RUMINAL DEGRADABILITY AND NUTRITIONAL VALUE OF *MORINGA OLEIFERA* LEAVES AND ITS FEEDING EFFECTS ON PRODUCTIVE PERFORMANCE OF LACTATING EWES

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SUMMARY

This study evaluated the effects of partially substituting Berseem clover (BC) with *Moringa Oleifera* leaves on lactating Barki ewes' performance. Three cannulated rams (51.50±4.0 kg, body weight) were used to evaluate the ruminal degradation of DM, NDF, and ADF for *Moringa Oleifera* leaves (ML) using the *in situ* nylon bag method. The true dry matter digestibility, total gas production (ml/400 mg DM), and fiber fractions degradability were investigated using *in vitro* batch culture. A lactation trial was conducted using fourteen multiparous lactating Barki ewes (averaging 43.5±0.4 kg, body weight) randomly assigned into two equal groups (seven each). The control group's basal diet consists of a concentrated feed mixture and Berseem clover (50:50). The treatment group was fed ML as a replacement for BC at a level of 15% (150 g/kg DM). Results showed no significant effect of ML on *in vitro* nutrient digestibility and milk production and composition. However, the treatment group showed numerically higher values for all nutrients' digestibility and produced more milk with a higher fat content. In addition, the plasma albumin and glucose concentrations both were increased significantly while the triglyceride level was reduced slightly ($p<0.05$) after ML substitution. In conclusion, moringa leaves could be utilized as a replacement for Berseem clover at a level of 150 g/kg DM without any adverse effect on the performance of lactating Barki ewes.

Keywords: *Moringa leaves, in situ degradability, milk and composition, lactating Barki ewes.*

INTRODUCTION

Moringa tree, also known as Moringa oleifera Lam., is a fast-growing tree that serves a variety of purposes and is notable for its resistance to drought. Its prospective values and qualities make it scientifically interesting. Moringa has several applications, including food, medicine, and animal feed. In Egypt, it is commonly cultivated for human consumption. Nevertheless, its cheap cultivation cost favors its usage as animal feed. Moringa oleifera is palatable and nutrient-rich forage, and the partial or entire substitution of berseem with moringa is strongly suggested in sheep feeding practices (Khayyal *et al*., 2015).

In recent years, researchers have become increasingly interested in studying the inclusion effects of moringa leaves in ruminants' rations (Aboamer *et al*., 2020; Ebeid *et al*., 2020, 2022; Kholif *et al*., 2015; Morsy *et al*., 2022). Moringa leaves might be used to counteract the lack of feed and poor quality of forages experienced during the dry season. According to Gebregiorgis *et al*. (2012), the addition of dried moringa leaves to the sheep's baseline diet, which consisted of Rhodes grass hay, during the dry season resulted in an increase in DM intake, body weight gain, and N retention. In addition, Sánchez *et al*. (2006) found that adding moringa as a protein supplement to low-quality rations improved DM intake, nutrient digestibility, and milk production without altering milk composition.

In an *in vitro* study, Morsy *et al*. (2022) used moringa leaves as a substitute for soybean meal in silage and reported a substantial improvement in DM, NDF, and ADF digestibility along with certain rumen kinetics such as pH, bacteria count, total gas production, total volatile fatty acids, acetate, and propionate; meanwhile, methane and carbon dioxide production generation were lowered.

Belhi *et al*. (2018) reported that, moringa leaves possess a substantial antioxidant potential that could effectively contribute to small ruminant nutrition due to their content of minerals and bioactive compounds. Furthermore, moringa leaves are an excellent source of several minerals, including iron, potassium, and calcium, along with multivitamins, all of which are necessary for body growth and milk production, in addition to producing milk with a high concentration of its constituents (Anwar *et al*., 2007). Additionally, moringa is abundant in vitamin C, which enhances iron absorption in animals (Anwar *et al*., 2007).

The use of moringa leaves as a replacement for alfalfa was shown to improve the milk yield and composition of Najdi ewes, as reported by Babiker *et al*. (2017). Also, Kholif *et al*. (2018) observed that replacing moringa leaves at a rate of 15% in lactating goats' diets increased milk yields, milk composition, and mono- and polyunsaturated fatty acids in milk.

Therefore, this research aimed to examine the *in situ* degradability of moringa leaves and the effect of replacing Berseem clover with moringa leaves (150 g/kg DM) on the nutrients' digestibility, blood metabolites, and production performance of lactating Barki ewes.

MATERIALS AND METHODS

In situ trials:

The *in situ* ruminal degradability of moringa leaves (ML) was evaluated using three cannulated rams (51.50±4.0 kg of body weight) as described by Orskov and McDonald (1979). *In situ* trials were conducted at the Marryot Research Station, Desert Research Centre, Ministry of Agriculture, 35 km south of Alexandria, Egypt. Rams were fed at a maintenance level of 40:60 concentrate to roughage as replicates. Moringa leaves were ground using a 1 mm Wiley mill and dried at 60 °C for 48 hours before incubation. Ankom *in situ* bags (SKU: R510) with a pore size of 50±10 µm were used. About 5.0 g was placed in a previously weighted, clean, dry, and numbered bag. The bags were incubated one hour after offering the morning meal. The incubation times were 2, 6, 8, 24, and 48 hours. After incubation, the bags containing residues were removed from the rumen, dipped in cold water to stop microbial activity, and washed until clear. Then, the bags were drained, dried for 72 hours at 60 °C, cooled in a desiccator, and weighed. Then, residues were analyzed for NDF (neutral detergent fiber) and ADF (acid detergent fiber) analysis according to Van Soest *et al*. (1991). To calculate soluble fraction (a), two bags were rinsed in water for 1 hour. When calculating the degradation rate for NDF and ADF, the 0 h is omitted from the calculation. The ruminal disappearance rate of tested feed at each incubation time was calculated as the difference between initial sample contents and residues after incubation, represented as a percentage of initial sample content. Data of ruminal disappearance characteristics of DM was fitted to the exponential equation following the procedure described by Orskov and Mcdonald (1979)**:** $y = a + b\ (1 - \exp(-k_d t))$, where: y = DM disappearance in rumen at time t, a = soluble fraction, b = slowly degradable fraction, k_d = constant rate of degradation of b (%/h) to determine the degradability coefficients. However, data of ruminal disappearance characteristics of fiber fractions (NDF and ADF) were fitted to the following equation $y = b\ (1 - \exp(-k_d t))$. Then ruminally degraded (RD) can be calculated as: $RD = a + b\ (k_d/(k_d + k_p))$, where k_p is the fractional outflow rate of solids from the rumen. This research assumed a 0.05 h^{-1} rumen outflow rate.

In vitro batch culture:

The *in vitro* study was conducted at Dairy Milk Production Laboratory, Dairy Sciences Department, National Research Centre, Egypt. The impact of substituting BC with ML on *in vitro* true dry matter and fiber fractions digestibility (IVTDMD) and total gas production was evaluated using a batch culture method. Rumen fluid was collected from 3 ruminally cannulated sheep before the morning feeding; rumen liquor was collected, squeezed through 4-layers of cheesecloth into a Schott Duran® bottle (L) with an O_2-free headspace, and immediately transported to the laboratory at 39°C where it was used as a source of inoculum. A mixture of tested ration in the ratio of 50:50 concentrate to roughage ratio was used as a substrate. Each treatment was tested in 6 replicates accompanied by blank vessels (no substrate). About 400 mg of the milled substrate was added to the incubation vessels of 100 mL capacity. Each vessel was filled with 40 mL of the incubation medium, which consists of rumen inoculum and buffer at 1:2 (v/v). Buffer was prepared according to Goering and van Soest (1970) procedure. After 24-hours of incubation at 39 °C, residuals were transferred into Ankom bags for future NDF analysis (Van Soest and Robertson, 1980). Ruminal pH was measured using a pH-meter electrode. Residuals were dried at 70 °C and analyzed. IVTDMD was calculated as

follows: IVTDMD = [100 – (W_3 – (W_1 x C_1)) x 100] / (W2 X DM), Where: W_1 = bag tare weight, W2 = sample weight, W3 = final bag weight after *in vitro* and sequential NDF treatment, C1 = blank bag correction (final oven-dried weight/original blank bag weight).

Lactation study:

The in-vivo study was carried out at the experiments station of Atomic Energy Authority, Inshas, Cairo, Egypt. Analysis of feed, feces, and milk samples was undertaken at Animal Milk Production Lab, Dairy Sciences Department, National Research Centre, Egypt.

Fourteen multiparous lactating Barki ewes (43.5±0.4 kg) after 15 days of parturition were randomly assigned into two groups (seven each). Ewes were fed experimental diets 15 days before parturition and 45 days postpartum. All diets met NRC (1975) recommendations. Ewes had two equal meals a day. Half of the concentrate feed mixture (CFM) was provided at 9:00 a.m. and 15:00 p.m. Berseem clover was offered daily at 10:00 a.m. Animals had continuous access to fresh water. Feedstuff ingredients had been analyzed according to the following standard laboratory chemical analyses: Dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), and ash contents according to AOAC (2005); neutral detergent fiber (NDF) and acid detergent fiber (ADF) as described by Van Soest *et al*. (1991). Mineral content of feedstuffs was analyzed using atomic absorption. The chemical composition (on DM basis) of feed ingredients is shown in Table 1.

Blood samples were withdrawn from the coccygeal vein of 3 ewes in each group on the final day of the experiment period to determine some biochemical analyses (plasma urea nitrogen, total protein, albumin, glucose, cholesterol, and triglyceride concentrations). Samples were obtained immediately after 3 hours of the morning feeding meal. Blood samples were collected in tubes with EDITA as an anti-coagulant then centrifuged at 4000 ×g for 15 min, and the obtained plasma was frozen at -20°C until analysis. The parameters were determined by the T80 UV/VIS Spectrometer (PG Instruments Ltd, UK) according to the standard protocols of the suppliers (brochures) and using a commercial kit (Biodiagnostic Co. for Diagnostic and Research Reagents; Dokki, Giza, Egypt).

Milk yield was recorded for every ewe once every two weeks, starting from the second week until six weeks of lactation. Twenty-four hours before hand milking, the lambs were kept away from their dams. Ewes were completely hand milked until stripping the udder. Representative milk samples of about 100 g/ewe were taken and stored at –20°C until analysis. Milk contents of fat, protein, lactose, solids-not-fat (SNF), total solids (TS), and some physical characteristics (density, freezing point, and pH) were determined using the LACTOSCAN SP MILK ANALYZER (Milkotronic Ltd- Bulgaria). Milk SNF and ash were calculated by the following equation: SNF % = TS % - Fat %; Ash % = TS % - Protein % - Fat % - Lactose %. Daily yields of fat, protein, lactose, ash, total solids, and solids-not-fat were computed for the individual milk yields from the sampling day of each ewe. Daily milk yield was standardized to 4% fat and 3.3% protein using the energy corrected milk (ECM) formula: ECM (kg/d) = (milk production × (0.383 × % fat + 0.242 × % protein + 0.7832) / 3.1138), (NRC, 2001).

Statistical Analysis:

Data were statistically analyzed using Two-Way Repeated Measures ANOVA (SPSS, 2011). The statistical model was as follows: $Y_{ijk} = \mu + R_i + T_j + (RT)_{ij} + e_{ijk}$, where Y_{ijk} = the k^{th} observation (k = 1… 20) for ration i in time j, μ = the overall mean, R_i = the effect of ration i (i = 1… 4), T_j = the effect of time j (j = 15, 30, 45, 60), $(RP)_{jk}$ = the interaction, and e_{ijk} = the experimental error.

RESULTS AND DISCUSSION

Feed ingredients:

Table (1) shows the chemical composition of the concentrated feed mixture (CFM), moringa leaves (ML), and Berseem clover (BC). As shown in the data, ML had a higher crude protein (CP) compared to BC (24.99 vs. 16.06%).

Instead, BC contains more ether extract and fiber (NDF and ADF). Berseem clover has almost twice as much fiber as ML. The NDF content for BC and ML was 54.19 and 36.73%, respectively. Furthermore, the ADF content was 40.34 and 21.68% for BC and ML, respectively.

Table (1): Proximate analysis of feed ingredients.

Items	Concentrated feed mixture	Moringa leaves	Berseem clover
Chemical composition, % (on DM basis)			
Dry Matter	89.15	93.05	90.86
Organic Matter	94.05	89.76	89.38
Crude protein	15.16	24.99	16.06
Ether extract	3.51	6.56	1.74
Neutral detergent fiber	42.08	26.73	54.19
Acid detergent fiber	24.63	21.68	40.34
Ash	5.95	10.24	10.62
Mineral content			
P, %		0.38	0.27
K, %		1.5	1.63
Ca, %		0.65	0.95
Mg, %		0.42	0.78
S, ppm		232.9	273.7
Fe, ppm		132	122
Mn, ppm		35	33
Zn, ppm		45	37
Cu, ppm		4.5	4.5

In situ ruminal degradability:

Table (2) represents the *in situ* DM, NDF, and ADF degradability for moringa leaves. The ruminally degraded fraction (RD) is a balance between the digestibility rate (k_d) and the passage rate (K_p) through the rumen. A k_d of 0.05 h^{-1} was used to calculate RD in this study. Results showed that ML had a high ruminal DM degradability of 76.3%. The high content of water-soluble fraction (a) in moringa leaves DM (45.0%) represents its high content of non-structural carbohydrates. In addition, the slow degradable fraction (b) for moringa leaves DM was about 52.1%, and it represents its contents from structural carbohydrates (cellulose and hemicellulose) that are slowly degraded at a rate (K_d) of (0.0760, fraction/h). However, the ruminal undegradable (RU) fraction of moringa leaves DM was only about 23.7%.

The ruminal degradability of ML fiber fractions (NDF and ADF) was approximately similar. The slow degradable fraction (b) was 71.9 and 73.9% for NDF and ADF, respectively. Also, the ruminally degraded fraction was 67.8 and 70.3% of NDF and ADF content, respectively.

The present findings agree with that found by Khayyal *et al*. (2015), who reveals that *in situ* ruminal digestibility coefficients of DM were lower ($P<0.05$) for berseem than moringa forage.

In vitro batch culture:

Table (3) shows *in vitro* true dry matter digestibility (IVTDMD), total gas production (TGP), and fiber fractions (NDF and ADF) digestibility for the experimental ration. Moringa leaves showed no significant influence on *in vitro* rumen digestibility. However, ML enhanced IVTDMD and NDF digestibility (NDFD) insignificantly. Total accumulated gas production (TGP) has only appeared to increase ($p<0.05$) when expressed as ml per 400 mg of dry matter (ml/400 mg DM). Our findings agree with those of Khayyal *et al*. (2015) who revealed that mixing berseem with moringa forage resulted in a significant improvement in all degradable fractions compared to berseem alone. Also, Li *et al*. (2019) found that diets containing *Moringa Oleifera* significantly increased the apparent digestibility of DM, CP, NDF, and ADF compared to the control diet.

On the other hand, the ML diet showed insignificantly decreased ADF digestibility (ADFD). The *in vitro* research showed that ML significantly affected rumen pH ($p=0.002$). The ML diet's rumen pH was somewhat lower than that of the control diet.

Table (2): *In situ* degradation of DM, NDF and ADF for moringa leaves.

Time (h)	Nutrients degradability*		
	DM	NDF	ADF
0	45.0	0	0
2	52.8	39.9	35.8
6	58.5	45.8	40.2
8	65.0	34.6	32.0
24	87.7	21.0	19.9
48	95.7	13.4	13.4
Fractions, %			
a	44.9	-	-
b	52.1	71.9	73.9
c	3.0	28.1	26.1
K_d, fraction/h	0.0760	0.8202	0.9695
Fractions, %			
RD	76.3	67.8	70.3
RU	23.7	32.2	29.7

DM: dry matter; NDF: neutral detergent fiber; ADF: Acid detergent fiber; a=soluble fraction, b=potentially degradable fraction; c=undegradable fraction; Kd=ruminal degradation rate (fraction/h); RD =Ruminally degraded, calculated a + b (K_d/(K_d + K_p)) where K_p =ruminal passage rates of .05/h.; RU =Ruminally undegradable, calculated (l - RD).

Table (3): *In vitro* true dry matter digestibility, total gas production, and fiber digestibility for the experimental rations.

Items	Treatments		SEM	p-value
	Control	Moringa leaves		
IVTDMD%	66.17	70.55	1.36	0.108
TGP, ml/400mg DM	52.38	55.80	1.68	0.366
TGP, ml/400 mg DMD	79.22	79.20	2.62	0.997
NDFD, %	25.13	31.71	2.61	0.244
ADFD, %	27.21	25.67	1.92	0.733
pH	7.17	6.98	0.04	0.002

ML: Moringa leaves; TGP: Total gas production; IVTDMD: In vitro true dry matter digestibility; NDFD: Neutral detergent fiber digestibility; ADFD: Acid detergent fiber digestibility.

Blood metabolites:

Blood plasma metabolites are often utilized to check the metabolic health of dairy cows. In the present investigation, moringa leaves (ML) substitution had no significant effect on plasma total protein, cholesterol, or urea (Table 4). However, albumin and glucose plasma concentrations were significantly increased ($p<0.05$) in ML-fed ewes compared to the control group.

Table (4): Blood metabolites of lactating ewes in the experimental groups.

Item	Treatments		SEM	p-value
	Control	Moringa leaves		
Protein, g dL^{-1}	6.30	6.06	0.21	0.620
Albumin, g dL^{-1}	4.36	5.32	0.21	0.007*
Urea, mg dL^{-1}	56.19	54.51	0.96	0.437
Glucose, mg dL^{-1}	32.50	51.79	3.56	<0.001*
Cholesterol, mg dL^{-1}	76.98	84.72	2.78	0.198
Triglyceride, mg dL^{-1}	45.88	39.07	1.77	0.040*

*SEM: Standard error of means; *The differences are significant at p<0.05.*

In addition, the ML-fed group had a significantly lower plasma triglyceride concentration compared to the control group. Results revealed that ML replacement had no deleterious impact on blood metabolites, which were within the normal range. Similar findings were observed by Kholif *et al*. (2018), who observed a significant increase in blood serum total protein, albumin, and glucose concentrations but a reduction in cholesterol and triglycerides in Nubian goats fed *Moringa Oleifera* foliage as a substitute for BC.

The increased albumin level in the ML group suggests better protein utilization, perhaps due to higher CP intakes and digestibility (Kholif *et al*., 2016). Also, glucose might increase in response to improved nutrient digestibility, propionic acid, and total VFA (Kholif *et al*., 2018). Results are in parallel with those found by (Kholif *et al*., 2018). The reduction in serum triglycerides may be related to ML's phenolic compounds. Gryglewski *et al*. (1987) reported that phenolic acids significantly reduced blood triglyceride concentration in rabbits.

Milk yield and composition:

Table (5) demonstrates the daily milk yield, energy corrected milk (ECM), and milk composition of lactating Barki ewes fed the experimental rations. Based on the data, it seems that ewes' milk yield and composition were not affected by moringa leaves replacement. However, daily milk yield, ECM, and milk fat content were all numerically higher in the ML-fed group compared to the control group ($p>0.05$). Furthermore, feeding ML had no significant effect on milk component yields. However, the ML-fed group had higher fat, protein, lactose, solid non-fat (SNF), and total solids (TS) yields than the control group.

These results are in parallel with those of Mendieta-Araica *et al*. (2011), who revealed no significant difference in milk composition of dairy cows fed ML as a replacement for commercial concentrate constituents. Also, Olvera-Aguirre *et al*. (2020) reported no significant effect of ML extracts on milk composition of Nadji ewes, while, a numerical increase in milk fat of 17% was observed.

The increase in the digestibility of nutrients and the efficiency with which feed is utilized lead to an increase in the energy supply needed to produce more milk (Brisibe *et al*., 2009). In addition, the moringa tree (leaves, pods, and seeds) provides important phytochemicals and phytosterols. These substances stimulate estrogen hormone synthesis, which encourages mammary gland duct growth (Mutiara *et al*., 2013).

Table (5): Milk yield, energy corrected milk (ECM), and milk composition in lactating ewes fed experimental rations.

Items	Treatments		SEM	*P value*
	Control	Moringa leaves		
Milk yield, g/h/day	490.2	642.8	46.48	0.129
ECM, g/h/day	600.5	784.6	63.69	0.176
Milk composition, %				
Fat	4.74	5.01	0.21	0.539
Protein	4.73	4.51	0.14	0.438
Lactose	6.76	6.80	0.17	0.912
Ash	0.96	096	0.03	0.944
SNF	12.46	12.26	0.33	0.772
TS	17.20	17.27	0.49	0.947
Milk constituent yields, g/day				
Fat	24.04	32.22	2.77	0.167
Protein	23.38	29.15	2.47	0.267
Lactose	33.09	44.07	3.56	0.152
SNF	61.14	79.44	6.53	0.189
TS	85.18	111.66	9.20	0.178

SEM: Standard error of means; ECM: Energy corrected milk; SNF: Solids non-fat; TS: total solid

CONCLUSION

Moringa leaves could be utilized as a replacement for Berseem clover at a level of 150 g/kg DM without any adverse effect on the performance of lactating Barki ewes.

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قابلية الهضم فى الكرش والقيمة الغذائية لأوراق المورينجا أوليفيرا وتأثيراتها الغذائية على الأداء الإنتاجى للنعاج الحلابة

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أجريت هذه الدراسة بهدف تقييم تأثير الاستبدال الجزئي للبرسيم المصري (BC) بأوراق المورينجا أوليفيرا (ML) على الأداء الإنتاجى لنعاج البرقى الحلابة. تم تقييم معاملات الهضم للمادة الجافة والألياف لأوراق المورينجا خلال تجربة هضم معملية بإستخدام الأكياس النايلون حيث تم استخدام ثلاث كباش مثبت بها كانيولا (بمتوسط وزن 4.0±51.50 كيلوجرام). كما تم تقدير معمل الهضم الحقيقى للمادة الجافة (IVTDMD) وانتاج الغاز الكلى (مللى/400 مليجرام مادة جافة) وكذلك معاملات الهضم لشقوق الألياف (NDFD & ADFD) من خلال تجربة هضم معملية. وتم إجراء تجربة مزرعية بإستخدام عدد أربعة عشر نعجة (متوسط وزن الجسم 0.4±43.5 كيلوجرام) بعد 15 يوم بعد الولادة تم تقسيمهم عشوائيا إلى مجموعتين (سبع حيوانات فى كل مجموعة). تم تغذية المجموعه الأولى على العليقة الكنترول والتى تتكون من مخلوط علف مركز والبرسيم المصرى بنسبة (50:50)، في حين غذيت المجموعة الثانية على أوراق المورينجا ML كبديل لـ BC عند مستوى 15٪ من كمية البرسيم المقدمه. أظهرت النتائج عدم وجود أى تأثير معنوى على معاملات الهضم أو إنتاج اللبن ومكوناته فيما أظهرت المجموعة المغذاة على المورينجا قيماً أعلى لمعاملات الهضم لجميع العناصر الغذائية وكانت تنتج محصول لبن يومى أعلى بنسبة دهن أعلى بالمقارنة بالمجموعه الكنترول. أيضا، أظهرت النتائج زيادة معنوية فى مستوي الألبيومين والجلوكوز فى بلازما الدم فى المجموعة المغذاه على أوراق المورينجا ML مع إنخفاض فى مستوى الجلسيريدات الثلاثية. وقد تبين من النتائج، أن إحلال أوراق المورينجا محل البرسيم المصري بنسبة 15٪ لم يكن له أى تأثير سلبي على الأداء الإنتاجي للنعاج الحلابة.

الكلمات المفتاحية: *أوراق المورينجا – معدل التكسير بالكرش – اللبن ومكوناته – أغنام برقى*