



Effect of Moringa-Supplemented Pelleted Diets on the Blood Oxidative Status and Vitamin C, Cholesterol and Glucose Concentrations of Ewes and Goats

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ABSTRACT

The present study examined changes in the oxidative state and the concentrations of vitamin C, glucose, and cholesterol in the serum of ewes and goats fed on alfalfa hay-based diet (AHD) supplemented with 25% *Moringa oleifera* (MOD) or *Moringa peregrine* (MPD) leaves. Thirty ewes (2 years old and 50-60 kg BW) and 30 goats (2 years old and 35-40 kg BW) were randomly allocated to 3 experimental groups, consisting of 10 ewes and 10 goats each. The 3 experimental groups of either ewes or goats were fed on one of the experimental diets (AHD, MOD, or MPD) for 6 weeks. Blood samples were collected every 2 weeks throughout the experimental period. Vitamin C concentration and the total antioxidant capacity of serum increased by > 33% in animals fed on *Moringa*-supplemented diets. Dietary supplementation with *Moringa* enhanced serum catalase activity by > 9% as a function of feeding period. Animals fed on *Moringa*-supplemented diets also showed reduced serum concentration of malondialdehyde (MDA) by > 45% compared with AHD-fed animals. Reductions in the serum concentrations of glucose by 14 to 41%, and that of cholesterol by 18 to 40% were observed in animals fed on *Moringa*-supplemented diets. It is worth mentioning that MOD supplementation showed the greatest positive effect on all tested blood parameters. The results of the present study indicate the importance of dietary supplementation of *Moringa* on the general animal health and immunity, which might be reflected in the improvement of shelf life of animal products (milk or meat).

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Authors' Contribution

EEB and FAJ were responsible for experimental designing, supervision of the project and paper edition. RSA and AAA-H contributed to execution of the experiment, statistical analysis and the paper edition. KAA and KG contributed to execution of the experiment, paper editing and final corrections.

Key words

Alfalfa hay, *Moringa oleifera*, *Moringa peregrine*, Small ruminants, Antioxidant

INTRODUCTION

In recent years, there has been an increased demand for healthy, nutritious, and appetizing foods. Under normal physiological conditions and during normal cell metabolism, a balance exists between the production of free radicals and endogenous antioxidant capacity that prevents free radicals from accumulating and disrupting normal cell functions. However, the quantity of free radicals produced can surpass cellular antioxidant capacities and cause oxidative stress (Weiss, 1998). High-producing dairy animals are susceptible to oxidative stress, which can be aggravated under certain environmental, physiological, and dietary conditions (Castillo *et al.*, 2005). Although oxidative stress may not be demonstrated clinically, it can have severe consequences on immune system functions, as it

contributes to increased incidences of postpartum diseases, including mastitis leading to lower milk quality (Castillo *et al.*, 2013). Feeding animals with diets rich in antioxidants can improve lipid metabolism and oxidative status.

Dietary intake of dried oregano plants was reported to positively affect some enzymatic and non-enzymatic antioxidant defences in goat milk and blood as well as the antioxidant capacity of the milk (Paraskevakis, 2015).

Moringa is a non-leguminous, fast growing, multipurpose tree and is characterised by its high economic importance and broad industrial and feed values. The tree is widely grown in dry tropical areas of the Middle East and Africa (Palada *et al.*, 2007). Furthermore, *Moringa* is characterised by its high protein and mineral content, trace amounts of anti-nutritional factors and ability to improve microbial protein synthesis in the rumen (Soliva *et al.*, 2005; Mendieta-Araica *et al.*, 2011; Nouman *et al.*, 2014). *Moringa oleifera* contains natural antioxidants such as vitamin C, tocopherols, flavonoids, and other phenolic compounds (Laandrault *et al.*, 2001; Iqbal and Bhangar,

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2006). These might help in controlling oxidative stress and improving animal health and immunity. Therefore, the main objective of this study was to determine the effect of supplementing alfalfa hay-based diet (AHD) with *M. oleifera* or *Moringa peregrine* on the oxidative status and serum concentrations of vitamin C, cholesterol, and glucose in Najdi ewes and Aardi goats.

MATERIALS AND METHODS

Materials

Fresh *M. oleifera* and *M. peregrine* leaves were manually harvested from mature trees (3–4 months old) at Durat-elizdihar Agricultural Farm, Gazan, Saudi Arabia. The leaves were air-dried under partial shade for 72 h by spreading on clean plastic sheets and turning them over several times. The dried leaves were ground to a fine powder and mixed with other feed ingredients into pelleted diets.

Animal management

Two-year-old Najdi ewes (30) with an average live body weight of 50–60 kg and 2-year-old Aardi goats (30) with an average live body weight of 35–40 kg were randomly allocated into 3 experimental groups with 10 ewes and 10 goats each. All animals were at the same stage of their second lactation. The animals were kept in semi-open sheds at AlKhalidiya Farm, Riyadh, KSA (latitude 24°23'22" N and longitude 45° 53'55" E). The experimental period consisted of 2 weeks of adaptation, followed by 6 weeks of data collection. The animals were fed ad libitum on one of the following pelleted experimental diets: Alfalfa hay based diet (AHD), *M. peregrine* supplemented diet (MPD), or *M. oleifera* supplemented diet (MOD). The feed ingredients of the experimental diets are shown in Table I.

All procedures described in this experiment were approved by the Faculty Research Ethics Committee at King Saud University (KSU-SE-21-19).

Blood sampling and serum analysis

Blood samples were collected at the beginning of the experiment (week 0) and every 2 weeks during the experimental period by jugular venipuncture into plain vacutainer 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 –20°C for further analyses. The total antioxidant capacity (TAC) and catalase activity of the serum samples were measured using an Antioxidant Assay Kit (Cayman, USA) and a Catalase Assay Kit (Cayman, USA), respectively. Malondialdehyde (MDA) and vitamin

C concentration in serum samples were measured using a thiobarbituric acid reactive substance (TBARS) assay kit (Cayman, USA) and an ascorbic acid colorimetric assay kit (BioVision, USA), respectively. The concentrations of glucose and cholesterol in serum samples were measured using Randox assay kit (Randox, Laboratories Ltd., UK).

Table I. Feed ingredients of the pelleted experimental diets.

Feed ingredient (%)	AHD	MPD	MOD
Alfalfa hay	40	15	15
M. peregrine	-	25	-
M. oleifera	-	-	25
Corn	25	25	25
Barley	21.8	17.9	27.6
Soya bean meal	10.2	14.1	4.4
Na Cl	1.0	1.0	1.0
Limestone	1.0	1.0	1.0
Na HCO ₃	0.8	0.8	0.8
Vitamin-mineral premix*	0.2	0.2	0.2

AHD, alfalfa hay based diet; MPD, *M. peregrine* supplemented diet; MOD, *M. oleifera* supplemented diet. *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; CoSO₄, 0.30 g; CuSO₄, 20.10 g; FeSO₄, 10.00 g; ZnO₂, 50.00 g; MnSO₄, 40.20 g; KI, 0.75 g.

Experimental design and statistical analysis

The data of each parameter were expressed as the mean of independent samples. Statistical comparisons of the results were performed via ANOVA using SPSS version 20. Significant differences ($P < 0.05$) between means were analysed by Duncan's Multiple Range Test according to Bryman and Cramer (2012).

RESULTS AND DISCUSSION

Serum vitamin C concentration

Figure 1 shows changes in serum vitamin C concentrations of ewes and goats fed on the experimental diets for 6 weeks. Although, serum vitamin C concentration of ewes and goats fed on MPD tended to increase, those animals fed on MOD exhibited a significant ($P < 0.05$) increase in serum vitamin C concentration reaching a level of 3.47 mg/100 ml in ewes (Fig. 1A) and 2.87 mg/100 ml in goats (Fig. 1B). *Moringa* leaves are rich in nutrients such as multivitamins which are essential for livestock growth and milk production (Mendieta-Araica *et al.*, 2011). Further, the nutrient absorption from *Moringa* leaves is far better than that from other leafy vegetables (Nouman *et al.*, 2014). Hence, this might be the reason

for the higher serum vitamin C concentration observed in ewes and goats fed on *Moringa*-supplemented diets, particularly MOD. As an antioxidant, vitamin C works as an electron donor by transferring an electron to the Cu compounds (Padayatty *et al.*, 2003). Vitamin C is an essential phytonutrient that contributes to the reduction of oxidative stress which occurs during metabolism of living cells. Diets supplemented with vitamin C-rich feed have been reported to increase the total antioxidant capacity and lower the concentration of active forms of thiobarbituric acid in the serum of goats (Abd-el-Rahman *et al.*, 2015).

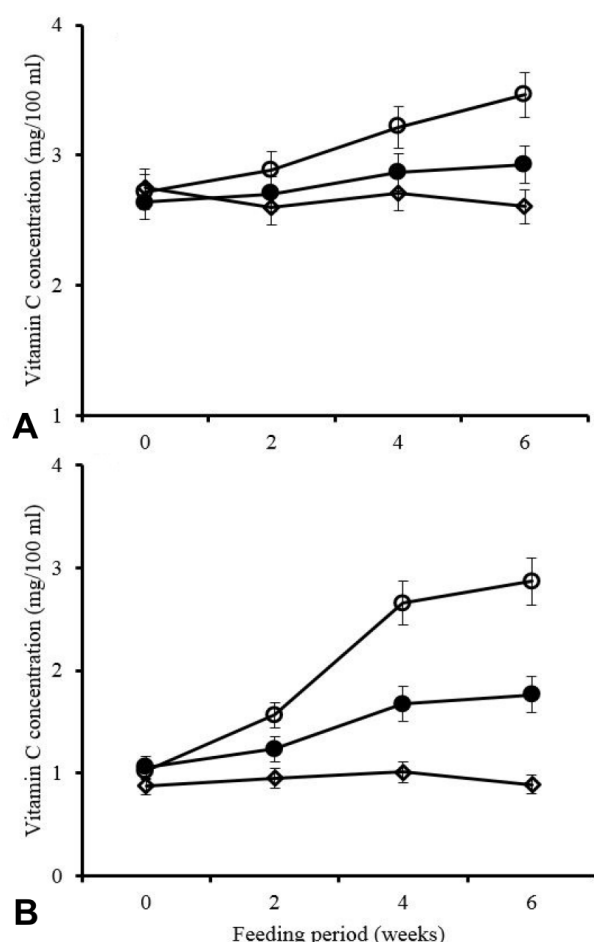


Fig. 1. Effect of *Moringa* supplemented pelleted diet on changes in serum vitamin C concentration of ewes (A) and goats (B) fed on alfalfa hay based diet (◇), diet supplemented with 25% *Moringa oleifera* (○) or *Moringa peregrine* (●) for six weeks.

Serum total antioxidant capacity (TAC)

Figure 2 shows changes in serum TAC of ewes and goats fed on MPD or MOD for 6 weeks. The measure of

TAC represents the cumulative quantity of all antioxidants present in plasma and body fluids, thus providing an integrated parameter rather than a simple summation of measurable antioxidants (Ghiselli *et al.*, 2000). Slight variations in serum TAC were observed prior to feeding of experimental diets in both ewes and goats (Fig. 2). Ewes fed on MOD (Fig. 2A) showed significantly ($P < 0.05$) higher serum TAC (19.64 mM/L) than did those fed on AHD (12.89 mM/L) and MPD (15.55 mM/L). Likewise, goats fed on MOD (Fig. 2B) showed significantly ($P < 0.05$) higher serum TAC (29.98 mM/L) than did those fed on AHD (26.23 mM/L) and MPD (28.56 mM/L). Serum TAC of ewes showed a progressive decline with the feeding period; however, the rate of decline was lower in ewes fed on MOD or MPD than in those fed on AHD (Fig. 2A). These results are in contradiction with the results of an earlier study reported by El-Far *et al.* (2014), in which serum TAC of ewes had increased with the feeding period of antioxidant-rich diet. However, serum TAC of goats fed on AHD showed a fluctuating pattern with the feeding period. Those fed on MOD or MPD exhibited a slight increase, with those fed on MOD exhibiting the highest value. The TAC results reported in this study are consistent with those of the previous studies that focused on antioxidant-rich diets (Abou-Zeina *et al.*, 2015). The significant increase in serum TAC observed in animals fed on *Moringa*-supplemented diets indicated the ability of MOD and MPD to improve the antioxidant status of animals. Interestingly, *Moringa oleifera* leaves have been reported to possess potent antioxidant activity against free radicals thus providing significant protection against oxidative damage (Sreelatha and Padma, 2009). Therefore, supplementation of diets of small ruminants with *Moringa* leaves, especially leaves of *M. oleifera* can improve serum oxidative status and consequently the immunity and health of the animals.

Serum catalase activity (CA)

Changes in serum CA of ewes and goats fed on the experimental diets for 6 weeks are shown in Figure 3. There was a slight variation in serum CA at the beginning of the experiment between groups fed different diets (week 0). Serum CA of ewes showed a slight progressive reduction with the feeding period. However, the decline rate was slower in ewes fed on MOD and MPD than in those fed on AHD. Serum CA of ewes at the end of the experimental period was 26.75, 24.09 and 20.88 nmol/min/L for MOD, MPD and AHD, respectively (Fig. 3A). On the other hand, serum CA of goats showed a progressive increase with feeding period for all experimental diets, with final values of 13.32, 11.99 and 10.42 nmol/min/L for MOD, MPD and AHD, respectively (Fig. 3B). The amino acid composition

of *M. oleifera* leaf fulfils the protein needs of the animal and boosts its immune system against diseases (Brisibe *et al.*, 2009). This might have led to enhanced production and activity of endogenous enzymes (such as catalase). With the progression of feeding period, serum CA of ewes decreased while that of goats increased for all experimental diets. AHD-fed ewes exhibited the highest rate of decline in serum CA, while goats showed the lowest rate of increase. The observed progressive increase in serum CA with feeding period in goats fed on diets supplemented with *Moringa* is consistent with previous report on goats fed on a diet rich in Greek oregano (Paraskevakis, 2015). Catalase is responsible for the detoxification of H_2O_2 (Paraskevakis, 2015). Therefore, the observed progressive increase in serum CA of goats could be due to the higher production of H_2O_2 , while the observed progressive reduction in serum CA of ewes could be attributed to the enzyme depletion or inhibition by increased production of free radicals (Gupta *et al.*, 2007).

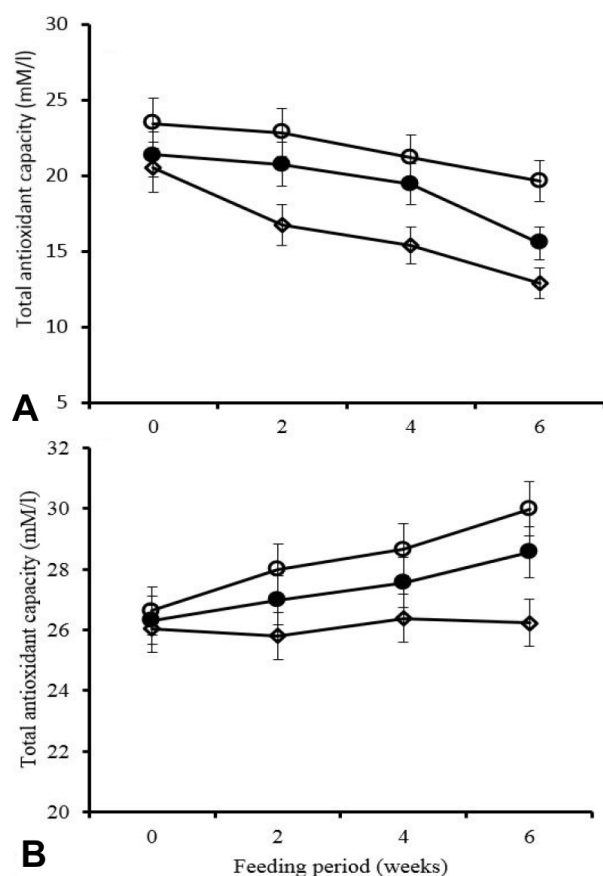


Fig. 2. Serum total antioxidant capacity of ewes (A) and goats (B) fed on alfalfa hay based diet (◇), diet supplemented with 25% *Moringa oleifera* (○) or *Moringa peregrine* (●) for six weeks.

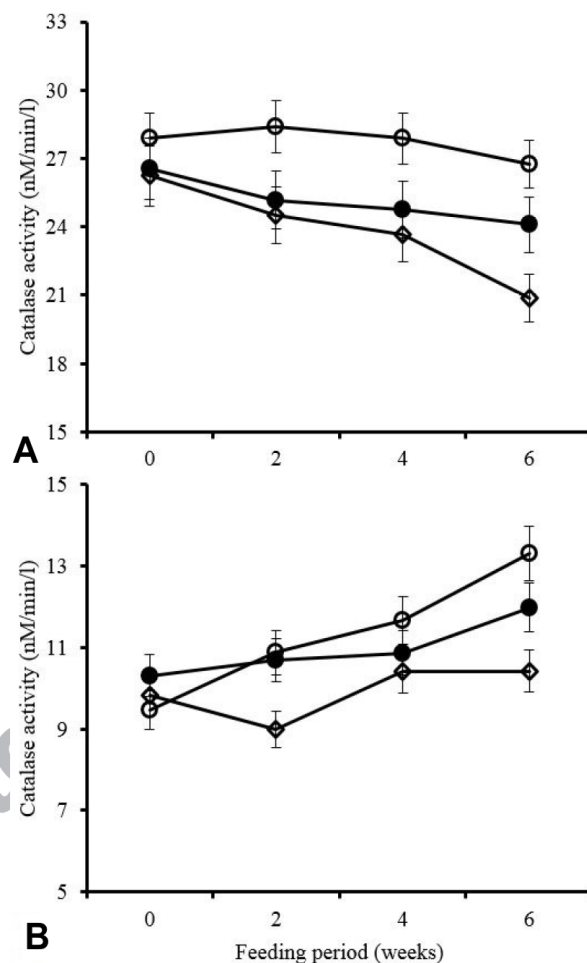


Fig. 3. Serum catalase activity of ewes (A) and goats (B) fed on alfalfa hay based diet (◇), diet supplemented with 25% *Moringa oleifera* (○) or *Moringa peregrine* (●) for six weeks.

Serum MDA concentration

Serum MDA concentration of ewes and goats fed on the experimental diets for 6 weeks is presented in Figure 4. At the beginning of the experiment (week 0), serum MDA concentration did not show any significant ($P < 0.05$) variation, and ranged from 4.30 to 5.09 nM and 3.00 to 3.10 nM for ewes and goats, respectively. During the experimental period, *Moringa*-supplemented diets significantly ($P < 0.05$) reduced serum MDA concentration in both ewes and goats, with MOD having the greatest effect. Serum MDA concentration in ewes had reduced from 3.31 nM when supplemented with AHD to 1.00 nM when supplemented with MOD (Fig. 4A), and from 3.95 nM when supplemented with AHD to 2.56 nM when supplemented with MOD in goats (Fig. 4B). The detection of oxidative stress depends on the quantification

of compounds such as MDA, which are formed by the degradation of products attacked by free radicals (Grotto *et al.*, 2009). Determination of thiobarbituric acid reactants (TBARS) is a widely used method for investigating overall lipid peroxidation (Antolovich *et al.*, 2002). *Moringa*-supplemented diet induced reduction in serum MDA concentration reported in this study is consistent with the findings reported by El-Far *et al.* (2014) on ewes fed on diets supplemented with *Nigella sativa* and *Zingiber officinale*. Supplementation of animals' diets with antioxidant-rich plants, such as *Moringa*, increases their antioxidative capacity and significantly influences animal oxidative metabolism. *M. oleifera* has been known for its higher total phenolic content and antioxidant activities compared with those in other plants such as spinach, cabbage, and broccoli (Pakade *et al.*, 2013). Furthermore, it has been reported that *Moringa* leaf extract suppresses the initiation and propagation of lipid peroxidation, and because of its phenolic content, may help suppress atherosclerosis by scavenging hydrogen oxide radicals (Chumark *et al.*, 2008). Therefore, supplementation of AHD with *M. oleifera* helped scavenge free radicals thereby improving the oxidative status and general health of both, ewes and goats and resulting in the observed significant ($P < 0.05$) decrease in serum MDA concentration. The reduction in serum MDA concentration reported herein could also be attributed to the higher serum vitamin C levels, antioxidant capacity, and catalase activity in ewes and goats fed on diets supplemented with *Moringa* than in those fed on AHD.

Serum glucose and cholesterol concentrations

Serum concentrations of glucose and cholesterol in ewes and goats fed on the experimental diets for 6 weeks are presented in Figure 5. At the beginning of the experiment (week 0), serum glucose and cholesterol concentrations were almost similar in all treatment groups. Serum glucose concentrations obtained at the beginning of the experiment (week 0) in both ewes and goats were within the normal range (50–75 mg/100 ml), and similar to those reported by Abdulaziz (2012) for ewes and goats. *Moringa*, particularly *M. oleifera*, supplementation in the diet of ewes and goats for 6 weeks significantly ($P < 0.05$) lowered serum glucose concentration from 60.09 mg/100 ml to 54.99 mg/100 ml (Fig. 5A) and 60.04 mg/100 ml to 50.00 mg/100 ml (Fig. 5B), respectively. A progressive decline in serum glucose concentration in both, ewes and goats fed with *Moringa*-supplemented diets was observed, while a progressive increasing trend was observed for those fed on AHD (Fig. 5). *Moringa* is potentially anti-diabetic because of its ability to inhibit α -amylase activity, which in turn regulates the amount of glucose released into

blood stream (Farooq *et al.*, 2007). It has also been reported that *Moringa* leaves significantly decreased blood glucose concentration in type 2 diabetes modelled Wistar rat and Goto-Kakizaki rat (Ndong *et al.*, 2007). Therefore, the observed reduction in serum glucose level in animals fed on *Moringa*-supplemented diets compared with the levels in their counterparts fed on AHD could be a consequence of the anti-diabetic effects of *Moringa*.

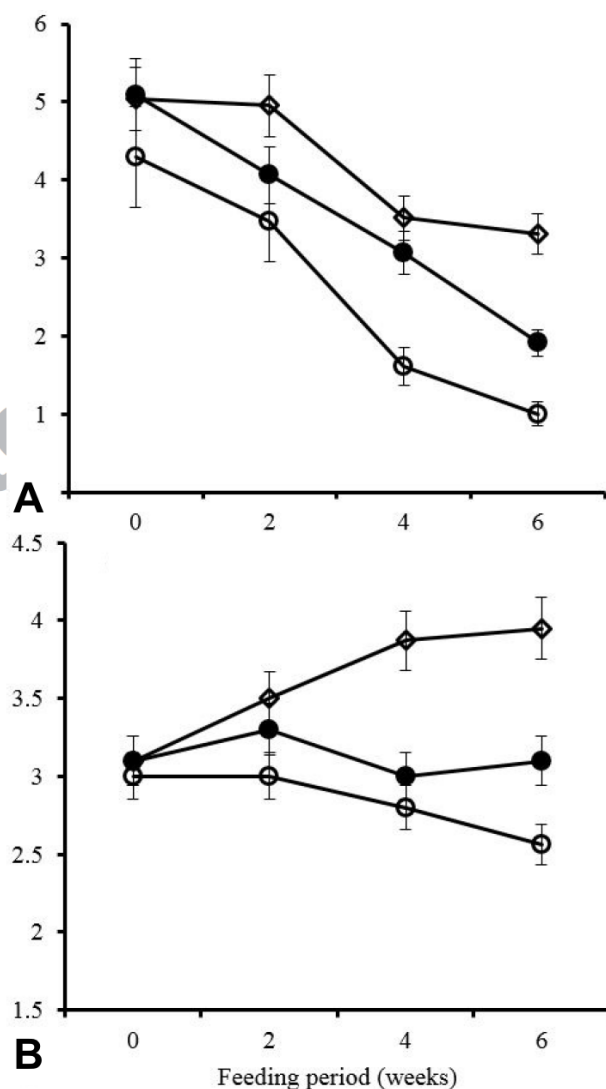


Fig. 4. Serum malondialdehyde (MDA) concentrations of ewes (A) and goats (B) fed on alfalfa hay based diet (◇), diet supplemented with 25% *Moringa oleifera* (○) or *Moringa peregrine* (●) for six weeks.

Figure 5 shows that ewes and goats fed on *Moringa*-supplemented diets for 6 weeks had a significantly ($P < 0.05$) lower serum cholesterol concentration (49.40

mg/100 ml and 41.78 mg/100 ml, respectively) than did their counterparts fed on AHD (60.68 mg/100 ml and 68.58 mg/100 ml, respectively). This effect could be attributed to the high phenolic content and antioxidant action of *Moringa* leaves, since phytochemicals and antioxidants can lower the synthesis and absorption of cholesterol (Saxena *et al.*, 2013). In addition, *Moringa* leaves are known for their bioactive phytoconstituent (β -sitosterol), which has been reported to have a cholesterol lowering effect in high-fat diet-fed rats (Ghasi *et al.*, 2000). Glucose is known as one of the precursors of cholesterol synthesis in the small intestine and liver (Iqbal *et al.*, 2012). Thus, the lower serum glucose concentrations reported herein in ewes and goats fed on diets supplemented with *Moringa* could be responsible for the observed lower serum cholesterol concentrations.

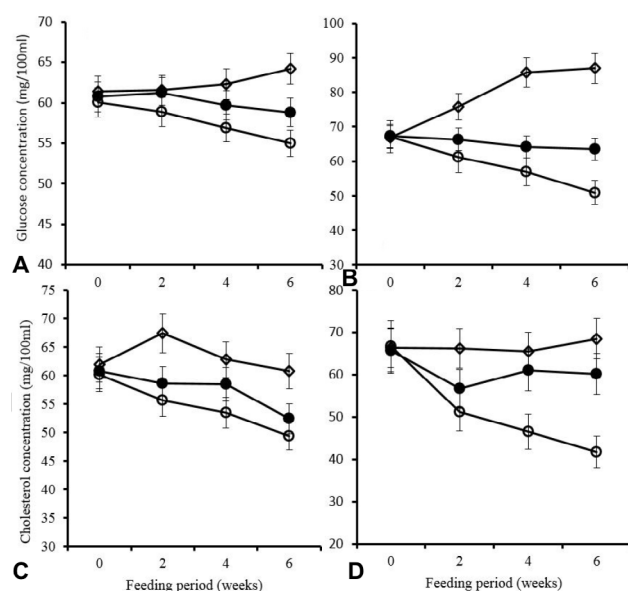


Fig. 5. Serum glucose and cholesterol concentrations of ewes (A, C) and goats (B, D) fed on alfalfa hay based diet ($\diamond$), diet supplemented with 25% *Moringa oleifera* ($\circ$) or *Moringa peregrina* ($\bullet$) for six weeks.

CONCLUSIONS

The obtained results suggest that incorporating *Moringa* leaves in small ruminants' diets at a level of 25% significantly improved the serum oxidative status by enhancing vitamin C content, total antioxidant capacity and catalase activity, along with the reduction of malondialdehyde concentration. Furthermore, serum glucose and cholesterol levels showed a significant progressive reduction with *Moringa*-supplemented diets

as a function of feeding period. Thus, partial inclusion of *Moringa* leaves as a non-traditional fodder at a level of 25% in small ruminants' diets could play a crucial role in promoting animal immune systems and improving general animal health and production.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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