Research Article

Effects of *Moringa oleifera* on Follicular Development, Blood and Metabolic Profiles of Subtropical Ewes during Peripartum

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Abstract | *Moringa oleifera* leaves supplementation has positive effects on productive and reproductive performances in animals and humans as well. The current study aimed to overcome the negative impact of the peripartum period on ovarian follicular development, and blood and metabolic profiles with *Moringa oleifera* (*M. oleifera*) supplementation. Fifteen pregnant Naimi ewes (49.0 ± 1.85 kg body weight) were allocated to three groups: control and two *M. oleifera* treated groups (50.0 and 100.0 g *M. oleifera* /kg diet). Body weights, milk composition, blood cell counts (red and white blood cells), and metabolic profile (total protein and blood urea nitrogen) were measured and evaluated. The ovarian follicular wave dynamics and corpora lutea (CL) development were followed. The results indicated that body weights significantly (P < 0.05) improved in ewes and lambs treated with *M. oleifera*. *M. oleifera* supplementation increased significantly (P < 0.05) fat (%) and milk energy (MJ/kg) whereas it lowered solid not-fat and lactose (%). Red blood cells (RBCs) and white blood cells (WBCs) during pre-and post-partum periods showed significant increases (P < 0.05) with *M. oleifera* supplementation. Total protein and blood urea nitrogen values were improved significantly (P<0.05) in *M. oleifera* treated groups. Moreover, the number of variable size follicles, as well as the number and diameter of CL were increased due to *M. oleifera* supplementation. It could be concluded that *M. oleifera* supplementation (50.0 and 100.0 g/kg diet) from eight weeks pre-partum to eight weeks post-partum of Naimi ewes improved the negative effects of the transition period of pregnant ewes.

Keywords | *M. oleifera*, milk, body weight, follicles, blood, metabolic

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INTRODUCTION

The peripartum stage which is known as the transition period is the most critical for the immediate and long-term performance of pregnant animals and their deliveries. This period in small ruminant species extends from three weeks pre-partum to three weeks post-partum (Tharwat et al., 2013). The decrease and disruptions of ovarian follicles growth and development and the quality of their containing oocytes, growth performance, and feed digestibility, metabolites, and hormones' profiles were observed during this period (Bragança et al., 2020; Ali et al., 2021). In addition, the decrease in feed intake during the pre-partum period can be reached up to 30-35%. Hence, restoration of such disruptions during the transition period would positively improve the productive and reproductive performances of small ruminant species.

Several strategies were suggested to alleviate the negative impacts of the transition period in small ruminant animals including diet formulation and additives (Hussein et al., 2015; Ali et al., 2021; Al-Mufarji and Mohammed, 2022; Al-Mufarji et al., 2022). Because of the negative influences of increased protein and urea in the diets on animals' fertility and health (McEvoy et al., 1997; Dawuda et al., 2002; Mohammed and Attaai, 2011; Mohammed et al., 2011, 2012) and the high cost of diets, it is important to search for useful supplements for productive and reproductive purposes (Senosy et al., 2017, 2018; Cherif et al., 2018; Mohammed, 2022). It has been observed that there is an increasing interest in the use of *M. oleifera* as feed supplements for ruminant animals (Al-Mufarji and Mohammed, 2022; Al-Masruri et al., 2022).

Several studies have been carried out to investigate the impact of *M. oleifera* on nutrient digestibility, blood and metabolic profiles, growth, and reproductive performances (Kholif et al., 2016, 2019; Al-Mufarji and Mohammed, 2022; Al-Masruri et al., 2022). *M. oleifera* supplementation in animals resulted in an improvement in growth performance (Paul et al., 2013; Warastomo et al., 2021; Fadiyimu et al., 2010, 2016, 2017; Abdel-Raheem and Hassan, 2021; Pandey et al., 2022), changes in milk production and composition (Kholif et al., 2016; 2019), blood profiles (RBCs, PCV, Hb, WBCs), plasma metabolites (total protein, albumin, blood urea nitrogen) (Fadiyimu et al., 2010, 2016, 2017; Meel et al., 2018), and reproductive performance (Barakat et al., 2015; Ajuogu et al., 2019). Little information was available on the effects of *M. oleifera* on blood and metabolic profiles, ovarian follicles, and CL development of Naimi ewes during the transition period in subtropics. Hence, we hypothesize that supplementation of *M. oleifera* would positively alter the blood, metabolic and biochemical status of Naimi ewes during the transition period and therefore improve productive and reproductive performances in subtropics.

MATERIALS AND METHODS

The experimental procedures were approved by the Ethical Clearance of the deanship of scientific research, the vice presidency for graduate studies and scientific research, King Faisal University, Saudi Arabia (Ref. No. KFU-REC-2022-JUN-EA1016). This experiment was carried out in the Research and Training Station of King Faisal University for 16 weeks.

Experimental design and animal management

Fifteen healthy pregnant Naimi ewes of 49.50 ± 1.85 kg and 2.5-3.0 years were randomly allocated into three equal groups (five animals per group) as control and two *M. oleifera* treated groups (50.0 and 100.0 g *M. oleifera*/ kg diet). The ewes were bred in semi housed system in a standard pen at a stocking rate of 1.75 m^2/ewe. The ewes were kept free inside the pen and given the routine vaccination of farm station. The ewes were daily fed one kg basal concentrate diet for the control group and a basal concentrate diet supplemented with 50.0 and 100.0g *M. oleifera* per head, as well as *ad-libitum* berseem hay. The given supplemented levels of *M. oleifera* were chosen according to a preliminary study for three weeks and a previous study (Ajuogu et al., 2019; Afzal, 2021; Al-Mufarji et al., 2022). The concentrate diet was offered twice at 08:00 am and 4:00 pm. Ewes were given access to drinking water *ad-libitum*. Body weights (kg) of ewes were recorded at 8 and 4 weeks pre-parturition and 4 and 8 weeks post-parturition whereas body weights (kg) of lambs were at lambing, 2, 4, 6, and 8 weeks of the study.

Collection and chemical analyses of milk samples

Three milk samples (50 mL) of control and *M. oleifera* treated ewes were collected biweekly in 100ml flasks for determination of protein, lactose, solids not-fat, fat, and ash (%) (MilkoScan™ Mars - Analyzer for milk). Milk energy was calculated using the equation (Economides 1986) as follows:

Calorific value (MJ/kg) = 1.94 + 0.43 x (where: x = fat %)

Blood collection and analysis

Five blood samples were collected of each ewe from control and *M. oleifera* treated groups every four weeks (8 and 4 weeks pre-parturition and 4 and 8 weeks post-parturition). The obtained blood samples were collected from jugular venipuncture and analyzed for hematological and chemical parameters through hematology analyzers (Abaxis Vetscan HM5) and chemistry analyzers (Skyla VB1; http://www.skyla.com/page/about/index.aspx?kind=103). The measured hematological parameters included red blood cells (RBCs, 10^{12}/L) and white blood cells (WBCs, 10^9/L). The presented plasma parameters included total protein (TP, g/dl) and blood urea nitrogen (BUN, mg/dl).

Ovarian and corpus luteum development

Ewes were investigated post-partum on days 3, 6, 9, 12, 15, and 18 days using an ultrasound scanner (Pie Medical, 100 LC, Holland). The ovaries of control and *M. oleifera* treated ewes were investigated for the ovarian follicular and corpora lutea waves and development. The numbers and sizes of follicles and corpora lutea were recorded. The

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ovarian follicles were categorized into small (2–2.9 mm), medium (3–4.9 mm), and large-sized follicles (≥ 5 mm). The numbers of CL were counted and the diameters were measured (Senosy et al., 2017, 2018).

Statistical analysis

Values of ovarian follicles and corpora lutea, body weights, milk chemical composition, red and white blood cells, total protein, and blood urea nitrogen of control and *M. oleifera* treated ewes (50.0 and 100.0 g *M. oleifera*/kg diet) are presented as mean. The values of *M. oleifera* treated and control groups were statistically analyzed using the General Linear Model (GLM) procedure of SAS (SAS, 2008) according to the following model:

$$Yij = \mu + Ti + eij$$

Where: μ = Mean, Ti = Effects of *M. oleifera* and eij= Standard error.

Duncan's Multiple Range Test (1955) was used to compare the means of the control and the two *M. oleifera* treated groups (50.0 and 100.0 g *M. oleifera* /kg diet).

RESULTS AND DISCUSSION

The effects of *M. oleifera* supplementation (50 and 100 g/kg diet) from 8 weeks pre-partum to 8 weeks postpartum period on body weights, milk composition, red and white blood cells, total protein and blood urea nitrogen, ovarian follicle dynamics, and CL development are presented in (Tables 1, 4 and Figures 1, 2). The results indicated the restoration of the negative effects of the peripartum period on body weights, metabolites, earlier ovarian follicle development reproduction, and through *M. oleifera* supplementation.

The solid not-fat and lactose values (%) were decreased significantly (P < 0.05) whereas fat (%) and energy (MJ/kg) values were increased in *M. oleifera* treated groups compared to the control group as recorded by Kholif et al. (2016, 2019); Choudhary et al. (2018). Furthermore, the recorded body weights of ewes (8 and 4 weeks pre-parturition and 4 and 8 weeks post-parturition) and lambs (2, 4, 6, and 8 weeks post-parturition post-parturition) were significantly higher (P < 0.05) in *M. oleifera* treated groups compared to control group. The potential properties of *M. oleifera* as a source of protein and fatty acids profiles have been confirmed according to Al-Mufarji and Mohammed (2022); Al-Masruri et al. (2022). We found that *M. oleifera* leaves contained carbohydrate (47.82%), fiber (28.35%), protein (28.28%), and fat (7.57) as indicated by (Jongrungruangchok et al., 2010; Moyo et al., 2011, 2012; Saini et al., 2014a, b). In addition, fatty acid profiles revealed saturated (3.76%), unsaturated (3.79), monounsaturated (2.39%), polyunsaturated (0.76%), and trans-fatty acids (0.64%).

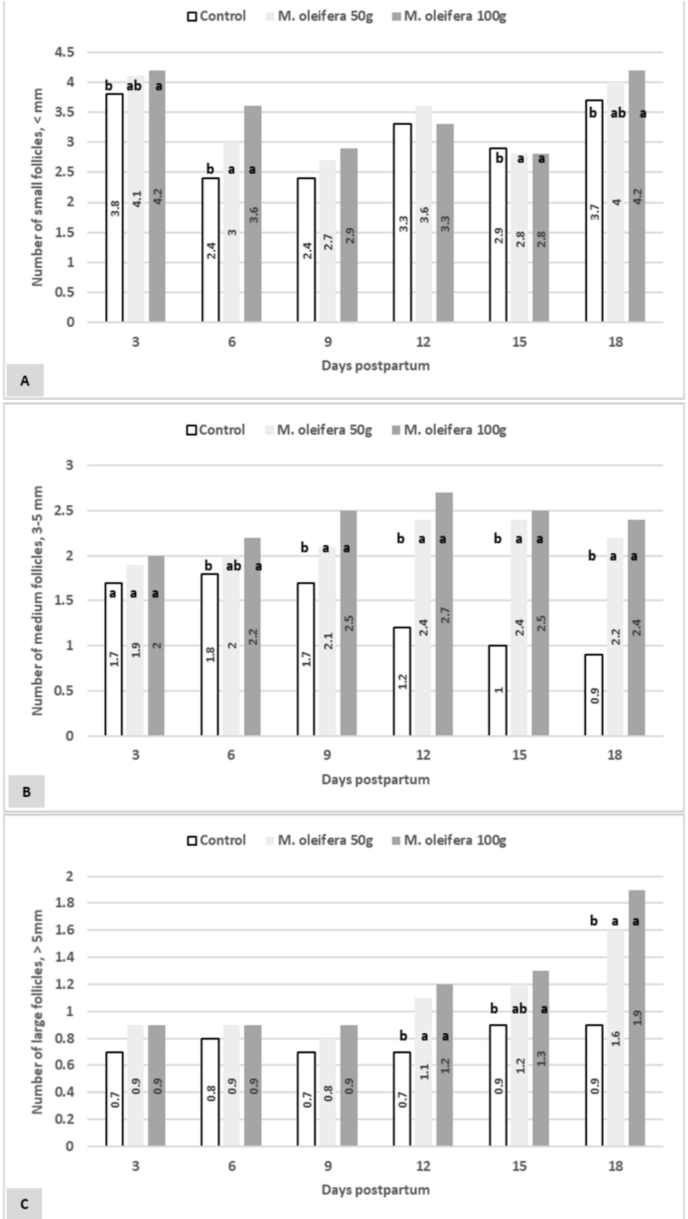

Figure 1: Effects of *Moringa oleifera* (50.0 and 100 g/kg diet) on ovarian follicle development postpartum of Naimi ewes.

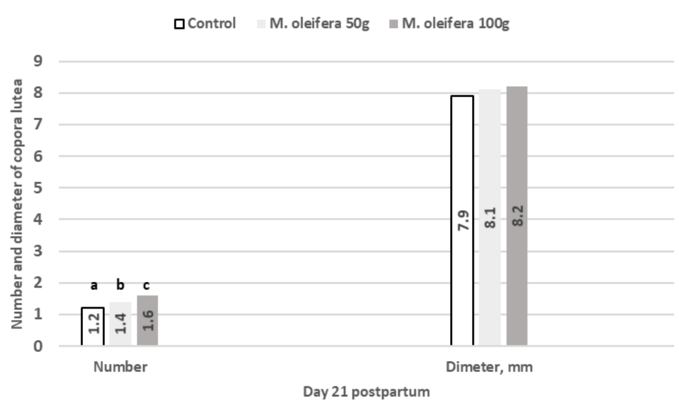

Figure 2: Effects of *Moringa oleifera* (50.0 and 100 g/kg diet) on number and diameter of corpora lutea postpartum of Naimi ewes.

Table 1: Effects of *Moringa oleifera* (50.0 and 100 g/kg diet) supplementation on milk constituents of Naimi ewes in subtropics.

Parameters	**Treatments**		
	Control	***M. oleifera* 50g**	***M. oleifera* 100g**
Solids not fat, %	$7.33^{a} \pm 0.730$	$7.06^{ab} \pm 0.70$	$6.89^{b} \pm 0.85$
Fat, , %	$3.31^{c} \pm 0.60$	$4.00^{b} \pm 0.56$	$5.54^{a} \pm 0.57$
Protein, %	2.82 ± 0.48	2.70 ± 0.56	2.76 ± 0.34
Lactose, %	$3.74^{a} \pm 0.50$	$3.19^{b} \pm 0.42$	$2.82^{c} \pm 0.46$
Milk energy content, MJ/kg	$3.36^{c} \pm 0.26$	$3.66^{b} \pm 0.24$	$3.89^{a} \pm 0.25$

$^{a, b, c}$ Values with different superscripts between groups significantly differ at $P < 0.05$.

Table 2: Effects of *Moringa oleifera* (50.0 and 100 g/kg diet) supplementation on body weight gain of ewes and lambs in subtropics.

Parameters	**Treatments**		
	Control	***M. oleifera* 50g**	***M. oleifera* 100g**
Ewes' body weight, kg	5	5	5
8-weeks pre-partum, kg	49.0 ± 1.40	48.50 ± 1.70	50.50 ± 2.47
4-weeks pre-partum, kg	$51.0^{b} \pm 1.38$	$50.60^{b} \pm 1.33$	$53.3^{a} \pm 2.17$
Parturition, kg	$45.80^{b} \pm 1.56$	$44.0^{b} \pm 1.54$	$48.8^{a} \pm 1.24$
4-weeks postpartum, kg	$47.10^{b} \pm 1.21$	$46.20^{b} \pm 1.47$	$51.20^{a} \pm 1.37$
8-weeks postpartum, kg	$48.80^{b} \pm 1.72$	$48.00^{b} \pm 1.14$	$53.20^{a} \pm 1.54$
Lambs' body weight, kg	6	7	6
Lambing	$3.56^{b} \pm 0.23$	$3.86^{ab} \pm 0.23$	$4.08^{a} \pm 0.24$
2-weeks	$5.50^{b} \pm 0.45$	$5.74^{b} \pm 0.26$	$6.38^{a} \pm 0.52$
4-weeks	$7.40^{b} \pm 0.50$	$7.70^{b} \pm 0.42$	$8.56^{a} \pm 0.41$
6-weeks	10.20 ± 0.54	10.64 ± 0.65	10.80 ± 0.25
8-weeks	12.40 ± 0.62	12.80 ± 0.67	12.90 ± 0.64

$^{a, b}$ Values with different superscripts between groups significantly differ at $P < 0.05$.

Table 3: Effects of *Moringa oleifera* (50.0 and 100 g/kg diet) supplementation on blood cells and hemoglobin values of ewes and lambs in subtropics.

Parameters	**Treatments**		
	Control	***M. oleifera* 50g**	***M. oleifera* 100g**
Red blood cells, 10^{12}/L			
8-weeks pre-partum, kg	11.70 ± 0.37	12.12 ± 0.37	12.14 ± 0.27
4-weeks pre-partum, kg	$12.10^{b} \pm 1.38$	$12.86^{a} \pm 0.34$	$12.90^{a} \pm 0.64$
Parturition, kg	$10.70^{b} \pm 0.30$	$11.94^{a} \pm 0.56$	$12.08^{a} \pm 0.70$
4-weeks postpartum, kg	$10.94^{b} \pm 0.25$	$12.52^{a} \pm 0.60$	$12.68^{a} \pm 0.57$
8-weeks postpartum, kg	$10.50^{b} \pm 0.25$	$12.92^{a} \pm 0.64$	$12.98^{a} \pm 0.46$
White blood cells, 10^{9}/L			
8-weeks pre-partum, kg	8.80 ± 0.51	9.00 ± 0.55	9.10 ± 0.46
4-weeks pre-partum, kg	$9.04^{b} \pm 0.69$	$9.40^{ab} \pm 0.26$	$9.46^{a} \pm 0.40$
Parturition, kg	$8.28^{b} \pm 0.55$	$9.12^{a} \pm 0.57$	$9.22^{a} \pm 0.57$
4-weeks postpartum, kg	$8.60^{b} \pm 0.68$	$10.04^{a} \pm 0.56$	$10.30^{a} \pm 0.34$
8-weeks postpartum, kg	$9.00^{b} \pm 0.63$	$10.54^{a} \pm 0.63$	$10.90^{a} \pm 0.46$

$^{a, b}$ Values with different superscripts between groups significantly differ at $P < 0.05$.

Supplementation of *M. oleifera* in small or large amounts contributed to improved feed efficiency, rumen fermentation, and growth performances (Paul et al., 2013; Warastomo et al., 2021; Fadiyimu et al., 2010, 2016, 2017; Abdel-Raheem and Hassan, 2021; Pandey et al., 2022). El-Hedainy et al. (2020) investigated the effects of 4 g *M. oleifera* seeds/ head for 45 days on the growth performance and body measurements of male Barki aged five months. They recorded the ability of *M. oleifera* seeds to improve the final body weights and daily gains of lambs compared to the control diet. Elghandour et al. (2017) observed significant decreases in methane (CH_4), ruminal ammonia-N, total protozoal number, and organic matter degradability with increases in CO_2 production, pH, and total bacterial number due to *M. oleifera* inclusion. the recorded modifications might explain the beneficial effects of *M. oleifera* treatment on milk composition and body weight gains.

Table 4: Effects of *Moringa oleifera* (50.0 and 100 g/kg diet) supplementation on total protein (g/dl) and blood urea nitrogen of ewes in subtropics.

Parameters	Treatments		
	Control	*M. oleifera* 50g	*M. oleifera* 100g
Total protein, g/dl			
8-weeks pre-partum, kg	7.76 ± 0.22	7.80 ± 0.19	7.74 ± 0.19
4-weeks pre-partum, kg	7.78^{b} ± 0.27	8.36^{a} ± 0.17	8.54^{a} ± 0.20
Parturition, kg	7.04^{b} ± 0.30	8.26^{a} ± 0.21	8.46^{a} ± 0.17
4-weeks postpartum, kg	7.42^{b} ± 0.24	8.46^{a} ± 0.15	8.64^{a} ± 0.27
8-weeks postpartum, kg	7.48^{b} ± 0.26	8.54^{a} ± 0.19	8.72^{a} ± 0.18
Blood urea nitrogen, mg/dl			
8-weeks pre-partum, kg	12.98 ± 0.84	13.46 ± 0.63	12.71 ± 0.45
4-weeks pre-partum, kg	13.49^{a} ± 0.94	12.15^{b} ± 0.57	12.06^{b} ± 0.70
Parturition, kg	14.11^{a} ± 0.83	12.64^{b} ± 0.55	12.62^{b} ± 0.64
4-weeks postpartum, kg	15.07^{a} ± 0.45	12.27^{b} ± 0.56	11.78^{b} ± 0.71
8-weeks postpartum, kg	15.45^{a} ± 0.64	11.71^{b} ± 0.64	11.59^{b} ± 0.50

$^{a, b}$ Values with different superscripts between groups significantly differ at $P < 0.05$.

The presented hematological parameters red blood cells (10^{12}/L), white blood cells (10^{9}/L), total protein (g/dl), and blood urea nitrogen (mg/dl) were improved in *M. oleifera* treated animals compared to control one (Tables 3, 4). The recorded values lie within the normal range for clinically-healthy small ruminants (Mohammed and Kassab, 2015; Kassab and Mohammed, 2013, 2014a, b; Kassab et al., 2017; Mohammed et al., 2021). The positive effects of *M. oleifera* on red blood cells, white blood cells, total protein, and blood urea nitrogen might be attributed to several factors including antioxidative properties, nutrient digestibility, rumen fermentation, and regulating pathways involved in the metabolism (Elghandour et al., 2017; Abdel-Raheem and Hassan, 2021; Giuberti et al., 2021; Al-Mufarji and Mohammed, 2022; Al-Masruri et al., 2022). Al-Mufarji and Mohammed (2022) and Al-Masruri et al. (2022) indicated that 25.0 and 50.0g organic *M. oleifera* daily supplementation to ewes resulted in improvement of body status through modulating blood and metabolites, liver, and kidney functions.

Ovarian wave follicular dynamics and CL development due to *M. oleifera* supplementation were presented in (Figures 1 and 2). *M. oleifera* contributed to accelerated and significantly increased ($P < 0.05$) follicular development and numbers and corpora lutea as well. The positive effects of *M. oleifera* treatment on oocyte quality and reproductive performance have been reported by (Barakat et al., 2015; Ajuogu et al., 2019). This could be attributed to the high level of beta-carotene, protein, and other fine chemicals (Sonbarse et al., 2020; Giuberti et al., 2021; Al-Mufarji and Mohammed, 2022), which improved the ovarian follicular dynamics and numbers and sizes of corpora lutea. The significant positive changes in body health due to *M. oleiferai* supplementation collectively resulted in a significant increase in ovarian folliculogenesis and corpora lutea (Al-Mufarji and Mohammed, 2022).

CONCLUSIONS AND RECOMMENDATION

The potential properties of *M. oleifera* as sources of protein and bioactive compounds restore the negative effects of the peripartum period in ewes of the subtropicsal areas. *M. oleifera* leaves supplementation (50 and 100 g/kg diet) from 8 weeks pre-partum to 8 weeks post-partum of Naimi ewes improved milk composition, improved body weights, blood cells (RBCs and WBCs), and metabolites (total protein and blood urea nitrogen), as well as earlier restoration of ovarian follicle development. Therefore, *in-vivo* and *in-vitro* studies are recommended to investigate the quality and viability of aspirated oocytes postpartum upon *M. oleifera* supplementation.

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NOVELTY STATEMENT

M. oleifera supplementation to pregnant ewes overcomes the negative effects of transition period.

AUTHOR'S CONTRIBUTION

Mohammed shared experimental design, wrote, and submit MS, and prepared Figures. Aiman Al Mufarji, Rashid Al-Zeidi, and Haitham Al-Masruri carried out the experiment and statistical analysis. Al-Hassan Mohammed prepared Figures and Tables and collected the references.

Conflict of interest

None of the authors has any conflict of interest to declare.

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