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REGULAR ARTICLES

Effect of partial replacement of alfalfa hay with *Moringa* species leaves on milk yield and composition of Najdi ewes

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Abstract The present study was carried out to investigate changes in milk yield and composition of Najdi ewes fed 25 % *Moringa* oleifera (MOD) or *Moringa peregrina* (MPD) leaf diets as a supplement to alfalfa hay diet (AHD). Thirty ewes (average 55 kg, 2 years old) were randomly sorted into three experimental groups with 10 animals each and were fed for a 6-week period with these diets (AHD, MOD, or MPD). Diets dry matter, crude protein, and crude fiber were comparable, while fat, nitrogen-free extract (NFE), neutral detergent fiber (NDF), acid detergent fiber (ADF), metabolizable energy (ME), total phenolic, and antioxidant activity varied ($p \leq 0.05$) between the diets. Feeding ewes with MOD increased ($p \leq 0.05$) the milk yield compared to those fed AHD while milk composition was similar ($p \leq 0.05$) between treatments. The concentration of malondialdehyde (MDA) in the ewes' milk and serum was lower ($p \leq 0.05$) for MOD, while the total antioxidant capacity, catalase activity, and vitamin C contents were increased ($p \leq 0.05$). The serum cholesterol and glucose of the ewes were lower ($p \leq 0.05$) for those fed MOD. Moringa diets increased ($p \leq 0.05$) average daily weight gain of lambs compared to those fed alfalfa diets. The results obtained showed that the inclusion of *Moringa*, especially *M. oleifera*, in the diet of Najdi ewes can improve milk yield and quality.

Keywords Alfalfa · *M. oleifera* · *M. peregrina* · Najdi sheep · Milk yield · Oxidative status

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Introduction

In most developing countries, one of the major constraints affecting the productivity of livestock especially in dry tropical areas is low quality and inadequacy of available feeds. The high prices of concentrates and legumes and the increase in competition between humans and livestock for protein ingredients in recent times limited the use of these sources as protein supplements (Odunsi 2003).

The use of tree parts as alternative feed resources for ruminant livestock is becoming increasingly important in many areas of the tropics and subtropics (Silanikove 2000). *Moringa* trees are multipurpose trees of economic importance with several industrial and feeding values. It is widely cultivated, especially in dry tropical areas of the Middle East and Africa (Palada et al. 2007). The advantages of using *Moringa* as livestock feeds include the following: the leaves are rich in minerals which are essential for animal weight gaining and milk production (Mendieta-Araica et al. 2011) and an excellent source of proteins that can improve the microbial protein synthesis in the rumen (Soliva et al. 2005).

Unlike other species belonging to the family Moringaceae, studies on the use of *Moringa oleifera* as livestock fodder have been carried out due to its high quality. Laandrault et al. (2001) reported that certain plants contained natural antioxidants such as vitamin C, polyunsaturated fatty acids, tocopherols, flavonoids, and other phenolic compounds, and *M. oleifera* is one of such plants (Isitua et al. 2015). The leaves of *M. oleifera* contained higher essential amino acids (like cysteine), fiber, and minerals (Yameogo et al. 2011; Ali et al. 2015) than *Moringa peregrina*. Various studies indicated the effectiveness of including *Moringa stenopetala* in the diet of sheep (Gebregiorgis et al. 2012). Despite the high nutritional qualities of *M. oleifera* and *M. peregrina* compared to other species of *Moringa*, and its ability to be easily cultivated in dry climatic conditions, there is a dearth of information on the effect of substituting *M. oleifera* and *M. peregrina* in the diet of lactating ewes. Consequently, it has been hypothesized that *Moringa* leaves can be used as a suitable roughage substitute in the diet of dairy sheep with possible enhancement of production performance. Thus, the aim of this study was to investigate the effect of *M. oleifera* and *M. peregrina* as substitutes for alfalfa hay on milk yield and quality of Najdi ewes.

Materials and methods

Experimental site

The experiment was conducted at Al-Khalidiya Agricultural Farm, Riyadh, Kingdom of Saudi Arabia, located at latitude 24° 23′ 22″ N and longitude 45° 53′ 55″ E. The mean annual ambient temperature is 31.6 °C, and the average monthly rainfall is 8.4 mm, with a marked dry season (April–September).

Feed preparation

Fresh *M. oleifera* and *M. peregrina* leaves were manually harvested from mature trees (3–4 months old) at Durt-elizdihar Agricultural Farm, Gazan, Saudi Arabia. The leaves were air-dried under partial shade by spreading on clean plastic sheets for 72 h and turned over several times. The dried leaves were ground to a fine powder and mixed with other ingredients into pellets using a pellet-plant at Al-Khalidiya Agricultural Farm, Riyadh, Kingdom of Saudi Arabia.

Chemical analysis of the feeding diets

The dry matter, crude protein, fat, crude fiber, neutral detergent fiber, acid detergent fiber, and ash contents of the experimental diets were determined according to the standard methods of the Association of Official Agricultural Chemists (AOAC 2005). All chemicals used in this study were of reagent grade. The metabolizable energy of the experimental diets was estimated using the energy equation in TMR proposed by Boguhn et al. (2003).

The total phenolic content of the experimental diets was analyzed using the Folin-Ciocalteu method (Singleton and Rossi 1965). The free radical scavenging activity of the diets was determined using 2,2,-diphenyl-2-picryl-hydrazyl (DPPH) according to Turkmen et al. (2005). All the measurements were carried out in triplicate.

Animals' management

Thirty ewes (average body weight 55± kg, 2± years of age) of second lactation (10–15± days) were randomly sorted into three experimental groups with 10 animals each and were fed daily 2 kg/head of the pelleted diets either alfalfa hay diet (AHD), *M. oleifera* diet (MOD), or *M. peregrina* diet (MPD) (Table 1) and had free access to clean fresh water. The animals were kept in semi-open sheds at Al-Khalidiya Farm, Riyadh, KSA. Each experimental group was assigned in a completely randomized design with 2 weeks adaptation period, and 6 weeks sample collection period. Ewes were hand-milked daily to record the daily milk yield, and the samples were immediately refrigerated at 4 °C. These samples were pooled into one sample per week throughout the collection period (6 weeks). The samples were transported to the laboratory and kept frozen at −80 °C for further analysis.

Blood samples were collected weekly before feeding by jugular vein puncture into plain vacationer tubes. The blood samples were centrifuged at 3000 rpm (Centrifuge 400R, Kendrow, Osterode, Germany) for 10 min to separate the sera. The separated serum samples were kept frozen at −80 °C for further analyses.

Analysis of milk and serum

Milk composition including fat, protein, lactose, total solids, solids-not-fat, and freezing point depression was analyzed

Table 1 Ingredients of the experimental diets (% dry-weight basis)

Ingredients	Diet		
	AHD	MPD	MOD
Alfalfa hay	40.0	15.0	15.0
M. oleifera	0.0	0.0	25.0
M. peregrina	0.0	25.0	0.0
Corn	25.0	25.0	25.0
Barley	21.8	17.9	27.6
Soya bean meal	10.2	14.1	4.4
NaCl	1.0	1.0	1.0
Lime stone	1.0	1.0	1.0
$NaHCO_3$	0.8	0.8	0.8
Vitamin-mineral premix[a]	0.2	0.2	0.2
Total	100.0	100.0	100.0

AHD alfalfa hay diet, *MPD M. peregrina* diet, *MOD M. oleifera* diet

[a] Vitamin-mineral premix provided the following per kilogram of diet: vitamin A, 500,000 IU; vitamin D3, 500,000 IU; vitamin E, 10,000 IU; $CoSO_4$, 0.30 g; $CuSO_4$, 20.10 g; $FeSO_4$, 10.00 g; ZnO_2, 50.00 g; $MnSO_4$, 40.20 g; KI, 0.75 g

using Milko Scan (Minor Type 78100, FOSS Electric, Denmark). For each experimental group, the values recorded during the 6 weeks of treatment were averaged. The energy content of milk was calculated according to the formula proposed by Tyrell and Reid (1965).

Total antioxidant capacity, malondialdehyde concentration, and catalase activity in the milk and serum samples were estimated using specific assay kits (Cayman, USA). For each experimental group, the values obtained during the six treatment weeks were averaged.

The concentration of ascorbic acid (vitamin C) was measured in milk and serum samples for each experimental group as an average value for the six treatment weeks using Ascorbic Acid Colorimetric Assay Kit (BioVision, USA). The serum glucose and cholesterol concentrations were measured using Randox Assay Kits (Randox, Laboratories Ltd., UK).

Experimental design and statistical analysis

The experimental design used was a completely randomized design. Each experimental period included 2 weeks of treatment adaptation and 6 weeks of data collection. Data of all parameters were statistically analyzed by one-way ANOVA using SAS (2007), software version 9.6. The significant differences ($p \leq 0.05$) between means were determined by Duncan's multiple comparison tests.

Results

Slight variations occurred in the dry matter, ash, crude protein, and crude fiber contents of the formulated diets (Table 2). Replacement of alfalfa hay with *M. oleifera* resulted in higher ($p \leq 0.05$) fat, nitrogen-free extract, metabolizable energy, total phenolic (TPC), and antioxidant activity, and lower ($p \leq 0.05$) NDF and ADF contents.

The milk yield of ewes fed on the different experimental diets showed comparable values during the adaptation period (Table 3). After that, the milk yield of ewes fed on *Moringa* diets was highly increased ($p \leq 0.05$) with a maximum increment rate obtained at the third week of sample collection for MOD (61.1 %) compared to AHD (51.6 %) and MPD (53.6 %). At the end of the sample collection period, ewes fed on MOD still showed higher ($p \leq 0.05$) increment rate in milk yield (49.7 %) compared to AHD (7.7 %) and MPD (34.9 %).

The milk composition did not show any significant ($p \geq 0.05$) differences among the treatments; however, a slight increase in milk fat, lactose, and energy output as well as the freezing point depression was observed in ewes fed on MOD compared to those fed on AHD or MPD (Table 4).

As shown in Table 5, replacing AHD with MOD reduced ($p \leq 0.05$) the malondialdehyde (MDA) concentrations in both milk and serum to 7.74 and 1.91 nmol/L, respectively. However, there was no significant ($p \geq 0.05$) difference in the MDA concentrations in milk and serum of ewes fed on AHD and MPD. A significant ($p \leq 0.05$) increase in total antioxidant capacity (TAC) and catalase activity was noted in the milk and serum of ewes fed on MOD. Vitamin C content was higher ($p \leq 0.05$) in the milk of ewes fed on MPD and MOD compared to those fed on AHD. However, serum vitamin C concentrations did not show significant ($p \geq 0.05$) variations between treatments.

Table 2 The chemical composition of the experimental diets (dry-weight basis)

Parameter	Diet		
	ADH	MPD	MOD
Dry matter (%)	91.20 ± 0.19	91.81 ± 0.41	91.74 ± 0.25
Ash (%)	8.07 ± 0.23	8.44 ± 0.21	8.98 ± 0.11
Crude protein (%)	18.66 ± 0.41	17.39 ± 0.32	17.24 ± 0.13
Fat (%)	2.96 ± 0.22^{b}	3.23 ± 0.23^{ab}	4.30 ± 0.25^{a}
Crude fiber (%)	11.83 ± 0.24	11.87 ± 0.16	10.13 ± 0.08
NDF (%)	27.00 ± 0.23^{a}	22.10 ± 0.18^{b}	20.90 ± 0.20^{b}
ADF (%)	16.00 ± 0.11^{a}	14.00 ± 0.13^{b}	11.20 ± 0.10^{c}
Nitrogen-free extract (%)	58.48 ± 0.36^{b}	59.07 ± 0.17^{ab}	60.35 ± 0.45^{a}
ME (MJ/kg DM)	7.10 ± 0.07^{a}	7.10 ± 0.07^{a}	7.30 ± 0.08^{b}
Total phenolics (mg GAE/g)	3.23 ± 0.12^{b}	4.50 ± 0.19^{a}	5.11 ± 0.23^{a}
Antioxidant activity (%)	56.89 ± 5.27^{c}	63.22 ± 0.72^{b}	67.96 ± 0.69^{a}

Values are means ± SD of three independent samples. Values having different superscripts in a row are significantly different at $p \leq 0.05$

AHD alfalfa hay diet, *MPD M. peregrina* diet, *MOD M. oleifera* diet, *ME* metabolizable energy, *NDF* neutral detergent fiber, *ADF* acid detergent fiber

Table 3 Average daily milk yield (kg) and rate of increase (%) of Najdi ewes fed 40 % alfalfa hay (AHD), 25 % *M. peregrina* (MPD), or 25 % *M. oleifera* diets (MOD)

Feeding period (weeks)	Diets					
	AHD	Rate of change (kg)	MPD	Rate of change (kg)	MOD	Rate of change (kg)
0	1.55 ± 0.07	–	1.66 ± 0.12	–	1.67 ± 0.24	–
1	1.84 ± 0.72	0.29^b	1.96 ± 0.31	0.30^b	2.38 ± 0.30	0.71^a
2	2.31 ± 0.06	0.76^b	2.39 ± 0.04	0.73^b	2.65 ± 0.01	0.98^a
3	2.35 ± 0.18	0.80^c	2.55 ± 0.34	0.89^b	2.69 ± 0.02	1.02^a
4	2.17 ± 0.28	0.62^c	2.41 ± 0.12	0.75^b	2.57 ± 0.08	0.90^a
5	2.16 ± 0.09	0.61^c	2.38 ± 0.08	0.72^b	2.56 ± 0.06	0.89^a
6	1.67 ± 0.17	0.12^c	2.24 ± 0.13	0.58^b	2.50 ± 0.15	0.83^a
Average yield	2.00 ± 0.22^a	–	2.23 ± 0.16^{ab}	–	2.43 ± 0.12^b	–

Values are means (±SD) of 10 samples. Values having different superscripts in a row are significantly different at $p \leq 0.05$

Significantly ($p \leq 0.05$) lower values for serum cholesterol and glucose levels were observed in ewes fed on MOD compared to those fed on AHD or MPD.

Discussion

The mean values of dry matter and ash contents of diet 3 were within the range reported by Adegun et al. (2011). Although the *Moringa*-substituted diets have a slightly lower crude protein than that of alfalfa hay diet but their values exceeded 11–13 % known to be capable of supplying adequate protein for maintenance and moderate growth in ewes. However, the values were lower than that reported by Moyo et al. (2012). According to Yousuf et al. (2007), animal diet is regarded to have low nutritional quality if it has relatively high fiber but low ether extract. Debela and Tolera (2013) reported that *Moringa* leaf meal can be considered as a good potential source of supplementary protein for ruminants. The amino acid supply of *Moringa* leaf meal protein concentrate is of particular nutritional importance as it provides the protein needs and boosts the immune system against diseases (Brisibe et al. 2009). Therefore, replacing the alfalfa diet with *Moringa* species could increase the nutritional quality of the ewes' diet because of their low fiber and high ether extract and less time for the rumen microorganisms to digest fiber. The high total phenolic content and antioxidant values of *Moringa* species diet especially *M. oleifera* could provide nutritional and health benefit to the ewes and consumers of the ewes' dairy products by delaying the rate of autoxidation of the fat.

Higher milk yield was observed in Najdi ewes fed on *Moringa* diets compared to those offered alfalfa hay throughout the experimental period. The values obtained were higher than the milk yield of Awassi ewes fed with olive cake and tomato pomace supplements as reported by Abbeddou et al. (2015). *M. oleifera* diet improved the milk yield due to its lower NDF and ADF and higher metabolizable energy contents. In addition, despite the slightly lower protein value of

Table 4 Effect of alfalfa hay diet replacement by *Moringa* species diets on milk composition of Najdi ewes

Parameter	Diet		
	ADH	MPD	MOD
Fat (%)	3.51 ± 0.21	3.72 ± 0.30	4.12 ± 0.23
Protein (%)	4.82 ± 0.10	4.80 ± 0.13	4.81 ± 0.14
Lactose (%)	3.63 ± 0.27	4.13 ± 0.19	4.21 ± 0.17
Total solids (%)	12.20 ± 0.32	12.71 ± 0.43	12.83 ± 0.51
Solid non-fat (%)	9.44 ± 0.13	10.00 ± 0.16	9.84 ± 0.14
Freezing point depression (%)	0.52 ± 0.03	0.54 ± 0.0.02	0.61 ± 0.05
Milk energy content (MJ/kg)	3.03 ± 0.14	3.20 ± 0.11	3.38 ± 0.16
Milk energy output (MJ/day)	6.06 ± 0.18^c	7.14 ± 0.14^b	8.21 ± 0.09^a

Values are means ± SD of 10 samples. Values having different superscripts in a row are significantly different at $p \leq 0.05$

AHD alfalfa hay diet, *MPD M. peregrina* diet, *MOD M. oleifera* diet

Table 5 Milk and serum oxidative status, vitamin C, cholesterol, and glucose concentrations of Najdi ewes fed 40 % alfalfa hay (AHD), 25 % *M. peregrina* (MPD), or 25 % *M. oleifera* diets (MOD)

Sample	Parameter					
	TBARS (nmol MDA)	TAC (mmol/L)	Catalase (nmol/min/ml)	Vitamin C (mg/100 ml)	Cholesterol (mg/100 ml)	Glucose (mg/100 ml)
Milk						
AHD	11.53 ± 1.91^{b}	3.72 ± 0.34^{b}	57.06 ± 3.92^{b}	4.01 ± 0.38^{b}	–	–
MPD	10.52 ± 1.61^{b}	4.50 ± 0.31^{b}	57.86 ± 3.64^{b}	4.72 ± 0.67^{a}	–	–
MOD	7.74 ± 1.01^{a}	5.05 ± 0.21^{a}	60.81 ± 4.35^{a}	6.09 ± 0.83^{a}	–	–
Serum						
AHD	2.92 ± 0.52^{a}	16.82 ± 1.01^{b}	23.93 ± 2.12^{c}	1.22 ± 0.15	61.12 ± 3.20^{a}	62.20 ± 3.22^{a}
MPD	2.25 ± 0.32^{a}	17.63 ± 2.11^{a}	25.80 ± 1.42^{b}	1.34 ± 0.28	61.21 ± 3.82^{a}	60.74 ± 2.85^{b}
MOD	1.91 ± 0.21^{b}	17.92 ± 0.23^{a}	28.23 ± 2.41^{a}	1.39 ± 0.58	58.50 ± 3.12^{b}	60.09 ± 3.41^{b}

Values are means (±SD) of 10 samples. Values having different superscripts in a column are significantly different at $p \leq 0.05$

TBARS thiobarbiturate acid reactive substances, *MDA* malondialdehyde, *TAC* total antioxidant capacity

Moringa diet as compared to alfalfa, *Moringa* protein has excellent rumen bypass characteristics (Sarwatt et al. 2004). Khalel et al. (2014) observed a significant increase in daily milk yield and improved milk composition in cows fed with *Moringa* as a protein supplement. Various factors such as type of feed, breeds of the animal, and the environmental conditions can affect the milk yield of the animal. The results showed that persistence of high milk production in ewes can be achieved by replacing their diet with *M. oleifera* especially in a dry environment where the milk yields of animals are generally associated with animal nutrition. According to Zarkadas et al. (1995), *Moringa* leaves enhanced lactation persistency because the leaves had a high concentration of amino acids.

The milk fat, lactose, freezing point depression, total energy, and energy output were relatively similar with a slight increase in ewes fed on *M. oleifera* diet. However, milk protein content was not affected by *Moringa* diets, probably because all diets, including control, provided adequate levels of fiber and protein. This is consistent with other studies that did not report any relationship between milk protein and percentage of dietary protein (Reyes-Sánchez et al. 2006). Further, Tufarelli et al. (2011) indicated that the diet can cause variation in milk fat without affecting the total solid contents. Ewes fed diet replaced with *M. oleifera* yielded slightly more milk fat and milk lactose compared to ewes fed on alfalfa diet. According to Gizachew et al. (2002) and Ali et al. (2015), *M. oleifera* leaves have high mineral contents most especially P, Ca, and Mg compared to native pasture. This might be responsible for the high milk fat and lactose contents of ewes fed on *M. oleifera* diet. The positive effects on milk fat and lactose contents in ewes fed on *M. oleifera* diet reported in the present study might be due to the higher metabolizable energy content.

Significantly lower MDA values in milk and serum of ewes fed on *M. oleifera* were observed as compared to those fed on *M. peregrina* and alfalfa hay diets. This response could be due to the high total phenolic content (5.11 mg GAE/g sample) and antioxidant capacity (67.96 %) of *M. oleifera* diet compared to other diets. Furthermore, the high level of milk catalase activity in ewes fed on *M. oleifera* will inhibit fat oxidation thereby retaining the nutritional characteristics of ewes' milk. The high antioxidant capacity of *M. oleifera* diet had a positive effect on the total antioxidant capacity of ewes' milk and serum. *Moringa* leaves had a high antioxidant capacity that has been assessed mainly for its ability to scavenge free radicals with a focus on biological activities and therapeutic uses (Mbikay 2012). Therefore, feeding ewes on *Moringa* diet rich in antioxidant compounds will improve milk quality thus satisfying the demands of milk manufacturers and consumers for healthier dairy products.

Although milk is not considered as a source of vitamin C, the results obtained from this study showed that ewes' milk contains an appreciable amount of vitamin C. Feeding ewes on *Moringa* species diets increased vitamin C content of their milk without affecting serum vitamin C concentration. *Moringa* leaves are rich in micronutrients such as iron, potassium, calcium, and multivitamins which are essential for livestock growth and milk production (Mendieta-Araica et al. 2011). Moreover, the absorption rate of these micronutrients is better compared to other leafy vegetables (Nouman et al. 2014) which might be responsible for the high milk vitamin C content in ewes fed on *Moringa* diets.

A significant decrease in serum glucose concentration was observed in ewes fed on *M. oleifera* diet compared to those fed on alfalfa diet. This could be attributed to the reported antidiabetic effects of *Moringa* (Farooq et al. 2007). The cholesterol is of nutritional interest because high levels of cholesterol in plasma are associated with an increasing risk of cardiovascular diseases. Replacement of alfalfa hay with *Moringa* leaves in the diet of ewes reduced the serum cholesterol level. The reduced serum cholesterol concentrations in ewes fed on *M. oleifera* diet are likely to be due to a functional effect of the phenolic acid contents of *Moringa*. It has been reported that phytochemicals can reduce the synthesis and absorption of cholesterol (Saxena et al. 2013). Moreover, glucose is one of the primary precursors for cholesterol synthesis in the small intestine or liver (Iqbal et al. 2012). Thus, the reduced level of serum glucose in ewes fed on *M. oleifera* diet may be responsible for the reduction of serum cholesterol concentration.

In conclusion, the results of the current study have shown that replacement of alfalfa hay by *Moringa* species in ewes' diet improved their milk yield and quality, with *M. oleifera* having significantly higher effects compared to *M. peregrina*. Also, replacement of alfalfa hay by *Moringa* in the ewes' diet improved the oxidative status of their milk and serum. It is worth to mention that the observed improvement was obtained by replacing 62.5 % of alfalfa hay in ewes' diet by *Moringa* leaves. Therefore, *Moringa*, especially *M. oleifera*, can be used commercially for feeding animals in arid and semiarid areas where cultivation of alfalfa is difficult due to water scarcity.

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Compliance with ethical standards All procedures described in this experiment were approved by the Faculty Research Ethics Committee at King Saud University.

Conflict of interest The authors declare that they have no conflict of interest.

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