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Moringa oleifera leaf meal as a protein source in lactating goat's diets: Feed intake, digestibility, ruminal fermentation, milk yield and composition, and its fatty acids profile

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ABSTRACT

Sixteen lactating Anglo-Nubian goats (36.2 ± 0.7 kg BW) were used in quadruplicated 4 × 4 Latin square design to evaluate the inclusion of *Moringa oleifera* leaf meal (MLM) in their diets. *M. oleifera* leaf meal inclusion rates were 0 (M0 or control, no MLM, only sesame meal), 10 (M10), 15 (M15) and 20% (M20), replacing sesame meal by 0 (control), 50, 75 and 100%, respectively. Goats fed on M15 and M20 diets showed increased feed intake of most nutrients ($P < 0.05$). Moreover, dry matter, organic matter, and fibre digestibilities were increased ($P < 0.05$) with M15 diet. Goats fed on M15 diet showed increased ($P < 0.05$) ruminal pH, volatile fatty acids and propionate concentrations compared to the control diet. Blood glutamic-pyruvic transaminase concentration was increased ($P < 0.05$), and urea-N and cholesterol concentrations were decreased ($P < 0.05$) in goats fed MLM diets. Milk yield and energy corrected milk were increased ($P < 0.01$) in goats fed MLM, and the greatest increase was observed in the group fed the M15 diet. Feeding MLM also affected milk composition increasing ($P < 0.05$) total solids and lactose contents. Milk components outputs were increased in goats fed MLM compared to control ($P < 0.01$). The relative percentage of saturated fatty acids was decreased ($P < 0.05$), and those of unsaturated (mono- or poly-) fatty acids and of conjugated linoleic acid were increased ($P < 0.05$) in the milk of goats fed M15 and M20 diets. *M. oleifera* can replace sesame meal as a protein source in diets for lactating goats. The inclusion of MLM increased feed intake, enhanced nutrient digestibility and ruminal fermentation, increased milk yield and modified milk fatty acid profile positively. An inclusion rate of 15% MLM (replacing 75% of sesame meal) in the diet was the most suitable level for lactating goats under the current experiment conditions.

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1. Introduction

The availability and price of concentrates, in particular of protein sources, are a serious problem for animal producers, especially for small farms stakeholders. Consequently, there is a need for alternative ingredients with high protein content and balanced amino acids profile, and with a suitable cost. Plants leaf meal, forage trees, saltbush and shrubs are good and cheap sources of protein (Mendieta-Araica et al., 2011a; Alsersy et al., 2015; Salem et al., 2006, 2015).

One of these potential tree forages is *Moringa oleifera* Lam (syns. *Moringa pterygosperm*, family *Moringaceae*), which grows throughout the tropics (Debela and Tolera, 2013). *M. oleifera* is an indigenous native tree from the Himalaya (Duke, 2001) but, at present, it is widely distributed almost worldwide (Soliva et al., 2005). *M. oleifera* can be grown in humid, hot, dry tropical and subtropical regions. It is a drought tolerant plant that can grow in all types of soils, except those that are waterlogged (Abdul, 2007), and can tolerate dry seasons lasting up to 6 months (Mendieta-Araica et al., 2013). The yield per ha varies widely depending on season, variety, fertilization, irrigation regimen, accession and ecological zone (Palada et al., 2007). Reported yields range from 43 to 115 tonnes of biomass ha^{-1} $year^{-1}$ (Foidl et al., 2001; Safwat et al., 2014), with about 4.2–24 tonnes ha^{-1} $year^{-1}$ of dry matter (DM) (Reyes-Sánchez et al., 2006; Nouman et al., 2014). Most of the production is located in India with 1.1–1.3 million tonnes $year^{-1}$ harvested from 38,000 ha (Patel et al., 2010). No information is available about the global and Egyptian production of *M. oleifera* leaves or seeds. In Egypt, *M. oleifera* is grown for human consumption; however, the low price of foliage encourages its use as animal feed. The price per kg DM varies considerably from 1 to 1.5 US$ for dried leaves up to 20–24 US$ for seeds. The price of branches with leaves and soft twigs as animal feed can be around 0.25–0.5 US$ per kg DM. *M. oleifera* leaf meal (MLM) contains from 179 to 268 g crude protein (CP)/kg DM (Sultana et al., 2015), with about 47% of bypass protein (Becker, 1995) and with adequate amino acid profile (Sánchez-Machado et al., 2010). The chemical composition of MLM can vary considerably depending on the proportions of small branches, twigs and leaf (Mendieta-Araica et al., 2011a), stage of maturity, time of sampling and *Moringa* species (Debela and Tolera, 2013), and agro-ecological zone where trees are growing (Sultana et al., 2015).

Experiments including *Moringa* fresh foliage in the diets of goats (Sultana et al., 2015), sheep (Fadiyimu et al., 2010) and cows (Mendieta-Araica et al., 2011b) have reported improved feed utilization and animal productive performance. Mendieta-Araica et al. (2011b) fed dairy cows fresh or ensiled *Moringa* foliage *versus* Elephant grass and reported unaffected live weights and milk yield and composition with increasing intake of fresh *Moringa*, in spite of higher CP and fibre digestibilities for ensiled *M. oleifera*. Moreover, Fadiyimu et al. (2010) included *M. oleifera* at different levels in diets for sheep, and reported decreased intake with increasing *M. oleifera* in the diet, but with increased nutrients digestibility.

Little information about *M. oleifera* as a protein source in the diet of ruminants is available. Therefore, the aim of this study was to evaluate the effects of replacing partially or completely a conventional protein source (sesame meal) with MLM in diets for lactating Anglo-Nubian goats on feed intake, digestibility, blood chemistry, and milk yield and composition.

2. Materials and methods

Goats were cared and handled in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999). The trial was conducted at a family owned commercial dairy farm near Behera governorate (Egypt) and at the Laboratory of Dairy Animal Production, National Research Centre (Egypt). The farm is located at latitude 31 °04'02.4" N and longitude 30 °31'42.19" E. The local climate is temperate-tropic humid with summer rains and with an annual average rainfall of 22 mm and mean annual temperature between 14 and 28 °C.

2.1. Moringa oleifera planting and preparation

Viable, clean and disease free *M. oleifera* seeds were obtained from The Egyptian Association of Moringa (National Research Centre, Egypt), and planted in density 100,000–150,000 seeds per ha. Before sowing, seeds were soaked in water for 24 h then kept in dark for 24 h for scarification and better germination. The land was irrigated biweekly with 1800 m^3 water ha^{-1} $month^{-1}$. Before starting the experiment, a uniformity cut was carried out 65 days after seeding, when plants reached a height of 65–70 cm (5–7 mm cutting height). Usually, *M. oleifera* is then cut after 40 days of regrowth resulting in 9 harvests per year and yielding 70–80 tonnes of fresh biomass ha^{-1} $year^{-1}$ (~23 tonnes DM ha^{-1} $year^{-1}$). For this particular experiment, *Moringa* biomass (composed of leaves and thin twigs, branches and stems) was harvested in a different way following a cut-and-carry approach so that the amount of *Moringa* biomass required to feed the goats was collected daily from the field every morning, mixed with the diet and immediately offered to the goats. The material collected was always from 40 (±5) days aftermaths. Additionally, *Moringa* material was sampled daily, composited weekly and dried at 60 °C in a forced-air oven for 48 h and stored for later chemical analysis.

2.2. Goats, feeding and experimental design

Sixteen lactating Anglo-Nubian goats (36.2 ± 0.7 kg of BW) were randomly assigned to four experimental groups. The experimental design was a quadruplicated 4 × 4 Latin square, with four treatments, four periods and four goats per treatment within each period (resulting in 16 replicates per treatment for the whole experiment). The four experimental treatments were randomly assigned to the four groups in the first period. The goats were housed individually in tie stalls with free access to water and fed on the experimental diets to meet their nutrient requirements according to NRC (2007) recommendations plus a 10% margin.

The basal diet fed to the goats contained 400 g of Egyptian berseem clover (*Trifolium alexandrinum*), 300 g of crushed yellow corn, 80 g of wheat bran and 20 g of minerals and vitamins per kg total mixed ration (DM basis). In the control diet (M0), the protein source was sesame meal included at 200 g/kg DM. In the other experimental diets, 50%, 75% or 100% of the sesame meal was replaced with MLM, by including 100 (M 10), 150 (M15) or 200 (M20) g of MLM per kg diet (on DM basis). Diets were fed to each goat individually at 08:00 and 16:00 h in two equal portions. Feed samples of berseem clover, concentrates mixture and MLM were taken daily, composited weekly and dried at 60 °C in a forced-air oven for 48 h and stored for later chemical analysis. The ingredient and nutrient contents of the four diets are in Table 1.

Each experimental period lasted 22 days; 15 days of adaptation to the new diet, and 7 days for measurements (feed intake, milk yield) and sample collection (sampling of feed and orts, faeces, ruminal fluid, blood, milk). Feed intake (recorded daily by weighing the offered diets and refusals from the previous day) and milk yield were measured daily. Faecal grab samples were collected twice daily at 07:00 and 15:00 h, dried at 60 °C in a forced-air oven for 48 h and pooled by goat within period. Acid insoluble ash was used as an internal indigestibility marker, and coefficients of digestion calculated according to Ferret et al. (1999).

Table 1
Ingredients and chemical composition of feeding stuffs and total mixed rations fed to the lactating Anglo-Nubian goats.

	Ingredients					Diets[a]			
	Berseem hay	*Moringa oleifera*	Crushed yellow corn	Sesame meal	Wheat bran	M0	M10	M15	M20
Ingredients (g/kg DM basis)									
Berseem hay						400	400	400	400
Moringa oleifera						0	100	150	200
Crushed yellow corn						300	300	300	300
Sesame meal						200	100	50	0
Wheat bran						80	80	80	80
Calcium carbonate						10	10	10	10
Salt						5	5	5	5
Minerals and vitamins mixture[b]						5	5	5	5
Chemical composition (g/kg DM basis)									
Dry matter (g/kg wet material)	875.0	320.2	866.0	898.1	871.4	859.2	801.4	772.5	743.6
Organic matter	857.9	889.0	890.3	869.3	852.2	852.3	854.3	855.2	856.2
Crude protein	143.7	241.2	90.8	259.0	129.7	146.9	145.1	144.2	143.3
Ether extract	49.9	47.3	45.2	119.9	56.2	62.0	54.7	51.1	47.5
Non-structural carbohydrates	213.1	263.9	540.0	211.3	204.4	305.9	311.1	313.7	316.4
Neutral detergent fibre	451.2	336.6	214.3	279.1	461.9	337.5	343.3	346.2	349.0
Acid detergent fibre	347.6	275.0	88.8	151.3	130.6	206.4	218.8	224.9	231.1
Acid detergent lignin	51.7	81.6	10.4	41.5	38.0	35.1	39.2	41.2	43.2
Cellulose	295.9	193.4	78.4	109.8	92.6	171.3	179.6	183.8	187.9
Hemicellulose	103.6	61.6	125.5	127.8	331.3	131.2	124.5	121.3	117.9
Total phenolic		45.15		14.33					
Tannins		25.63		5.24					

[a] *Moringa oleifera* added at 0% (M0), 10% (M10), 15% (M15) and 20% (M20), replacing 0%, 50%, 75% and 100% of sesame meal, respectively.
[b] Contained: Ca (141 g/kg), P (87 g/kg), Mg (45 g/kg), S (14 g/kg), Na (120 g/kg), K (6 g/kg), Fe (944 mg/kg), Zn (1613 mg/kg), Cu (484 mg/kg), Mn (1748 mg/kg), I (58 mg/kg), Co (51 mg/kg), Se (13 mg/kg), vitamin A (248,000 U/kg), vitamin D3 (74,000 UI/kg), vitamin E (1656 IU/kg).

Dried feed, feed orts and faecal samples were ground through a 1 mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA), and analyzed for DM (#930.15), ash (#942.05), N (#954.01), and ether extract (EE; #920.39), according to AOAC (1995) official methods. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin were analyzed according to Van Soest et al. (1991). Non-structural carbohydrates (NSC), cellulose, hemicelluloses and organic matter (OM) were calculated. Tannin concentrations in MLM and sesame meals were determined according to Makkar (2003), and total phenolic content were determined chromatographically according to Meier et al. (1988).

2.3. Sampling and analysis of rumen fluid

On the last day of each experimental period, ruminal contents were sampled at 0, 3, and 6 h post morning feeding to determine the pH and concentration of fermentation end-products. Rumen contents (~100 mL) were collected once at each sampling time from the ventral sac by using a stomach tube, and then composite samples taken from each goat were strained through 4 layers of cheesecloth. The pH of ruminal fluid was measured immediately using a pH metre (HI98127 pHep®4 pH/Temperature Tester, Hanna® instrument, Italy).

A subsample of 5 mL was preserved in 5 mL of 0.2 M HCl for ammonia-N analysis and 0.8 mL of rumen liquor was mixed with 0.2 mL of a solution containing 250 g of metaphosphoric acid/L for total volatile fatty acids (tVFA) analysis. Samples collected at 3 h post-feeding were analyzed for the individual VFA. All samples were stored at −20 °C until laboratory analyses. The concentration of ruminal ammonia-N was determined according to AOAC (1995). Total VFA concentration in samples was determined by titration, after steam distillation of a 4 mL sample, by the method of Annison (1954). The percentages of the individual VFA were measured by gas-liquid chromatography (Varian 3700; Varian Specialties Ltd, Brockville, Ontario, Canada).

2.4. Sampling and analysis of blood serum

On the last day of each experimental period, blood samples (10 mL) were taken 4 h after feeding from the jugular vein of each goat into a clean dry tube, without anticoagulants. Blood samples were centrifuged at 4000 × *g* at 4 °C for 20 min. Serum was separated into 2 mL clean dried Eppendorf tubes and frozen at −20 °C until analysis.

Blood serum samples were analyzed for concentrations of total protein, albumin, urea-N, glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT), glucose, creatinine, cholesterol and triglycerides using specific kits (Stanbio Laboratory, Boerne, Texas, USA) following manufacturer instructions. Globulin concentration was calculated.

2.5. Milk sampling, milk composition and fatty acids analysis

During the last 7 days of each experimental period, goats were milked by hand twice daily at 09:00 and 21:00 h, and samples (10% of recorded milk yield) were collected at each milking. A mixed sample of morning and evening milkings was taken daily. Milk samples were analyzed for total solids, fat, protein, and lactose using infrared spectrophotometry (Foss 120 Milko-Scan, Foss Electric, Hillerød, Denmark). The ash content of milk was determined after heating a milk sample in a muffle furnace at 550 °C for 8 h. Fatty acids in milk were determined as described previously in Kholif et al. (2014) using methyl esters prepared by base-catalysed methanolysis of the glycerides (KOH in methanol) according to International Standards (ISO-IDF, 2002) on a Perkin-Elmer chromatograph (model 8420, Beaconsfield, Perkin Elmer, Beaconsfield, UK) equipped with a flame ionization detector.

Average yields (g/d) of each milk component were calculated for each individual goat by multiplying milk yield by the component content (g/kg) of milk. The gross energy content in milk was calculated according to Tyrell and Reid (1965) as:

$$\text{Milk energy content (MJ/kg)} = 4.184 \times 2.204 \times [41.63 \times \text{fat (g/kg)} + 24.13 \times \text{protein (g/kg)} + 21.60 \times \text{lactose (g/kg)} - 117.2]/10000.$$

The milk energy output (MJ/d) was then calculated as milk energy (MJ/kg) × milk yield (kg/d).

Energy corrected milk (ECM) was calculated according to Sjaunja et al. (1991) as:

$$\text{ECM (kg/d)} = \text{milk yield (kg/day)} \times [38.3 \times \text{fat (g/kg)} + 24.2 \times \text{protein (g/kg)} + 16.54 \times \text{lactose (g/kg)} + 20.7]/3140.$$

2.6. Statistical analyses

Data on nutrient intake, digestibility, blood chemistry parameters and milk yield and composition (including fatty acids profile) were analyzed using a quadruplicated 4 × 4 Latin square design, with four periods and four experimental diets (M0, M10, M15 and M20). Four lactating goats were used within each period and treatment. Each goat was an experimental unit, thus resulting in 16 replicates per treatment (4 per period). The statistical model included a random effect of goat (G_k) with period (P_i) and diet (D_j) as fixed effects: $y_{ijk} = \mu + P_i + D_j + G_k + E_{ijk}$, where y_{ijk} is each individual observation for a given variable, μ is the overall mean and E_{ijk} is the residual random term. Statistical analyses were performed using PROC MIXED of SAS (SAS Inst. Inc. Cary, NC, 2004). In the case of ruminal fermentation variables measured at different times (0, 3 and 6 h post-feeding), a model with repeated measures in time on the same experimental unit was used. The interactions time × diet were never significant, and only diet effects within each sampling time will be reported. The Tukey test was used for the multiple comparisons of means, and polynomial (linear and quadratic) contrasts were used to examine the dose responses to increasing levels of MLM in the diet. Significance was declared at a level of $P < 0.05$ and trend of $P \leq 0.10$.

3. Results

3.1. Feed intake and nutrient digestibility

Feed intake by goats was significantly ($P < 0.0001$) increased with diets containing MLM. Goats fed on MLM diets consumed more (significant linear and quadratic trends, $P < 0.05$) feed nutrients (except for EE and hemicelluloses) than goats fed the control diet. The goats fed the M15 diet showed the greatest feed and nutrient intakes. Moreover, the digestibility of DM ($P < 0.0001$), OM ($P < 0.0001$), NDF ($P = 0.0157$), ADF ($P < 0.0001$) and cellulose ($P < 0.0001$) was increased when goats were fed MLM diets. However, feeding goats on MLM decreased ($P < 0.0001$) CP digestibility. No differences ($P > 0.05$) were observed for EE, NSC and hemicelluloses digestibilities (Table 2).

Total digestible nutrients (quadratic, $P = 0.0049$), digestible CP (quadratic, $P < 0.0001$), digestible energy (quadratic, $P = 0.0054$), metabolizable energy (quadratic, $P = 0.0054$) and net energy of lactation (quadratic, $P = 0.0049$) were decreased in M20 compared to M0 diet (Table 2).

3.2. Ruminal fermentation kinetics and blood parameters

For ruminal pH, goats fed on M10 and M20 diets had increased ($P < 0.05$) ruminal pH at the time of feeding, with no differences ($P > 0.05$) after 3 and 6 h. Goats fed the M15 diet showed increased ($P < 0.05$) tVFA at all sampling times, compared to the goats of the control group. Ruminal ammonia-N concentrations determined at 3 and 6 h after feeding were decreased ($P < 0.05$) in goats fed MLM diets compared to the control diet (Table 3).

No significant diet effects ($P > 0.05$) were observed on ruminal acetic (C2) and butyric (C4) acid concentrations at 3 h after feeding. However, the concentration of propionic acid (C3) was increased in the rumen of goats fed the M15 diet ($P = 0.0209$) compared to M0 diet. Consequently, the C2/C3 ration was decreased ($P = 0.0091$) in the rumen of goats fed MLM diets (Table 3).

Feeding MLM diets to goats had no significant effects ($P > 0.05$) on serum total proteins, albumin, globulin, glucose, creatinine, GOT and triglycerides concentrations (data not shown). However, the serum concentration of GPT was increased (quadratic, $P = 0.0141$) in goats fed the M20 diet compared to the control diet. Decreased urea-N (linear, $P = 0.0339$; quadratic, $P < 0.0001$) and cholesterol (linear, $P = 0.0009$; quadratic, $P < 0.0001$) concentrations were observed in the serum of goats fed MLM diets (Table 3).

3.3. Milk yield and composition

Milk yield (linear, $P = 0.0001$; quadratic, $P = 0.0352$), energy corrected milk yield (linear, $P < 0.0001$; quadratic, $P = 0.0299$), milk energy output (linear, $P < 0.0001$; quadratic, $P = 0.0259$), and milk constituent (fat, protein, lactose) outputs ($P < 0.001$) were significantly increased when goats were fed MLM diets compared to the control diet. The greatest milk yields were observed in goats fed the M15 diet. Total solids (linear, $P = 0.0402$) and lactose (linear, $P = 0.001$) milk contents were increased in goats fed the M15 diet compared to those fed the control diet (Table 4).

The milk fatty acid profile was significantly modified when goats were fed MLM diets (Table 5). The relative percentages of C14:1 ($P = 0.0017$), C18:1^{n9T} ($P = 0.0017$), C18:1^{n9C} ($P = 0.0037$), C18:2 *trans*-10, *cis*-12 ($P = 0.0005$) and C18:2 *cis*-9, *trans*−11 ($P = 0.0032$) were increased, and the concentration of C16:0 ($P = 0.0011$) was decreased, in the milk of goats fed M15 and M20 diets. Consequently, the relative percentages of total unsaturated (TUFA; $P = 0.001$), monounsaturated (MUFA; $P = 0.0014$), and polyunsaturated fatty acids (PUFA; $P = 0.0051$), and of total conjugated linoleic acid (CLA; $P = 0.0004$), and the TUFA/total saturated fatty acids ratio, (TSFA; $P = 0.0014$) were increased in the milk of goats fed the M15 and M20 diets compared to the control diet. On the contrary, the percentage of TSFA was decreased ($P = 0.001$) in the milk of goats fed MLM diets (Table 5).

4. Discussion

In the current study, MLM contained about 24% CP (~47% of bypass protein; Becker, 1995). Although this content is less than in sesame meal (26%), soybean meal (~40–44% CP), cottonseed meal (~40% CP) and sunflower seed cake (~35% CP), which are mostly used as protein concentrates in ruminant nutrition, the MLM may be considered as a good potential source of supplementary protein for ruminants (Debela and Tolera, 2013). The amino acid supply from this protein concentrate may be of particular nutritional significance as it may cover goat's protein needs and boost the immune system against diseases (Brisibe et al., 2009). In general, dairy goats require about 16% CP (NRC, 2007), and thus MLM may be included as

Table 2
Feed intake, nutrients digestibility and nutritive value of diets with different levels of *Moringa oleifera* replacing sesame meal as a protein source and fed to lactating Anglo-Nubian goats ($n = 16$).

	Diets*				SEM	*P* value		
	M0	M10	M15	M20		Control *vs.* Moringa	Linear	Quadratic
Intake (g/d)								
Dry matter	730.8^{c}	792.8^{b}	848.7^{a}	801.1^{b}	8.39	<0.0001	<0.0001	0.0004
Organic matter	622.8^{c}	677.3^{b}	725.8^{a}	685.9^{b}	7.18	<0.0001	<0.0001	0.0002
Crude protein	107.3^{c}	115.0^{b}	122.4^{a}	114.8^{b}	1.21	<0.0001	<0.0001	0.0186
Ether extract	45.3^{a}	43.4^{b}	43.4^{b}	38.0^{c}	0.42	<0.0001	0.0028	<0.0001
Non-structural carbohydrates	223.5^{c}	246.6^{b}	266.2^{a}	253.5^{b}	2.64	<0.0001	<0.0001	<0.0001
Neutral detergent fibre	246.6^{c}	272.2^{b}	293.8^{a}	279.6^{b}	2.91	<0.0001	<0.0001	<0.0001
Acid detergent fibre	150.8^{c}	173.5^{b}	190.9^{a}	185.1^{a}	1.91	<0.0001	<0.0001	<0.0001
Cellulose	125.5^{c}	142.4^{b}	156.0^{a}	150.6^{a}	1.54	<0.0001	<0.0001	<0.0001
Hemicelluloses	95.5^{bc}	98.7^{b}	102.9^{a}	94.4^{c}	1.04	<0.0001	0.0367	0.0409
Digestibility (g absorbed/kg ingested)								
Dry matter	574.5^{c}	590.9^{bc}	644.1^{a}	612.5^{b}	7.11	<0.0001	0.1103	0.0014
Organic matter	609.5^{b}	627.6^{ab}	645.0^{a}	611.6^{b}	4.87	<0.0001	0.0117	0.2516
Crude protein	625.2^{a}	620.5^{ab}	605.9^{b}	584.9^{c}	4.76	<0.0001	0.4873	<0.0001
Ether extract	602.4	597.4	591.6	593.4	6.51	0.6564	0.5934	0.4174
Non-structural carbohydrates	566.0	560.7	560.2	558.9	6.10	0.7718	0.4626	0.4871
Neutral detergent fibre	572.9^{b}	586.5^{ab}	608.5^{a}	590.0^{ab}	7.46	0.0157	0.2069	0.2663
Acid detergent fibre	566.1^{b}	580.8^{b}	607.6^{a}	584.3^{b}	5.52	<0.0001	0.0660	0.1169
Cellulose	602.5^{b}	611.2^{b}	645.5^{a}	620.3^{b}	5.73	<0.0001	0.2900	0.0610
Hemicelluloses	578.7	571.6	582.9	569.3	8.20	0.6238	0.5427	0.5606
Digestible nutrients and energy value†								
Total digestible nutrients (g/kg DM)	569.0^{a}	563.9^{ab}	562.8^{ab}	555.1^{b}	31.30	0.0262	0.2484	0.0049
Digestible crude protein (g/kg DM)	91.8^{a}	90.0^{a}	87.4^{b}	83.8^{c}	0.69	<0.0001	0.0706	<0.0001
Digestible energy (MJ/kg DM)	10.5^{a}	10.4^{ab}	10.4^{ab}	10.3^{b}	0.059	0.0266	0.2194	0.0054
Metabolizable energy (MJ/kg DM)	10.6^{a}	10.5^{ab}	10.5^{ab}	10.4^{b}	0.059	0.0295	0.2652	0.0054
Net energy of lactation (MJ/kg DM)	5.4^{a}	5.3^{ab}	5.3^{ab}	5.2^{b}	0.033	0.0258	0.2416	0.0049

* *Moringa oleifera* added at 0% (M0), 10% (M10), 15% (M15) and 20% (M20), replacing 0%, 50%, 75% and 100% of sesame meal, respectively.
† Calculated according to (NRC, 2001).
^{abc}Means in the same row with different superscripts significantly differ ($P < 0.05$).
SEM = Standard error of the mean.

a protein supplement to match such requirements (Moyo et al., 2010).

4.1. Feed intake and nutrient digestibility

Partial or total replacement of sesame meal with MLM increased feed intake and, by extension, the ingestion of most nutrients and nutritional fractions, suggesting that diets containing MLM may be more palatable than the diet containing sesame meal alone. The low degradability of MLM protein in the rumen may also affect intake, as an increased provision of undegradable protein can increase feed intake (M'hamed et al., 2001; Kholif et al., 2014). This hypothesis may be supported by the gradual increased intake with increasing MLM inclusion in diets. However, goats fed the M20 diet showed slight decreased intake and digestibility compared to those fed the M15 diet. This may be due to tannins and phenolic compound concentrations observed in MLM compared to sesame meal. Tannins in feeds may reduce feed palatability, slow digestion rate, and contribute to develop conditioned aversions (Frutos et al., 2004). The reaction between feed tannins and salivary proteins causes an astringent sensation reducing palatability (Salem et al., 2013).

The improved nutrients digestibility in M15 goats may be related to improved ruminal fermentation. Frutos et al. (2004) and Salem et al. (2006) reported that less than 5% is the acceptable level of tannins in feeds without negative effects on digestibility. Rumen microorganisms have the ability to tolerate low and moderate concentrations of the secondary metabolites of plants including phenolic compounds (Varel et al., 1991) and tannins (Frutos et al., 2004) without any negative effects on rumen fermentation.

Mendieta-Araica et al. (2011a) replaced soybean meal at 20% of diet DM with the same amount of MLM and reported unaffected DM, OM, NDF and ADF intakes and digestibility with significant higher CP intake and digestibility for the soybean meal treatment. Sultana et al. (2015) partially and completely replaced the concentrate feed mixture from the diets of Black Bengal goats with MLM and found that partial replacement increased nutrients intake and NDF digestibility compared to the complete replacement and control.

4.2. Ruminal fermentation kinetics

Feeding goats on MLM increased ruminal tVFA concentrations. Lu et al. (2008) reported that an optimum dietary fibre level in the diet enhanced cellulolytic activity in the rumen and increased salivation during eating and ruminating. Ruminal pH in goats fed MLM diets (5.99–7.06) were within the range considered acceptable for fibre digestion (Ørskov and Ryle, 1990). Increased tVFA can be interpreted as a result of improved digestion with MLM diets compared to control.

Table 3
Ruminal fermentation and blood serum parameters in lactating Anglo-Nubian goats ($n = 16$) fed diets with different levels of *Moringa oleifera* replacing sesame meal as a protein source.

	Diets*				SEM	*P* value		
	M0	M10	M15	M20		Control *vs.* Moringa	Linear	Quadratic
pH								
0 h	6.88^{b}	7.06^{a}	7.02^{ab}	7.05^{a}	0.041	0.0103	0.0031	0.1063
3 h	5.83	6.05	6.04	5.99	0.071	0.1253	0.0348	0.5977
6 h	6.36	6.40	6.48	6.56	0.075	0.2604	0.7251	0.0573
Ammonia-N (g/L)								
0 h	21.0	20.6	20.5	20.0	0.39	0.3458	0.4766	0.0974
3 h	29.7^{a}	27.5^{b}	25.7^{c}	24.9^{c}	0.43	<0.0001	0.0010	<0.0001
6 h	25.5^{a}	24.6^{a}	22.8^{b}	20.0^{c}	0.31	<0.0001	0.0424	<0.0001
Total volatile fatty acids (tVFA; mmol/L)								
0 h	6.12^{b}	6.69^{ab}	7.11^{a}	6.88^{a}	0.179	0.0025	0.0300	0.0354
3 h	11.99^{b}	12.89^{ab}	13.51^{a}	12.74^{ab}	0.316	0.0148	0.0509	0.4338
6 h	9.72^{b}	10.08^{ab}	10.95^{a}	10.60^{ab}	0.293	0.0258	0.3919	0.0599
Individual VFA at 3 h after feeding								
Acetic (C2; mmol/L)	7.18	6.94	7.82	7.22	0.245	0.1743	0.5180	0.5990
Propionic (C3; mmol/L)	3.33^{b}	3.58^{ab}	4.27^{a}	3.74^{ab}	0.148	0.0209	0.2786	0.1695
Butyric (C4; mmol/L)	1.798	1.790	1.678	1.795	0.199	0.9651	0.9796	0.9961
Other (mmol/L)	0.010	0.019	0.013	0.020	0.0063	0.6128	0.3239	0.4631
C2/C3	2.15^{a}	1.94^{b}	1.83^{b}	1.93^{b}	0.042	0.0091	0.0114	0.0715
Blood serum parameters								
Urea-N (mg/dL)	44.40^{a}	40.78^{ab}	38.25^{bc}	34.73^{c}	1.170	<0.0001	0.0339	<0.0001
Cholesterol (mg/dL)	128.44^{a}	121.13^{bc}	123.50^{ab}	116.50^{c}	1.450	<0.0001	0.0009	<0.0001
Glutamic-pyruvic transaminase (Units/mL)	18.69^{b}	20.06^{ab}	21.44^{ab}	22.38^{a}	0.957	0.0494	0.3153	0.0141

* *Moringa oleifera* added at 0% (M0), 10% (M10), 15% (M15) and 20% (M20), replacing 0%, 50%, 75% and 100% of sesame meal, respectively.
^{abc}Means in the same row with different superscripts significantly differ ($P < 0.05$).
SEM = Standard error of the mean.

Ruminal ammonia-N were above the range (0.60–1.59 mM ammonia-N) considered by Satter and Slyter (1974) to be sufficient for microbial protein synthesis. Decreased ruminal ammonia-N concentration with MLM fed goats compared to control fed goats may be related to the lower protein degradability of *Moringa* in the rumen. Tannins have an ability to bind to dietary protein, thus protecting it from rumen degradation and increasing

Table 4
Milk yield and composition in lactating Anglo-Nubian goats ($n = 16$) fed diets with different levels of *Moringa oleifera* replacing sesame meal as a protein source.

	Diets*				SEM	*P* value		
	M0	M10	M15	M20		Control *vs.* Moringa	Linear	Quadratic
Milk								
Yield (g/d)	818.6^{b}	906.9^{a}	943.0^{a}	901.5^{a}	14.52	<0.0001	0.0001	0.0352
Energy corrected milk (g/d)	777.6^{b}	872.3^{a}	917.5^{a}	865.0^{a}	14.57	<0.0001	<0.0001	0.0299
Milk energy content (MJ/kg)	3.04^{b}	3.08^{ab}	3.12^{a}	3.07^{ab}	0.016	0.0126	0.0898	0.4623
Milk energy output (MJ/d)	2.47^{b}	2.78^{a}	2.93^{a}	2.76^{a}	0.046	<0.0001	<0.0001	0.0259
Milk composition (g/kg)								
Total solids	122.0^{b}	124.6^{ab}	126.3^{a}	124.6^{ab}	0.85	0.0098	0.0402	0.2130
Fat	35.9	35.5	35.6	35.0	0.35	0.3099	0.4041	0.0973
Protein	37.8	38.1	38.2	38.6	0.37	0.4912	0.5900	0.1521
Lactose	41.6^{b}	44.1^{a}	45.7^{a}	44.2^{a}	0.66	0.0007	0.0010	0.0912
Ash	6.8	7.0	6.8	6.8	0.09	0.4130	0.1739	0.8456
Milk component yield (g/d)								
Total solids	99.65^{b}	112.88^{a}	119.13^{a}	112.39^{a}	1.859	<0.0001	<0.0001	0.0102
Fat	29.05^{b}	32.01^{a}	33.36^{a}	31.21^{ab}	0.618	0.0002	0.0015	0.3780
Protein	30.84^{b}	34.47^{a}	36.00^{a}	34.77^{a}	0.670	<0.0001	0.0004	0.0135
Lactose	34.19^{c}	39.90^{b}	43.09^{a}	39.95^{b}	0.784	<0.0001	<0.0001	0.0042
Ash	5.56^{b}	6.30^{a}	6.36^{a}	6.19^{a}	0.125	0.0001	0.0001	0.1079
Feed efficiency								
Milk (milk/DMI)	1.12	1.15	1.11	1.13	0.022	0.7137	0.4425	0.9440
Energy corrected milk (ECM; ECM/DMI)	1.07	1.10	1.08	1.09	0.022	0.7544	0.2824	0.9718

* *Moringa oleifera* added at 0% (M0), 10% (M10), 15% (M15) and 20% (M20), replacing 0%, 50%, 75% and 100% of sesame meal, respectively.
^{abc}Means in the same row with different superscripts significantly differ ($P < 0.05$).
DMI = Dry matter intake; SEM = Standard error of the mean

Table 5
Fatty acids profile (g/100 g total fatty acids) in milk of lactating Anglo-Nubian goats fed on diets with different levels of *Moringa oleifera* replacing sesame meal as a protein source.

	Diets*				SEM	*P* value		
	M0	M10	M15	M20		Control *vs.* Moringa	Linear	Quadratic
C4:0	4.16	4.55	4.20	4.48	0.184	0.4105	0.1871	0.5815
C6:0	1.31	1.51	1.54	1.55	0.080	0.2122	0.1248	0.2009
C8:0	2.71^{ab}	2.31^{b}	3.10^{a}	2.73^{ab}	0.135	0.0327	0.0788	0.2188
C10:0	6.27	5.72	6.17	6.41	0.324	0.5227	0.2799	0.3393
C12:0	3.24	3.58	3.16	3.55	0.133	0.1521	0.1213	0.4241
C14:0	10.24	9.46	9.33	10.04	0.500	0.5473	0.3112	0.7655
C14:1	0.153^{b}	0.198^{b}	0.598^{a}	0.488^{a}	0.0495	0.0017	0.5438	0.0021
C15:0	1.29	1.33	1.37	1.34	0.034	0.4017	0.3565	0.4768
C16:0	28.12^{a}	26.54^{a}	22.03^{b}	20.99^{b}	0.721	0.0011	0.1727	0.0004
C16:1	1.18	1.27	1.54	1.50	0.162	0.3990	0.7234	0.2151
C17:0	1.185^{a}	0.873^{ab}	0.483^{b}	0.700^{ab}	0.1271	0.0379	0.1328	0.0791
C18:0	12.50	11.66	11.39	12.63	0.631	0.4797	0.3829	0.5072
C18:1^{n9T}	23.52^{b}	27.08^{a}	29.91^{a}	28.77^{a}	0.634	0.0017	0.0074	0.0043
C18:1^{n9C}	2.13^{ab}	1.61^{b}	2.72^{a}	2.46^{a}	0.126	0.0037	0.0259	0.0087
C18:2 *trans*−10, *cis*−12	0.548^{b}	0.858^{a}	0.923^{a}	0.908^{a}	0.0316	0.0005	0.0004	0.0018
C18:2 *cis*−9, *trans*−11	0.133^{b}	0.145^{b}	0.148^{ab}	0.163^{a}	0.0031	0.0032	0.0308	0.0008
C18:3^{n3}	0.130	0.163	0.175	0.143	0.0116	0.1180	0.0957	0.8013
C18:3^{n6}	0.313	0.323	0.345	0.278	0.0315	0.5401	0.8299	0.3400
C20:0	0.888	0.850	0.873	0.898	0.0311	0.7325	0.4268	0.4792
TSFA	71.89^{a}	68.37^{ab}	63.65^{c}	65.31^{bc}	0.746	0.0010	0.0157	0.0019
TUFA	28.10^{c}	31.64^{bc}	36.35^{a}	34.70^{ab}	0.744	0.0010	0.0153	0.0018
MUFA	26.98^{c}	30.15^{bc}	34.76^{a}	33.21^{ab}	0.756	0.0014	0.0252	0.0024
PUFA	1.12^{b}	1.49^{a}	1.59^{a}	1.49^{a}	0.058	0.0051	0.0042	0.0393
Total CLA	0.68^{b}	1.00^{a}	1.07^{a}	1.07^{a}	0.033	0.0004	0.0004	0.0012
ω-6/ω-3	2.46	2.01	1.98	1.99	0.277	0.5859	0.2948	0.5067
TUFA/TSFA	0.392^{c}	0.463^{bc}	0.572^{a}	0.532^{ab}	0.0173	0.0014	0.0264	0.0026

* *Moringa oleifera* added at 0% (M0), 10% (M10), 15% (M15) and 20% (M20), replacing 0%, 50%, 75% and 100% of sesame meal, respectively.
^{abc}Means in the same row with different superscripts significantly differ ($P < 0.05$).
TSFA = total saturated fatty acids, TUFA= total unsaturated fatty acids, MUFA= mono unsaturated fatty acids, PUFA= poly unsaturated fatty acids, CLA= conjugated linoleic acid (C18:2 *trans*−10, *cis*−12 and C18:2 *cis*−9, *trans*−11), SEM = Standard error of the mean.

protein flow to the small intestine of the host (Salem et al., 2013).

Soliva et al. (2005) studied the ruminal fermentation of *M. oleifera* leaves in comparison with soybean meal and rapeseed meal. They reported unaffected ruminal pH values and tVFA, and decreased ammonia-N concentration when *M. oleifera* leaves were degraded and fermented.

4.3. Blood chemistry

Decreased serum urea-N may be a result of decreased ruminal ammonia-N. Although GPT concentration was increased in the blood of goats fed MLM diets, the observed GOT and GPT concentrations were within the normal physiological range (Stanek et al., 1992).

The reduced blood cholesterol concentrations observed in MLM goats may be due to a functional effect of the phenolic acids in MLM. Saxena et al. (2013) stated that phytochemicals can reduce the synthesis and absorption of cholesterol. Moreover, reduced blood cholesterol and lipid levels are some of the biological activities of phenolic acids (Gryglewski et al., 1987). Astuti et al. (2011) reported that rations containing *M. oleifera* lowered serum cholesterol concentration. Khalel et al. (2014) found that blood glucose, total protein, albumin and globulin were higher for cows fed *Moringa* rations at 20 and 40% than those fed on diet free of *Moringa*. However, blood cholesterol and urea were decreased with *Moringa* diets, with no effects on GOT and GPT.

4.4. Milk yield and composition

The major finding of the current study was that milk yield, and milk energy and nutrient outputs were markedly increased by replacing sesame meal with MLM in the goats' rations. Milk yield was increased by 10 to 15% when goats were fed MLM diets. The increased milk production is a result of the increased feed intake and enhanced nutrient digestibility and ruminal fermentation observed in goats fed MLM. Sarwatt et al. (2004) explained the increased milk production recorded in cows fed *Moringa* as a result of a positive effect on the rumen environment and fermentation, with an increased amount of microbial biomass and of feed undegraded protein reaching the abomasum. Khalel et al. (2014) also reported a significant increase in daily milk yield and improved milk composition in cows offered *Moringa* as a protein supplement.

In the current study, ruminal concentrations of propionic acid were increased in MLM fed goats. Propionate is the precursor for gluconeogenesis and lactose synthesis, and increased glucogenic precursors have a favourable effect on milk yield (Rigout et al., 2003). Although the content of milk lactose is fairly constant with small variations, in our study milk lactose content was significantly increased in goats fed MLM. Conversely, fat and protein concentrations in milk were not affected by including *Moringa* in the diets, probably because all diets, including control, provided adequate levels of fibre and protein (Huhtanen, 1994). However, Mendieta-Araica et al. (2011a) reported

decreased milk yield without any effect in milk composition when soybean meal was replaced with MLM in the diets of dairy cows, as the protein and energy intakes were increased when cows were fed the soybean meal concentrate.

4.5. Milk fatty acid profile

Milk fatty acid profile was significantly affected by the inclusion of MLM in the diets of goats. Feeding MLM decreased TSFA in milk (up to 13% with M15 diet) and increased TUFA (up to 29%, with M15 diet) and total CLA contents (up to 47–58% with MLM diets). The increased PUFA in total milk fatty acids observed with diets containing MLM is important for both human and animal health, as they are precursors of long chain *n*-3 PUFA that are considered as important bioregulators of many cellular processes and linked to the development and functionality of the immune system (Khotimchenko, 2005). Moyo et al. (2010) reported that *Moringa* is rich in PUFA, and its inclusion in the diet of ruminants is recommended as it prevents the occurrence of diseases and promotes good health. Moreover, MLM decreased the ω-6/ω-3 ratio in milk, which is considered beneficial for the consumers. Human nutritionists urge consumers to increase intake of *n*-3 PUFA at the expense of *n*-6 PUFA.

The reason of the differences in milk fatty acids profile may be based on the distinct fatty acids profile in both MLM and sesame meal. Milk fatty acids originated mainly from plasma uptake (~60%) or by *de novo* synthesis in the mammary gland from acetate and 2-hydroxybutyrate derived from rumen fermentation, involving acetyl CoA carboxylase enzymes and fatty acid synthetase (Mesquita et al., 2008; Kholif et al., 2014). Ruminants do not synthesize PUFA; consequently their concentration in milk depends on the amount absorbed in the small intestine.

5. Conclusion

M. oleifera can replace sesame meal as a protein source in diets for lactating goats. The inclusion of *Moringa* leaf meal increases feed intake, enhances nutrient digestibility and ruminal fermentation and increases milk yield. Milk fatty acid profile is positively modified in goats fed *Moringa* leaf, as the relative percentage of unsaturated fatty acids and CLA are increased whereas saturated fatty acids are decreased. An inclusion rate of 15% MLM (replacing 75% of sesame meal) in the diet was the most suitable level for lactating goats under the current experiment conditions.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest

All authors declare that there are no present or potential conflicts of interest among the authors and other people or organizations that could inappropriately bias their work.

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