group than in other forage groups may be due to the low palatability of reed forage, which results in a low voluntary intake, even when the animals ingest young plants (Ahmed et al., 2011; Shehata et al., 2006).

Additionally, both the higher fiber contents in reed plants and low nitrogen retention limited DMI. Moreover, the higher NDF (57.26%) contents of the reed plant are always negatively correlated with DMI (Mokhtarpour and Jahantigh, 2018). However, the improved forage intake of rams that were fed on sesban and alfalfa may be attributed to its lower NDF and ADF and higher CP with more palatable parts and thus increased DMI (Tekliye et al., 2018). Similar results of feed intake were observed with growing lambs (Farghaly et al., 2022b).

The higher nutrient digestibility of all forage groups could indicate the benefits of combining concentrates with fodder, such as alfalfa, sesban, and reed plants. Additionally, the digestibility of sesban and reed plants makes it equivalent to the digestibility value of alfalfa maybe due to the moderate tannin levels, which can provide adequate levels of both rumen degradable and by-pass protein to ruminants (Mekoya et al., 2009; Nsahlai et al., 1999). Mekoya et al. (2009) reported that replacing concentrate mixture with sesban at 47.5 levels and 95% as a source of protein in lamb diets increased DM, organic matter and N digestibility. Tekliye et al. (2018) reported that supplementing sesban leaves to ration significantly enhanced apparent nutritional digestibility.

The digestibility of NDF and ADF was significantly ($P < 0.05$) higher in the reed forage and alfalfa groups than that in the sesban group. The improved structural carbohydrate digestibility of the reed group may be attributed to reducing the intake level, which decreased the passage rate and exposed the feed to microbial attack for a long time (Fenner et al., 1967). Moreover, the higher protozoa count in the rumen liquor of the reed group may participate in improving the fiber fraction digestibility. McSweeney and Mackie (2012) reported that protozoa were responsible for 30%–40% of the overall fiber digestion under specific conditions. The same authors stated that protozoa were also involved in lipid hydrolysis and were thought to account for 30% – 40% of total ruminal activity. El-Talty et al. (2015) observed that replacing berseem with reed in diets of growing lambs increased the digestibility of DM, OM and CP.

The higher DCP value of alfalfa and sesban rations may be due to their increasing nitrogen content. Similar results have been reported by Zaki et al. (2015) who attribute the improved DCP value of ration-contained concentrate feed mixture with sesban due to an increased CP digestibility. Jayanegara et al. (2010)

found that the addition of sesban leaves to concentrate-based diets dramatically reduced *in vitro* methane generation, implying enhanced protein utilization. Ahmed et al. (2017) found that the feeding value expressed as TDN and DCP was improved with feeding sesban and corn silage compared with other treatments.

The decrease in nutritive value of reed plants when compared with other forages groups may be attributed to the variation of maturity phase and parts of the plants that used in this study. Al-Sodany et al. (2013) stated that the nutritive value and nutrient content of reed forage were better in younger plants than the older ones and in leaves than stems, which had a strong relationship with plant digestibility. Furthermore, many previous studies approved that the nutritive value in terms of TDN and DCP of sesban and reed forage could be improved by ensilage or ensilage with addition of other forage plants (Ahmed et al., 2017; Shehata et al., 2006; Zaki et al., 2015).

Nitrogen Retention

Reed treatment lowered N intake, N digested and N retained. The improvement in N intake, N digested and N retained in rams fed alfalfa or sesbania may be related to higher forage intake from these groups than that observed from reed group. Additionally, the improved digestible crude protein (DCP) and rumen fermentation parameters from alfalfa or sesban groups could be associated with improvement of N retention from these groups (Zaki et al., 2015). Such results indicate that the tested forages were equal to alfalfa. El-Talty et al. (2015) observed a decreased N intake when berseem clover was replaced by reed in diets of growing lambs as a result of the low CP concentration in reed compared to berseem clover. Moreover, they stated that N balance had the same trend as daily body gain. In the present experiment, the experimental animals were adult rams, with no daily growth.

Rumen Fermentation Parameters

The treatment did not affect the ruminal pH, and all values were greater than the optimum level (5.6) for ruminal fiber degrading microbial activities and growth (Ryle and Ørskov, 1990). Preventing a decline in ruminal pH is important in order to avoid a change in ruminal microbiota from predominantly fibrolytic to amylolytic microbial communities (Tajima et al., 2001).

The observed $\text{NH}_3\text{-N}$ concentrations were greater than the level (8.5 mg $\text{NH}_3\text{-N}$ dL^{-1}) for optimum rumen microbial proliferation and activity (Jones and Jones, 2012). It could be noticed that the $\text{NH}_3\text{-N}$ values and VFA concentrations in rumen fluid decreased with feeding reed forage compared with other groups, which may be attributed to its lower CP content. El-Kholany et al. (2018) found that the differences in rumen $\text{NH}_3\text{-N}$ concentrations among treatments were correlated with CP content differences in different roughage feeds. Moreover, Fenner et al. (1967) found that NH_3 production rate and level were directly related to the N- solubility of the source of the feed and the proportion of protein of the all-roughage ration. Additionally, the results showed a significant effect of sampling times for all treatments on $\text{NH}_3\text{-N}$ and VFA concentrations which reached to peak at 4 h post feeding. This peak was noticed by Shehata et al. (2006) who found that the peak pH values, $\text{NH}_3\text{-N}$ and VFA NH_3 concentrations appeared at 3 h post feeding, which was noticed when Zaraibi bucks were fed with

reed forage in silage or hay forms. Moreover, the reduced NH_3 levels in the rumen at 8 h post-feeding probably reflect the uptake of this nutrient by the rapidly growing microbial population. The significant effects among all treatment times on $\text{NH}_3\text{-N}$ and VFA may be related to forage digestion by microorganisms in rumen after feeding and their absorption by rumen cells and their use by microorganisms to produce micro-protein. This action is important for rumen nitrogen recycling and protein synthesis efficiency (McSweeney and Mackie, 2012).

The total protozoa count was decreased by approximately 24.6% in the sesban group compared with the alfalfa and reed forage groups. The results obtained are consistent with the findings of Newbold et al. (1997) who stated that sesban was toxic to rumen protozoa, which was due to the saponin-containing component of the plant (Kholif, 2023). The numerically increased total protozoa count that was higher at 4 h post feeding than before feeding or 8 h post feeding was confirmed by Zaki et al. (2015) when feeding the first and second cuts of sesban *ad libitum*. Similarly, Nhan (1998) found that the protozoa number of growing goats fed with *Sesbania grandiflora* was 4.38 and 4.56 10^6 mL^{-1} for 0 and 4 h post feeding, respectively. Aziz et al. (2018) reported an increased protozoa number and improved rumen liquor parameters with the increased roughage ratio in the diets.

Conclusion

The chemical analysis and nutritive value and rumen fermentation of sesban and reed forage in the present study were close to that of alfalfa. Replacing alfalfa with sesban and reed did not affect feed utilization; however, sesban showed enhanced results compared to reed forage as sources of protein and energy for rams feeding during the Egyptian summer season. More experiments with growing and lactating animals under different conditions are recommended to evaluate different dietary levels of sesban and reed plants.

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CRedit authorship contribution statement

Mohsen M. Farghaly, Soliman M. Mousa, Mohamed A. Abd El-Monaime, Abdelraheem I. Suliman, Ahmed E. Kholif, Hatem A. Hamdon: Funding, Supervisin. **Mohsen M. Farghaly, Hatem A. Hamdon:** Conceptualization, Investigation, performed most of the experiments, Dana analysis, Original draft preparation. **Mohsen M. Farghaly, Soliman M. Mousa, Mohamed A. Abd El-Monaime, Abdelraheem I. Suliman, Ahmed E. Kholif, Hatem A. Hamdon:** Performed some of the experiments. **Ahmed E. Kholif, Einar Vargas-Bello-Pérez:** Manuscript editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Abdel-Shafy H.I., Kamel A.H. (2016). Groundwater in Egypt Issue: Resources, Location, Amount, Contamination, Protection, Renewal, Future Overview. *Egypt J Chem.* 59 (3): 321–362. doi:10.21608/ejchem.2016.1085
- Ahmed M.E., Abdel-Gawad A.M., Shehata E.I., Tawfik S.A. (2011). Influence of Using Reed Forage in Different Forms as Fresh, Silage and Hay on Blood Profile and Carcass Quality of Growing Rahmani Sheep. *Egypt J. Sheep Goats Sci.* 6 (2): 25–35. doi: 10.21608/EJSGS.2011.27028
- Ahmed M.E., Abdelhamid A.M., Ibrahim F.A., Grawish S.I.M. (2017). Response of Lactating Zaraibi Goats to Diets Containing Sesbania and Kochia Silages as a New and High Source of Protein. *Int J Curr Res Biosci Plant Biol.* 4 (10): 52–62. doi: 10.20546/ijcrbp.2017.410.005
- Ahmed M.E., Shehata E.I., Ammou F.F.A., Khalifa E.I., El-Zolaky O.A. (2009). Productive and Reproductive Performance of Rahmani Sheep Fed Rations Containing Reed Forage (*Arundo donax* L.) either Fresh Hay or Silage. *Egyptian J Sheep Goat Sci.* 4 (1): 45–54
- Al-Snafi A.E. (2015). The Constituents and Biological Effects of *Arundo donax* - A Review. *International Journal of Phytopharmacy Research* 6 (1): 34–40.
- Al-Sodany Y., El-Sheikh M., Baraka D., Shaltout K. (2013). Elements Accumulation and Nutritive Value of *Phragmites australis* (Cav.) Trin. ex Steudel in Lake Burullus: A Ramsar Site, Egypt. *Catrina Int J Environ Sci* 8 (1): 51–63. doi: 10.12816/0010675
- Ammar H., Kholif A.E., Soltan Y.A., Almadani M.I., Soufan W., Morsy A.S., Ouerghemmi S., Chahine M., de Haro Marti M.E., Hassen S., Selmi H., Horst E.H., Lopez S. (2022). Nutritive Value of *Ajuga iva* as a Pastoral Plant for Ruminants: Plant Phytochemicals and *in vitro* Gas Production and Digestibility. *Agriculture* 12 (8): 1199. doi: 10.3390/agriculture12081199
- AOAC. (2019). Official Methods of Analysis of AOAC International, 18th Edition, Official Methods of Analysis of AOAC International. AOAC International, Washington DC. doi: 10.1093/9780197610138.001.0001
- Aziz H., Nassar M., Badway H., Abd Elrahman M. (2018). Rumen Fermentations and Rumen Ciliate Protozoa of Goat Kids Fed Diets with Different Concentrate: Roughage Ratio. *Egyptian Journal of Nutrition and Feeds* 21 (3): 667–683. doi: 10.21608/ejnf.2018.75770
- Baran M., Váradyová Z., Kráčmar S., Hedbávný J. (2002). The Common Reed (*Phragmites australis*) as a Source of Roughage in Ruminant Nutrition. *Acta Vet Brno* 71 (4): 445–449. doi: 10.2754/avb200271040445
- Chibnall A.C., Rees M.W., Williams E.F. (1943). The Total Nitrogen Content of Egg Albumin and Other Proteins. *Biochemical Journal* 37 (3): 354–359. doi: 10.1042/bj0370354
- de la Cruz A.A. (1983). Caloric Values of Marsh Biota. Mississippi-Alabama Sea Grant Consortium Publ. No. MASGP -83-006, Mississippi
- Dehority A.B. (2018). Laboratory Manual for Classification and Morphology of Rumen Ciliate Protozoa, 1st Edition, Laboratory Manual for Classification and Morphology of Rumen Ciliate Protozoa. CRC Press, Boca Raton, FL, USA. doi: 10.1201/9781351073912
- El-Kholany M., Maged G., Sadik W., Mehany A. (2018). Effect of Using Different Types of Silages Mixtures in Dairy Goat Diets on Milk Production, Feed Conversion, Rumen Fermentation, Nutritional Value and Blood Components. *Journal of Animal and Poultry Production* 9 (2): 115–121. doi:10.21608/jappmu.2018.38935
- El-Mogazy M.M., Ahmed M.E., Abu El-Naser I.A.M. (2017). Response of Zaraibi Goat Bucks to Rations Containing Different Ratios of Sesbania Hay as a New and High Source of Protein. *Egyptian J Sheep Goat Sci.* 12 (03): 65–75. doi:10.12816/0044929
- El-Talty Y.I., Abdel-Gwad M.H., Mahmoud A.E.M. (2015). Effect of Common Reed (*Phragmites australis*) Silage on Performance of Growing Lambs. *Asian J Anim Sci.* 9 (1): 1–12. doi:10.3923/ajas.2015.1.12
- Farghaly M.M., Youssef I.M.I., Radwan M.A., Hamdon H.A. (2022a). Effect of Feeding *Sesbania Sesban* and Reed Grass on Growth Performance, Blood Parameters and Meat Quality of Growing Lambs. *Trop Anim Health Prod.* 54 (1): 1–13. doi: 10.1007/s11250-021-03006-5
- Farghaly M.M., Youssef I.M.I., Radwan M.A., Hamdon H.A. (2022b). Effect of Feeding *Sesbania Sesban* and Reed Grass on Growth Performance, Blood Parameters and Meat Quality of Growing Lambs. *Trop Anim Health Prod.* 54 (1): 1–13. doi: 10.1007/S11250-021-03006-5/TABLES/6
- Fass. (2010). Guide for the Care and Use of Agricultural Animals in Research and Teaching, 3rd Edition, The Federation of Animal Science Societies. Federation of Animal Science Societies, Champaign, IL, USA, 169 pp.
- Fenner H., Dickinson F.N., Barnes H.D. (1967). Relationship of Digestibility and Certain Rumen Fluid Components to Level of Feed Intake and Time of Sampling after Feeding. *J Dairy Sci.* 50 (3): 334–344. doi: 10.3168/jds.S0022-0302(67)87420-4
- Gebreyowhans S., Zegeye T. (2019). Effect of Dried *Sesbania Sesban* Leaves Supplementation on Milk Yield, Feed Intake and Digestibility of Holstein Friesian X Zebu (Arado) Crossbred Dairy Cows. *Trop Anim Health Prod.* 51 (4): 949–955. doi: 10.1007/s11250-018-1779-0
- Gomase Pravin, Gomase Priti, Anjum S., Shakil S., Shahnavaj K.M. (2012). *Sesbania Sesban* Linn: A Review on Its Ethnobotany, Phytochemical and Pharmacological Profile. *Asian J Biomed Pharm Sci.* 2 (12): 11–14.
- Gouda G.A., Kholif A.E., Hamdon H.A., Kassab A.Y., Patra A.K. (2022). Utilization of Waste Date Palm Leaves Biomass Ensiled with Malic or Lactic Acids in Diets of Farafra Ewes under Tropical Conditions. *Animals* 12 (11): 1432. doi:10.3390/ani12111432
- Jayanegara A., Goel G., Makkar H.P.S., Becker K. (2010). Reduction in Methane Emissions from Ruminants by Plant Secondary Metabolites : Effects of Polyphenols and Saponins. In: Sustainable Improvement of Animal Production and Health (Odongo N.E., Garcia M., Viljoen G.J., eds), FAO, Rome, Italy, pp. 151–157.
- Jones M., Jones G. (2012). *Animal Nutrition*, 7th Edition, IGCSE Biology. Pearson Education Limited, UK, pp. 64–83 doi: 10.1017/cbo9780511862793.008
- Khalili H., Varvikko T. (1992). Effect of Replacement of Concentrate Mix by Wilted *Sesbania (Sesbania Sesban)* Forage on Diet Digestibility, Rumen Fermentation and Milk Production in Friesian X Zebu (Boran) Crossbred Cows Fed Low Quality Native Hay. *Anim Feed Sci Technol* 36 (3–4): 275–286. doi: 10.1016/0377-8401(92)90062-B
- Kholif A.E. (2023). A Review of Effect of Saponins on Ruminant Fermentation, Health and Performance of Ruminants. *Vet Sci.* 10 (7): 450. doi: 10.3390/vetsci10070450
- Kholif A.E., Hamdon H.A., Gouda G.A., Kassab A.Y., Morsy T.A., Patra A.K. (2022). Feeding Date-Palm Leaves Ensiled with Fibrolytic Enzymes or Multi-Species Probiotics to Farafra Ewes: Intake, Digestibility, Ruminant Fermentation, Blood Chemistry, Milk Production and Milk Fatty Acid Profile. *Animals* 12 (9): 1107. doi: 10.3390/ani12091107
- Lee M.A. (2018). A Global Comparison of the Nutritive Values of Forage Plants Grown in Contrasting Environments, *Journal of Plant Research*. Springer Tokyo. doi: 10.1007/s10265-018-1024-y
- MAFF (1975). Energy Allowances and Feeding Systems for Ruminants. British Ministry of Agriculture, Fisheries and Food, London, UK, 85 pp.
- Mahmoud A.E.M., Abbas M.S., Cieslak A., Szumacher-Strabel M. (2017). Evaluation of Chemical Composition and *in vitro* Dry and Organic Matter Digestibility of Some Forage Plant Species Derived from Egyptian Rangelands. *J Anim Plant Sci.* 27 (5): 1573–1581
- Manaye T., Tolera A., Zewdu T. (2009). Feed Intake, Digestibility and Body Weight Gain of Sheep Fed Napier Grass Mixed with Different Levels of *Sesbania Sesban*. *Livest Sci.* 122 (1): 24–29. doi: 10.1016/j.livsci.2008.07.020

- McDonald P, Edwards R.A., Greenhalgh J.F.D., Morgan C.A., Sinclair L.A., Wilkinson R.G., Edwards M., Morgan G., Wilkinson S. (2010). Animal Nutrition, 7th Edition. London, UK
- McSweeney C., Mackie R. (2012). Commission on Genetic Resources for Food and Agriculture. Micro-Organisms and Ruminant Digestion: State of Knowledge, Trends and Future Prospects, Commission on Genetic Resources for Food and Agriculture. FAO, Rome, Italy, 61 pp.
- Mekoya A., Oosting S.J., Fernandez-Rivera S., Tamminga S., van der Zijpp A.J. (2009). Effect of Supplementation of *Sesbania Sesban* to Lactating Ewes on Milk Yield and Growth Rate of Lambs. *Livest Sci.* 121 (1): 126–131. doi: 10.1016/j.livsci.2008.06.002
- Metoui M., Essid A., Bouzoumita A., Ferchichi A. (2019). Chemical Composition, Antioxidant and Antibacterial Activity of Tunisian Date Palm Seed. *Pol J Environ Stud.* 28 (1): 267–274. doi: 10.15244/pjoes/84918
- Mohsin F., Al-Hamdani A. (2012). Using *Sesbania Sesban* to Improve Some Soils Affected by Salt in Anbar Governorate-Iraq. *Iraqi Journal of Desert Studies* 4 (1): 5–11
- Mokhtarpour A., Jahantigh M. (2018). Effect of Supplementing Common Reed (*Phragmites australis*) with Urea on Intake, Apparent Digestibility and Blood Metabolites of Baluchi Sheep. *Iran J Appl Anim Sci.* 8 (4): 623–628
- Müller B., Sterner V.A.C., Papp L., May Z., Orlóci L., Gyuricza C., Sági L., Solti Á., Fodor F. (2022). Alkaline Salt Tolerance of the Biomass Plant *Arundo donax*. *Agronomy* 12 (7): 1589. doi: 10.3390/agronomy12071589
- Newbold C.J., el Hassan S.M., Wang* J., Ortega M.E., Wallace R.J. (1997). Influence of Foliage from African Multipurpose Trees on Activity of Rumen Protozoa and Bacteria. *Br J Nutr.* 78 (2): 237–249. doi: 10.1079/bjn19970143
- Nhan N.T.H. (1998). Effect of *Sesbania grandiflora*, *Leucaena leucocephala*, *Hibiscus rosa-sinensis* and *Ceiba pentadra* on Intake, Digestion and Rumen Environment of Growing Goats. *Livest Res Rural Dev.* 10 (3): 8–14
- Nikiel C.A., Eltahir E.A.B. (2021). Past and Future Trends of Egypt's Water Consumption and Its Sources. *Nat Commun.* 12 (1): 4508. doi: 10.1038/s41467-021-24747-9
- Nour A.M., Tag-El-Din A.E., Nour A.A., El-Nagar A. (1995). Utilization of Reeds Bean Straw and Berseem Straw in Feeding Lactating Friesian Cows. *Alexandria Journal of Agricultural Sciences* 45: 83–93.
- NRC. (2007). Nutrient Requirements of Small Ruminants, Nutrient Requirements of Small Ruminants. National Academies Press, Washington, D.C. doi: 10.17226/11654
- NRC. (2001). Nutrient Requirements of Dairy Cattle. Nutrient Requirements of Dairy Cattle. doi:10.17226/9825
- Nsahlai I. V., Bryant M.J., Umunna N.N. (1999). Utilization of Barley Straw by Steers: Effects of Replacing Urea with Protein, Source of Protein and Quantity of Rumen Degradable Nitrogen on Straw Degradation, Liquid and Particle Passage Rates and Intake. *J Appl Anim Res.* 16 (2): 129–146. doi: 10.1080/09712119.1999.9706274
- Ryle M., Ørskov E.R. (1990). Energy Nutrition in Ruminants, Energy Nutrition in Ruminants. Springer Netherlands, Dordrecht, 149 pp. doi:10.1007/978-94-009-0751-5
- Sabra H.A., Hassan S.G., Mohamed M.I. (2010). Effect of *Sesbania Sesban* (*Sesbania egyptiaca*) Supplementation on the Reproductive Performances of Baladi Sheep as Compared to Berseem (Egyptian Clover). *J Reprod Infertil.* 1 (3): 66–70.
- Shehata E., Ahmed M.E., Ammou F.F.A., Soliman A.A.M., Aiad K.M., Abdel-Gawad A.M. (2006). Comparison of Feeding Reed as Hay or Silage with Feeding Berseem Hay or Maize Silage to Dairy Zaraibi Goat. *Egyptian Journal of Sheep, Goat and Desert Animal Sciences* 1 (1): 233. doi: 10.21608/EJSGS.2006.28417
- Soliva C.R., Amelchanka S.L., Kreuzer M. (2015). The Requirements for Rumen-Degradable Protein per Unit of Fermentable Organic Matter Differ between Fibrous Feed Sources. *Front Microbiol* 6 (JUN). doi: 10.3389/fmicb.2015.00715
- Soltan Y.A., Morsy A.S., Lucas R.C., Abdalla A.L. (2017). Potential of Mimosine of *Leucaena leucocephala* for Modulating Ruminant Nutrient Degradability and Methanogenesis. *Anim Feed Sci Technol.* 223: 30–41. doi: 10.1016/j.anifeedsci.2016.11.003
- Tajima K., Aminov R.I., Nagamine T., Matsui H., Nakamura M., Benno Y. (2001). Diet-Dependent Shifts in the Bacterial Population of the Rumen Revealed with Real-Time PCR. *Appl Environ Microbiol.* 67 (6): 2766–2774. doi: 10.1128/AEM.67.6.2766-2774.2001
- Tekliye L., Mekuriaw Y., Asmare B., Mehret F. (2018). Nutrient Intake, Digestibility, Growth Performance and Carcass Characteristics of Farta Sheep Fed Urea-Treated Rice Straw Supplemented with Graded Levels of Dried *Sesbania Sesban* Leaves. *Agric Food Secur.* 7 (1): 1–10. doi: 10.1186/s40066-018-0226-9
- van Soest P.J., Robertson J.B., Lewis B.A. (1991). Methods for Dietary Fiber, Neutral Detergent Fiber and Nonstarch Polysaccharides in Relation to Animal Nutrition. *J Dairy Sci* 74 (10): 3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2
- Warner A.C. (1964). Production of Volatile Fatty Acids in the Rumen: Methods of Measurement. *Nutr Abstr Rev.* 34 (34): 339–352.
- Zaki A., Osman A., Ibrahim F., Soliman E. (2015). Effect of Using *Sesbania Sesban* and Its Mixtures with Some Summer Fresh Grasses on Lambs Productive Performance in New Reclaimed Soil. *Journal of Animal, Poultry & Fish Production* 3 (1): 7–16. doi:10.21608/japfp.2015.7427