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Antioxidant potential of Moringa leaves for improvement of milk and serum quality of Aardi goats

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ABSTRACT

The effects of *Moringa* leaves as a partial replacer of alfalfa hay on oxidative status of goats' blood and milk were investigated in this study. Three dietary treatments including alfalfa (AHD), 25% *Moringa oleifera* Lam. (MOD) or 25% *Moringa peregrina* Forssk. (MPD) diets were used in this study. Thirty goats were randomly assigned into 3 experimental groups with 10 experiment units (10 goats) each using completely randomized design. The experiment consisted of 2-week adaptation and 6-week collection data. Both MPD and MOD showed higher fat, nitrogen free extract, total phenolics, antioxidant activity than AHD. Malondialdehyde concentration of milk and serum was lower in goats fed MPD and MOD. Catalase activity, vitamin C contents and total antioxidant capacity were higher in the milk and serum of goats fed diets containing MOD or MPD than that fed AHD. Replacement of alfalfa hay in goats' diet with *M. oleifera* or *peregrina* leaves resulted in reduction of serum cholesterol and glucose levels. The results suggest that utilization of *M. oleifera* or *peregrina* leaves as a non-traditional fodder could improve both animal and consumer oxidative status and immune systems through the transfer of bioactive compounds, particularly antioxidants and vitamin C from fodder to milk.

1. Introduction

One of the major constraints hampering ruminant production in many developing countries, particularly in dry and tropical areas, is poor quality feed due to unavailability and the high cost of feedstuffs, especially protein sources. In these regions, the growth of high-quality fodder crops like alfalfa is difficult due to environmental conditions such as water scarcity. Animals are fed by farmers with crop byproducts and hay of low quality that are deficient in nitrogen, minerals and vitamins but high in lignocellulose resulting in digestibility issues and voluntary intake (Gerbregiorgis et al., 2012). As these feeds may be deficient in protein and they can result in lower energy levels, improper growth, low quality meat and reduced milk production and quality (Gerbregiorgis et al., 2012). Consequently, there is a need for alternative cheap protein sources with a balanced amino acid profile that can be both fast produced and abundant under dry conditions. Fodder trees and shrubs are some of the alternative cheap and good protein sources that have been employed effectively in small ruminant production during the dry season (Aregheore, 2002; Moyo et al., 2012).

Moringa species are multipurpose trees from non-leguminous group. They are fast growing trees of economic and industrial importance and a potential source of animal feed. Middle East and African countries are reported to have wide cultivation of these trees (Palada et al., 2007). *Moringa oleifera* foliage has been reported to be a potential cheap source of protein for animals feeding (Sarwatt et al., 2004). The use of *M. oleifera* as animal feed in dry and tropical regions has several advantages such as the ability to be cultivated under drought conditions, high protein and mineral contents, improving microbial protein synthesis in the rumen, high content of naturally occurring antioxidants such as vitamin C, tocopherols, flavonoids and phenolic compounds and also negligible amount of anti-nutritive compounds (Soliva et al., 2005; Mendieta-Araica et al., 2011; Nouman et al., 2014).

Several studies have reported efficient use of *M. oleifera* in improving goat production such as West African dwarf goat (Asaolu et al., 2012), Sudan Nubian goat (Babeker and Abdalbagi, 2015), Bengal goat (Sultana et al., 2015) and Anglo-Nubian goat (Kholif et al., 2015). However, the use of other Moringa species such as *M. peregrina* has not been investigated. *Moringa oleifera* and *M. peregrina* leaves cultivated in Saudi Arabia are rich in major amino acids (like cysteine), minerals and fiber contents (Yameogo et al., 2011) owing to high nutritional quality of *Moringa* species and suitability of these plants for cultivation in dry

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Table 1
Experimental diets formulation (% DW).

Ingredients	Diet[T]		
	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
NaHCO3	0.8	0.8	0.8
Vitamin-mineral premix*	0.2	0.2	0.2
Total	100.0	100.0	100.0

[T] AHD alfalfa hay diet, MPD *Moringa. peregrina* Forssk. diet, MOD *Moringa oleifera* Lam. diet.

* Vitamin-mineral premix/Kg diet: vitamin A, 500,000 IU; vitamin D3, 500,000 IU; vitamin E, 10,000 IU; CoSO4, 0.30 g; CuSO4, 20.10 g; FeSO4, 10.00 g; ZnO2, 50.00 g;MnSO4, 40.20 g; KI, 0.75 g.

climatic conditions. Therefore, this study was carried out to investigate the effects of replacing alfalfa hay with dry *Moringa oleifera* Lam or *Moringa peregrina* Forssk leaves on milk and serum quality of Aardi goat.

2. Materials and methods

2.1. Materials

Manually harvested fresh (3–4 months old) mature *Moringa* trees were obtained from a farm in Gazan, Saudi Arabia. The two *Moringa* species (*M. oleifera* Lam. and *M. peregrine* Forssk) were identified by specialized botanist in the farm. The leaves obtained from the species were spread on plastic sheets and air dried for 3 days under partial shade accompanied with turning over. The leaves were ground to a powder form and added to other ingredients to form feed pellets. All chemicals used for analysis were of analytical grade.

2.2. Experimental design and husbandry

The experimental farm was located at latitude 24°23′22″ N and longitude 45°53′55″ E having a mean annual temperature of 31.6 °C and an average monthly rainfall of 8.4 mm accompanied with a marked dry season from April to September. Experimental procedures for animal study adopted in the present research were approved by the King Saud University Faculty Research Ethics Committee in November 2013. Two-year-old female Aardi goats (30) with an average weight of 38.0 ± 1.80 kg were randomly assigned into 3 experimental groups with 10 animals each using an entirely randomized design.

All goats were at the same stage of their second lactation. The animals were kept in semi-open sheds and each experimental period consisted of 2 weeks adaptation to the treatment and 6 weeks of data collection. The animals were fed *ad libitum* with three different diets: alfalfa hay (AHD), *M. peregrina* Forssk (MPD) or *M. oleifera* Lam. (MOD) formulated according to the nutritional requirements of goats (NRC, 1981) as shown in Table 1.

2.3. Analysis of experimental diets

The proximate composition (dry matter, crude protein, fat, crude fiber, nitrogen-free extracts and ash) of the experimental diets was obtained according to AOAC (2005) method. The analysis of total phenolic was carried out according to Folin–Ciocalteu as reported by Singleton and Rossi (1965) and antioxidant activity was evaluated using 2, 2,-diphenyl-2-picryl-hydrazyl (DPPH) method (Turkmen et al., 2005).

2.4. Sampling and analysis of milk and serum

Goats were hand-milked daily and milk samples from each goat were pooled into one sample per week and immediately kept frozen at −20 °C for further analysis. The collection of blood samples from experimental goats was done on weekly basis prior to feeding from jugular vein using plain vacutainer tubes.

The collected blood samples were subjected to centrifugation (Centrifuge 400R, Kendrow, Osterode, Germany) at 3000 rpm for 10 min to separate the serum. Serum samples collected during 6 weeks were pooled and stored at −20 °C for further analyses. The contents of fat, protein, lactose, solids-not-fat, total solids percentages and freezing point depression were carried out for milk samples on weekly basis using a Milko Scan (Minor Type 78,100, FOSS Electric, Denmark). The mean value for six weeks was obtained for each group of animals. Milk energy content was obtained following Tyrell and Reid (1965) formula.

The milk and serum total antioxidant capacities (TAC) were analyzed using an Antioxidant Assay Kit (Cayman, USA). Milk and serum catalase activity was measured using a Catalase Assay Kit (Cayman, USA). Milk and serum malondialdehyde (MDA) concentration for each group of animals was measured using a thiobarbituric acid reactive substance (TBARS) Assay Kit (Cayman, USA). Milk and serum vitamin C content was measured using an Ascorbic Acid Colorimetric Assay Kit (BioVision, USA). Serum glucose and cholesterol concentrations were measured using Randox Assay Kits (Randox, Laboratories Ltd., UK).

2.5. Statistical analysis

In all instances three samples were analyzed to generate an average value, and data were analyzed using analysis of variance (ANOVA) procedure following a completely randomized design using the Generalized linear Model (GLM) procedure of SAS (version 9.2, 2008). The differences among means were determined using Duncan multiple range test. Significant difference was accepted at $P \leq .05$.

3. Results and discussion

In this study alfalfa hay was partialy replaced by *M. oleifera* Lam. or *M. peregrina* Forssk. in the pelleted total mixed ration of goats. Interestingly, *M. oleifera* produces leaves during the dry season and during times of drought, and is an excellent source of green vegetable when little other feed is available (FAO, 2014). However, alfalfa hay was partialy replaced by *M. oleifera* or *M. peregrina leaves* in the current study due to the reported moderate palatability of *M.oleifera* leaves for ruminants (Garcia et al., 2008).

Table 2
Experimental diets chemical composition of (dry-weight basis).

Parameters	Diets		
	AHD	MPD	MOD
Dry matter (%)	$91.20^{a} \pm 0.19$	$91.81^{a} \pm 0.41$	$91.74^{a} \pm 0.25$
Ash (%)	$8.07^{a} \pm 0.23$	$8.44^{a} \pm 0.21$	$8.98^{a} \pm 0.11$
Crude protein (%)	$18.66^{a} \pm 0.41$	$17.39^{a} \pm 0.32$	$17.24^{a} \pm 0.13$
Fat (%)	$2.96^{b} \pm 0.22$	$3.23^{ab} \pm 0.23$	$4.30^{a} \pm 0.25$
Crude fiber (%)	$11.83^{a} \pm 0.24$	$11.87^{a} \pm 0.16$	$10.13^{a} \pm 0.08$
Nitrogen free extract (%)	$58.48^{b} \pm 0.36$	$59.07^{ab} \pm 0.17$	$60.35^{a} \pm 0.45$
Total phenolic (mg GAE/g sample)	$3.20^{b} \pm 0.12$	$4.50^{a} \pm 0.19$	$5.11^{a} \pm 0.23$
Antioxidant activity* (Radical inhibition %)	$56.89^{c} \pm 5.27$	$63.22^{b} \pm 0.72$	$67.96^{a} \pm 0.69$

Means (triplicate samples) within the same row with different superscript letter (s) significantly differ at $P \leq .05$, according to Duncan multiple range test; AHD: alfalfa hay diet; MPD: *Moringa peregrina* Forssk. diet; MOD: *Moringa oleifera* Lam. diet; GAE: Gallic acid equivalent.

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Table 3
Effect of diet treatments on milk composition of Aardi goats.

Parameters	Diets		
	AHD	MPD	MOD
Fat (%)	$3.91^{b} \pm 0.21$	$3.34^{b} \pm 0.12$	$4.29^{a} \pm 0.10$
Protein (%)	$3.51^{a} \pm 0.06$	$3.35^{a} \pm 0.04$	$3.56^{a} \pm 0.06$
Lactose (%)	$4.51^{a} \pm 0.07$	$4.62^{a} \pm 0.04$	$4.73^{a} \pm 0.03$
Total solids (%)	$12.60^{a} \pm 0.21$	$11.91^{b} \pm 0.10$	$13.11^{a} \pm 0.22$
Solid non-fat (%)	$8.80^{a} \pm 0.19$	$8.61^{a} \pm 0.25$	$8.93^{a} \pm 0.17$
Freezing point depression	$0.57^{b} \pm 0.07$	$0.58^{b} \pm 0.04$	$0.61^{a} \pm 0.03$
Milk energy content (MJ/kg)	$3.07^{a} \pm 0.87$	$2.82^{b} \pm 0.79$	$3.23^{a} \pm 0.93$

Means (triplicate samples) within the same row with different superscript letter (s) significantly differ at P ≤ .05, according to Duncan multiple range test; AHD: alfalfa hay diet; MPD: *Moringa peregrina* Forssk. diet; MOD: *Moringa oleifera* Lam. diet.

3.1. Chemical composition of the experimental diets

The experimental diets used in the current study were analyzed for their chemical composition and the results are presented in Table 2. The diets were statistically similar in their crude protein content. However, in MOD the fat, nitrogen free extract and total phenolic content (TPC) were significantly ($P \leq .05$.) higher than those of AHD, but did not differ from those of MPD. The variation in protein and nitrogen free extract between diets is mainly to keep the different diets isonitrogenous and iso-caloric. The antioxidant activity of the diets ranged between 56.89 and 67.96% with the lowest ($P \leq .05$.) value obtained for AHD and highest ($P \leq .05$.) value for MOD. The crude protein content of the experimental diets did not differ significantly ($P \geq .05$) and falls within the range recommended for lactating goats (NRC, 1981). The crude protein content of the diets is comparable with the values reported by Kakengi et al. (2005) and Sultana et al. (2015), but is lower than the values reported by Sànchez et al. (2006) and Fadiyimu et al. (2010). The high total phenolic content of *Moringa* containing diets could improve goats' health as well as preventing oxidation and increasing shelf life of goat products such as milk. Reports have shown that *M. oleifera* leaves can be used to scavenge free radicals owing to their high antioxidant activity (Mbikay, 2012).

3.2. Effect of the dietary treatment on milk composition

The effect of the dietary treatment on milk composition of Aardi goat over the 6 weeks period is shown in Table 3. Goats' milk protein, lactose and solid non-fat levels were not affected by the type of diet (Table 3). However, milk fat and freezing point depression were higher ($P \leq .05$.) for MOD, while milk total solid and milk energy contents were lower ($P \leq .05$.) for MPD. The milk energy content obtained in this study was compatible with the value of Anglo-Nubian goats (Kholif et al., 2015). Results from this study thus showed that it is recommendable to substitute alfalfa hay with *Moringa* species in lactating goats' diet without any compromise on milk composition. The results obtained in the present study are consistent with findings reported by Babiker et al. (2016) for Najdi ewes.

3.3. Effect of the dietary treatment on oxidative status and vitamin C content of milk

The effect of substituting alfalfa hay with *Moringa* species on oxidative status and vitamin C content of pooled milk and serum samples, in addition to serum cholesterol and glucose levels of Aardi goats is presented in Table 4. Milk and serum samples collected from goats fed with MOD had significantly ($P \leq .05$.) lower MDA values (6.82 and 2.04 nmol/mL, respectively) than that of milk and serum from goats fed with AHD (8.73 and 3.47 nmol/mL). The decrease in MDA values may be due to high phenolic contents (Babiker et al., 2018) and antioxidant activity of *M. oleifera* compared to alfalfa.

The high antioxidant activity in MOD may provide a health benefit to the goat by scavenging free radicals and also retarding the oxidation of milk fat. The catalase activity and total antioxidant capacity (TAC) of both milk and serum samples collected from MOD fed goats were significantly ($P \leq .05$.) higher compared to those fed AHD or MPD. Goats fed the MPD or MOD showed significantly ($P \leq .05$.) higher vitamin C content in milk and serum, but significantly ($P \leq .05$) lower serum cholesterol and glucose levels. The positive contribution of *M. oleifera* diet to the TAC of goats' milk may be employed in the production of healthy milk and dairy products. Previous studies reported that *M. oleifera* leaves possessed high antioxidant activity that can provide health benefit (Mbikay, 2012).

The high catalase contents in milk and serum of goats fed *M. oleifera* or *peregrina* diets may also contribute to the reduction in the MDA value by catalyzing the decomposition of hydroperoxides, thereby protecting the milk from further spoilage by oxidation as reported for Najdi ewes (Babiker et al., 2016). This could help in increasing the shelf life of goat's milk and dairy products. The experimental diets showed significant effects on both milk and serum vitamin C content, especially in goats fed with MOD, where vitamin C can be absorbed and circulates freely in plasma to enter into all tissues and body fluids (Iqbal et al., 2004). *Moringa oleifera* and *peregrina* leaves are rich in vitamins, Fe, K and Ca when compared to other leafy forages such as *Trifolium alexandrinum* (Berseem). (Khalel et al., 2014; Babiker et al., 2018), and these nutrients can quickly be absorbed and improve milk quality (Mendieta-Araica et al., 2011; Nouman et al., 2014). Goats' milk is deficient in vitamin C, but partial inclusion of *M. oleifera* or *M. peregrina* in their diet can result in the production of milk rich in vitamin C. Similar increase in vitamin C was observed when Najdi ewes were fed similar diets (Babiker et al., 2016).

Serum glucose level of goats fed *M. oleifera* diet was within the normal range (50–75 mg/100 mL) required for a healthy goat (Pal et al., 2010). Partial inclusion of *M. oleifera* in the goats' diet significantly ($P \leq .05$.) lowered the serum glucose level compared to those fed the AHD or MPD. *Moringa oleifera* is known as one of the highly potential antidiabetic plants possibly potentiating the insulin action (Farooq et al., 2007). The significant decrease in serum cholesterol level in samples from goats fed MPD or MOD may have resulted from functional effects associated with phenolic and saponin contents. Saponins and antioxidants can reduce cholesterol synthesis and its absorption (Saxena et al., 2013). The herein presented results are consistent with

Table 4
Effect of diet treatments on milk and serum oxidative status, vitamin C, cholesterol and glucose concentrations of Aardi goats.

Parameters	Milk			Serum		
	AHD	MPD	MOD	AHD	MPD	MOD
TBARS (nmol/ml MDA)	$8.73^{b} \pm 1.84$	$6.73^{a} \pm 1.62$	$6.82^{a} \pm 1.29$	$3.47^{a} \pm 0.21$	3.07a ± 0.46	$2.04^{b} \pm 0.31$
TAC (mmol/l)	$10.61^{c} \pm 0.79$	$12.05^{b} \pm 0.76$	$14.82^{a} \pm 5.72$	$26.47^{c} \pm 0.55$	$28.37^{b} \pm 0.57$	$29.67^{a} \pm 0.49$
Catalase (nmol/min/ml)	$50.97^{c} \pm 5.41$	$51.98^{b} \pm 5.72$	$54.27^{a} \pm 5.10$	$10.17^{b} \pm 0.23$	$10.60^{b} \pm 0.31$	$12.82^{a} \pm 0.86$
Vitamin C (mg/100 ml)	$1.50^{b} \pm 0.22$	$2.54^{b} \pm 0.81$	$3.69^{a} \pm 0.24$	$0.88^{c} \pm 0.12$	$1.70^{b} \pm 0.39$	$2.73^{a} \pm 0.65$
Cholesterol (mg/100 ml)	–	–	–	$65.51^{a} \pm 3.81$	$55.48^{b} \pm 1.72$	$54.87^{b} \pm 3.42$
Glucose (mg/100 ml)	8-	–	–	$85.58^{a} \pm 3.72$	$79.96^{b} \pm 5.46$	$68.37^{c} \pm 4.23$

Means (triplicate samples) within the same row with different superscript letter(s) significantly differ at P ≤ .05, according to Duncan multiple range test. AHD: alfalfa hay diet; MPD: *Moringa peregrina* Forssk. diet; MOD: *Moringa oleifera* Lam. diet; TBARS: thiobarbituric acid reactive substances; TAC: total antioxidant capacity.

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the serum cholesterol reducing effects of *M. oleifera* diets reported by Astuti et al. (2012). Cholesterol synthesis may occur in the small intestine or the liver, where glucose acts as a precursor (Iqbal et al., 2012). Thus, *M. oleifera* diet induced low serum glucose level in the goats may be responsible for the observed low serum cholesterol level. The reported results from this study on serum glucose and cholesterol values for goats fed the tested diets, corresponds with report on Najdi ewes (Babiker et al., 2016). Therefore, partial replacement of alfalfa hay with *M. oleifera* in goats' diet will help to reduce the serum glucose level and consequently serum cholesterol level of the goats.

Based on the obtained results, we conclude that incorporating 25% *M. oleifera or peregrina* leaves into the goat diet significantly improved the oxidative status of milk and serum by improving the content of vitamin C, total antioxidant capacity, and catalase activity and reducing the thiobarbituric acid reactive substance (TBARS) concentration. These positive results suggest that inclusion of *M. oleifera or peregrina* leaves as fodder crops substitute in animal feed, especially in dry areas such as Saudi Arabia where the growth of alfalfa is difficult due to water scarcity, may improve both animals and consumers oxidative status and immunity through the transfer of antioxidants and vitamin C from fodder to milk.

Conflict of interest

The authors declare that they have no conflict of interest.

Author approval

All authors contributed equally to the work equally and all support its submission to your journal (SAJB).

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