

# METABOLIC ATTRIBUTES, MILK PRODUCTION AND OVARIAN ACTIVITY OF EWES SUPPLEMENTED WITH A SOLUBLE SUGAR **OR A PROTECTED-FAT AS DIFFERENT ENERGY SOURCES DURING POSTPARTUM PERIOD**

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#### Abstract

The effects of two dietary supplemental energy sources on metabolic attributes, milk production and ovarian activity of ewes during early to mid-postpartum period were studied using thirty multiparous lactating ewes (Rahmani × Barki) weighing 43.10±1.22 kg and 3-6 years old. The ewes were assigned to three groups (n=10 per group). All ewes received the same diet supplemented with isocaloric and isonitrogenous protected-palm oil (50 g/ewe/d, F-group) or a sugar cane molasses (140 g/ewe/d, M-group) or without supplementation (control, C-group), for 60 days starting 2 weeks postpartum. Results showed that, during the experimental period, both energy sources reduced (P<0.05) body weight loss of ewes compared with the control (2.57 kg in F-group, 0.911 kg in M-group and 4.71 kg in C-group). The metabolic profiles of ewes were affected by the sources of energy, the highest (P<0.05) concentration of serum triglycerides was in the F-group, whereas the highest (P<0.05) concentration of serum insulin was in the M-group. The lowest serum glucose concentration was (P<0.05) in the F-group (73.20 mg/dL) and the highest in the C-group (76.85 mg/dL), whereas it was intermediate in the M-group (74.69 mg/dL). Ewes in the F-group had (P<0.05) the highest milk yield and energy-corrected milk (531.72 g/d and 554.40 g/d, respectively) compared with those in the M-group (491.76 g/d and 525.12 g/d, respectively) and C-group (429.96 g/d and 462.00 g/d, respectively). The highest (P<0.05) number of corpora lutea (CL; ovulation activity) was in the F-group (0.45), whereas it was not different between the M-group (0.25) and the C-group (0.15). In conclusion, during early to mid-postpartum period, protected-fat supplementation increased serum triglycerides concentration which was effectively used as an energyvielding nutrient for improving milk production. It could also be suggested that specific fatty acid in protected-fat improved quality of the ovulatory follicle and thus occurrence of ovulation.

Key words: energy source, lactation, postpartum ewes, ovulation

In ewes and goats, lactation accounts for about 50% of daily energy expenditure, inducing negative energy balance, altering their metabolism and lowering available energy required for the maintenance of lactation and for other important reproductive processes (Blache et al., 2008). Optimum productivity of lactating animals could be achieved by adjusting energy content and/or energy sources (Goetsch et al., 2011). A large body of published research showed possible interactions between dietary energy sources and different physiological processes (Palmquist, 1994; Martin et al., 2004; Van Knegsel et al., 2007); nutrients such as fatty acids, sugars and amino acids can signal the amount of energy that is readily available from digestion and from body stores, in addition to their direct influences on the hypothalamo-pituitary-ovarian axis (Scaramuzzi et al., 2006). Ewes supplemented with long chain fatty acids in the form of Ca-soaps palm oil had improvements in the numbers and sizes of the pre-ovulatory follicles, ovulation rates (El-Shahat and Abo-El maaty, 2010), conception and lambing rates (Hashem and El-Zarkouny, 2014) and milk production (Otaru et al., 2011). On the other hand, feeding high-sugar diets often increases dry matter intake, butyrate concentration in the rumen, and milk fat yield. These nutritional characteristics of sugars may allow using high-sugar feedstuffs as an alternative energy source to increase dietary energy density (Oba, 2011). Additionally, sugars such as sucrose, galactose, glucose and fructose have pivotal roles in different biological functions in ruminants (Onions et al., 2009); a sugar such as glucose is certainly required by ruminants. It is an important nutrient for the synthesis of milk lactose and milk fat. Further, high circulating glucose concentration was suggested as a positive metabolic signal to the reproductive axis (Zabuli et al., 2010). The other important soluble sugar for ruminants is fructose which could be used as an alternative energy source for ovine ovaries (Campbell et al., 2010). Accordingly, during lactation, feeds high in available fat, protein, starch, and/or sugar are commonly supplemented for increasing energy density of the diet and for providing specific nutrients that are expected to be critical nutrients for optimal production and reproduction (Oba, 2011; Morales et al., 1989). Indeed, fats and carbohydrates possess different metabolic pathways resulting in different energy-generating substrates, and therefore, portioning of the energy toward different physiological events (Van Knegsel et al., 2007). Therefore, this study aimed to evaluate metabolic attributes, milk production and ovarian activity of ewes in postpartum period supplemented with two sources of energy (protected fat of palm oil vs. sugar cane molasses).

## Material and methods

The present study was conducted at the Agricultural Experimental Station, Faculty of Agriculture, Alexandria University (Latitude 31°20'N, 30°E). The procedures imposed on the animals were carried out meeting the International Guiding Principles for Biomedical Research Involving Animals (1985).

#### Animals and management

Thirty lactating Rahmani × Barki ewes, 3-6 years old and weighing  $43.10\pm1.22$  kg with body condition score (BCS) of  $2.8\pm0.1$  (scale ranging from 1=emaciated to 5=obese, Jefferies, 1961) at allocation were used. The BCS of the ewes was taken by a trained person, who mastered the technique. All ewes were kept outdoors with shelters during the day, and housed in a semi-open barn at night. The animals lambed within one week in December, and the experiment was carried out during the period from mid-December to mid-February (60 days experimental period). All ewes had a single birth (lamb), and the sex ratio was 4 males: 6 females in the supplementation groups and 5 males: 5 females in the control group.

#### **Experimental design**

Ewes were equally (n=10 per group) allocated into three experimental groups, ewes of each group were kept in a separated pen, with a common trough, according to the type of supplementation. The amounts of concentrate diet and roughage were calculated to cover the daily nutritional requirements of sheep (NRC, 2001). The chemical analyses of the concentrate diet, green clover (Trifolium alexandrinum), protected-fat and molasses were shown in Table 1. The experimental groups were: 1) the control-group (C-group) received no supplementation, 2) the fat-supplemented group (F-group) in which ewes received additional 50 g/ewe/d of protected-fat of palm oil and 3) the molasses-supplemented group (M-group) in which ewes received 140 g/ewe/d of sugar cane molasses. The percentages of fatty acids composition of the protected-fat used were, according to producer's statement (Megalac, Volac Ingredients Sdn. Bhd., Malaysia): myristic acid, <2; palmitic acid, 48; stearic acid, 5; oleic acid, 36 and linoleic acid, 9. Both additives were well mixed with the respective concentrate diet; molasses was daily sprinkled and mixed well with the concentrate mixture ration. The concentrate diet was firstly presented to the animals to ensure that the amount of supplementation was consumed. The amount of both additives was calculated to provide equal amounts of metabolizable energy (isocaloric). Nutritional treatment lasted for 60 days starting 2 weeks postpartum.

Chemical composition		Diet					
	concentrate	green clover	protected-fat	molasses			
Organic matter (g/kg)	865.2	853.8	840.0	897.6			
Crude protein (g/kg)	164.0	173.6	00.00	25.8			
Ether extract (g/kg)	25.0	12.4	840.0	5.00			
Nitrogen free extract (g/kg)	481.9	394.0	00.00	851.6			
Crude fiber	194.3	273.8	00.00	15.2			
Neutral detergent fiber (g/kg)	453.6	497.8	-	-			
Acid detergent fiber (g/kg)	281.2	324.7	-	-			
ME (MJ/kg DM)	11.52	115.8	30.35	11.67			

Table 1. Chemical analysis (g/kg) of the experimental feeds on dry matter (DM) basis

\*ME = Metabolizable energy of all feeds was calculated by the equation of NRC (2001).

### Ewes weighing and blood sampling

Weight of each ewe was recorded at the beginning and at the end of the nutritional treatment, ewes were forbidden access to feed and water 12 h before weighing. Blood samples were collected by means of a jugular venipuncture in non-heparinized tubes. Samples were collected two weeks after the start of the nutritional treatment and at biweekly intervals thereafter. All samples were collected in the morning before access to feed. Serum was separated by centrifugation of samples at 700 ×g for 20 min, and was frozen at -20 °C for later analyses. Concentrations of serum glucose, triglycerides, total protein and urea were determined using commercial kits (Stanbio Laboratory, 1261 North Main Street, Boerne, Texas 78006, USA). Concentrations of insulin and triiodothyronine (T<sub>3</sub>) were also measured in the same serum samples. Both hormonal assessments were carried out using ELISA technique by commercially available kits (DRG International Inc., 41 Mountain Avenue, Springfield, New Jersey 07081, USA). Sensitivities of the methods were 1.76  $\mu$ l U/mL and 0.2 ng/mL for insulin and T<sub>3</sub>, respectively. The corresponding intra- and inter-assay coefficients of variations were 2.1–5.7% and 5.6–5.0 %, respectively.

## Determination of milk yield and milk composition

Milk production was determined biweekly during the experimental period using the weigh-suckle-weigh technique as described by Ouedraogo et al. (2000). Lambs were removed from their dams and kept in separate pens 20 h prior to milking process. Milk yield was determined by milking out the ewes by hand into 1000 mL graduated plastic beaker, and weighed thereafter. Values of milk yield produced after 20 h separating period were multiplied by a correction factor of 1.2 to get the milk yield for 24 h (Otaru et al., 2011). Samples of 50 mL were collected and immediately analyzed for milk composition by Master ECO ultrasonic milk analyzer (Ultrasonic Milk Analyzer Master ECO, Belovo, Bulgaria). The yield of energy-corrected milk (ECM) was calculated according to the NRC (2001) equation: ECM (g/d) = milk yield (g/d) × {(0.0929 × % of milk fat) + (0.0563 × % of milk protein) + (0.0395 × % of milk lactose)}/0.749Mcal/kg.

### Monitoring of ovarian activity

Ovarian activity was monitored using an ultrasound scanner equipped with 5 MHz linear array, B-mode, real-time endorectal probe (Pie Medical Equipment B.V., Maastricht, Netherlands). The scanning was carried out while the ewe was in a standing position. The probe was fitted to a plastic rod (1×30 cm) to facilitate the insertion of the probe into the rectum. The probe was lubricated by a hydrosoluble gel and sheathed with polyvinyl chloride pipe (2×35 cm) to avoid damage of the rectal mucosa. The probe was gently inserted about 20 cm through the rectum after feces removal until the anechoic content of the bladder was visible on the screen, and then the probe was rotated 90° clockwise and 180° counterclockwise across the reproductive tract until the uterine horns and both ovaries were scanned (Gonzalez-Bulnes et al., 2010). Different structures observed in the ovaries including follicles  $\geq 2$  mm and corpora lutea (CL) were counted and measured throughout the experimental period, starting 2 weeks following the beginning of the treatment and at biweekly

intervals thereafter. Follicles were classified according to their sizes to one of three categories (Hashem et al., 2015): small ( $\geq$ 2–3 mm), medium ( $\geq$ 3–<5 mm) and large follicles ( $\geq$ 5 mm, ovulatory follicles). Data were used to estimate: total number of follicles (TNF), follicle population (small, medium and large), diameter of the ovulatory follicle (DOF, mm) and numbers of corpora lutea (no. of CL) and their diameters (Diameter of CL, mm). The mean number of CL per ewe was used as an indicator for the ovulation activity (as CL is a resultant of the ovulation process).

#### Statistical analyses

The numbers of follicles and their classifications (according to their diameters) and numbers of corpora lutea were subjected to the square root transformation before performing analysis of variance (Harvey and Damon, 1987). The fixed effects of treatment (energy source), time (sampling or measuring times) and their interactions on repeated measurements including metabolic attributes, total number of follicles and their classification, yield and composition of milk were analyzed using the MIXED procedure for repeated measurements of SAS (2001). The statistical model was:

$$y_{iik} = \mu + T_i + D_i + (TD)_{ii} + e_{iik}$$

where:

 $y_{iik}$  is animal's performance,

 $\mu$  is the overall mean,

 $T_i$  is the fixed effect of the i<sup>th</sup> treatment (i = 1, 2, 3),

 $D_i$  is the fixed effect of the j<sup>th</sup> sampling time (j = 1, 2, 3, 4),

 $(TD)_{ii}$  is the interaction between treatment and sampling time,

 $e_{iik}$  is the residual error.

Results of animal body weight were also analyzed using the MIXED procedure but with two measuring times (J = 1, 2) representing initial (day 0) and final (day 60) body weight.

The change in animal body weight (different between initial and final body weights) was analyzed using Generalized Linear Model (GLM).

$$y_{ii} = \mu + T_i + e_{ii}$$

where:

 $y_{ii}$  is animal's performance,

 $\mu$  is the overall mean,

 $T_i$  is the fixed effect of the i<sup>th</sup> treatment (i = 1, 2, 3),

 $e_{ii}$  is the residual error.

Differences among the treatment groups were detected using the Duncan new multiple range test. All results were expressed as least square means  $\pm$  standard error of mean (LSM $\pm$ SEM). Differences between means were considered significant or tended to be significant at P<0.05 and P<0.1, respectively.

### Results

## Effects on body weight and blood metabolites

Data for body weight, body weight loss and metabolic profile of ewes supplemented with different energy sources are presented in Table 2. The initial body weight of ewes in the three experimental groups was in the same range (44.78 kg in C-group, 44.21 kg in F-group and 45.50 kg in M-group; P>0.05), however, the final body weight of ewes was highest (P<0.05) in the M-group (44.58 kg) and lowest in the C-group (40.07 kg) and was intermediate in the F-group (41.64 kg). Both supplemental energy sources significantly (P<0.05) decreased body weight loss of ewes compared with control, with superior effect due to molasses supplementation (0.911 kg in M-group, 2.57 kg in F-group and 4.71 kg in C-group). Concentrations of serum glucose did not differ between the M-group (74.69 mg/dL) and the C-group (76.85 mg/dL), but it recorded the lowest value (P < 0.05) in the F-group (73.20 mg/dL). On the other hand, ewes in the F-group had the highest (P<0.05) concentrations of serum triglycerides (8.74 mg/dL) compared with the M-group (7.40 mg/dL) and C-group (7.07 mg/dL). Concentrations of serum total protein, urea and T, did not differ (P>0.05) among the experimental groups. In contrast, ewes supplemented with molasses had the highest (P<0.05) concentration of serum insulin (34.73  $\mu$ lU/ mL) compared with control (25.44 µlU/mL) and fat-supplemented (25.83 µlU/mL) groups.

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Parameter*		Treatment						
	C-group	F-group	M-group	SEM	P-value			
Live weight** (kg)								
initial	44.78	44.21	45.50	1.34	0.794			
final	40.07 b	41.64 ab	44.58 a	1.15	0.018			
weight change	4.71 a	2.57 b	0.911 b	0.60	0.001			
Metabolic status								
$T_3 (ng/mL)$	0.583	0.535	0.601	0.04	0.568			
insulin (µlU/mL)	25.44 b	25.83 b	34.73 a	2.86	0.031			
glucose (mg/dL)	76.85 a	73.20 b	74.69 ab	0.95	0.019			
triglycerides (mg/dL)	7.07 b	8.74 a	7.40 b	0.39	0.001			
total protein (g/dL)	7.23	7.22	7.07	0.14	0.689			
urea (mg/dL)	73.95	74.25	72.17	1.88	0.380			

Table 2. Effect of protected-fat (F-group) or molasses (M-group) supplementation vs. control (C-group) on body weight changes and metabolic status of lactating ewes (LSM±SEM)

\*Values of metabolic hormones and blood serum metabolites are the average of biweekly consecutive samples taken during dietary supplementation period.

\*\*Initial and final weights refer to the weight of ewe at the beginning and the end of the dietary supplementation.

a, b - means with unlike superscripts within the same row differ significantly (P<0.05).

Effects of supplementing the energy sources on milk production and milk composition are presented in Table 3. Dietary supplementation with different energy sources improved (P=0.010) milk yield compared with the control ewes. The highest milk ( $\pm$ 12.0) and energy-corrected milk yields ( $\pm$ 22.7) were in the protected fat-supplemented ewes (531.72 and 554.40 g/d) followed by the molasses-supplemented ewes (491.76 and 525.12 g/d, respectively) and the control ewes (429.96 and 462.00 g/d, respectively). The percentage of lactose and total solids-not-fat was significantly higher (P<0.05) in the M-group than in the other two groups.

Parameter	Treatment					
	C-group	F-group	M-group	SEM	P-value	
Milk yield (g/day)	429.96 b	531.72 a	491.76 ab	19.0	0.010	
Energy corrected milk yield (g/day)	462.00 b	554.40 a	525.12 ab	22.7	0.042	
Milk composition (%)						
fat	3.50	3.26	3.17	0.16	0.405	
protein	4.52	4.57	4.69	0.04	0.131	
lactose	5.89 ab	5.79 b	6.08 a	0.06	0.029	
ash	0.79	0.76	0.74	0.11	0.215	
total solids-not-fat	11.28 b	11.13 b	11.52 a	0.12	0.048	
total solids	14.78	14.48	14.60	0.20	0.590	
Fat yield (g/day)	14.73	16.18	15.58	1.06	0.715	
Protein yield (g/day)	19.39	22.19	22.80	0.95	0.087	
Lactose yield (g/day)	25.23	28.50	29.89	1.25	0.104	

Table 3. Effect of protected-fat (F-group) or molasses (M-group) supplementation vs. control (C-group) on milk yield and milk composition (LSM±SEM)

a, b – means with unlike superscripts within the same row differ significantly (P<0.05).

#### Effects on ovarian activity

Effects of supplementing the energy sources on ovarian activity including total number of follicles and their size classification and number of corpora lutea and their diameters during the experimental period are shown in Table 4. Total number of follicles tended to increase (P=0.089) in molasses-fed ewes compared to the other two groups. Neither molasses nor protected-fat had an effect on the number of small ( $\geq$ 2–3 mm) or medium (>3–<5 mm) follicles. However, numbers of the ovulatory follicles ( $\geq$ 5 mm) were greater (P<0.05) in both supplemental ewes compared with the control ewes. In addition, the highest significant (P<0.02) number of CL ( $\pm$ 0.07) was for the F-group (0.45) compared with the M-group (0.25) and the C-group (0.15).

Parameter	Treatment					
	C-group	F-group	M-group	SEM	P-value	
Total number of follicles/ewe	1.32	1.45	1.89	0.19	0.089	
Follicle distribution						
small (≥2–<3 mm)	0.47	0.47	0.71	0.11	0.366	
medium (>3-<5 mm)	0.42	0.35	0.42	0.08	0.777	
large (ovulatory follicle) (≥5 mm)	0.42 b	0.75 a	0.83 a	0.19	0.040	
Diameter of large follicles (mm)	5.53	6.13	5.75	0.22	0.179	
No. of corpora lutea (CL)/ewe	0.15 b	0.45 a	0.25 b	0.07	0.021	
Diameter of CL (mm)	11.63	11.30	10.65	0.26	0.445	

Table 4. Post-partum ovarian activity of ewes supplemented with protected-fat (F-group) or molasses (M-group) vs. control (C-group) (LSM±SEM)

a, b – means with unlike superscripts within the same row differ significantly (P<0.05).

#### Discussion

The major aim of the present study was to assess the efficiency of two different supplemental energy sources belonging to different nutritional classes (fats and carbohydrates), and thus different metabolic pathways in improving energy balance and metabolism of postpartum ewes and thus subsequent productivity. In the present study, metabolic profile of ewes throughout postpartum period was affected greatly by the source of energy. As set out in Table 2, serum insulin concentration was increased in molasses-supplemented group. Molasses is a soluble disaccharide, sucrose, which is comprised of glucose and fructose. Sugar is generally considered to be a carbohydrate fraction that ferments rapidly in the rumen into different volatile fatty acids, as butyrate in the case of molasses feeding, while a subtle amount of sugars can escape to be absorbed in the intestine (Oba, 2011; Soder et al., 2011). Volatile fatty acids such as butyrate and propionate have a stimulatory effect on insulin release (McAtee and Trenkle, 1971), which explains the increased serum insulin concentration observed in the molasses-supplemented group. On the other hand, unchanged glucose concentration during the molasses supplementation period could be attributed to the increased cellular glucose uptake under the effect of insulin. This finding is in accordance with that of Patton et al. (2004) who found that greater insulin concentration in blood of cows supplemented with a mixture of glucogenic precursor and fat was associated with greater blood glucose clearance, and consequently improved energy status. The other speculative reason for the unchangeable glucose in the molasses-supplemented ewes could be ascribed to the inability of molasses to produce enough moles of propionate during rumen microbial fermentation, which is known to be the main glucose precursor in ruminants via the liver gluconeogenesis (Van Knegsel et al., 2007). In this study, protected-fat supplementation increased circulating triglycerides. Similarly, it was previously observed that the addition of palm oil in the diet of dairy sheep influences the metabolism of lipids and causes an increase in serum cholesterol and triglyceride concentrations (Bianchi et al., 2014;

Hashem and El-Zarkouny, 2014). Overall, results of the present study showed that both dietary supplemental energy sources enhanced metabolism of ewes by providing additional source of energy as triglycerides, or by increasing insulin which acts as a signal for good metabolic status (Scaramuzzi et al., 2006). Such changes in metabolism of ewes encourage the use of available energy-yielding metabolites in different productive processes as denoted by the improved milk production and reduced body weight loss in supplemented ewes compared with non-supplemented ewes whose glucose level was higher with no associated production.

The present results showed that, although both supplementations enhanced milk production and reduced body weight loss, the effects were not in the same trend. Protected-fat was more efficient in increasing milk and energy-corrected milk yields than reducing body weight loss, whereas molasses supplementation showed an opposite trend. The increase in milk yield of dairy animals fed dietary fats has been observed in previous studies (Appeddu et al., 2004; Palmquist et al., 1993). In the current study, the increase in milk production was associated with an elevated concentration of serum triglycerides. Chiofalo et al. (2005) recorded a reduction in serum triglycerides of sheep during lactation due to the utilization of this metabolite by the mammary gland. The mammary gland has abundant lipoprotein lipase and readily extracts blood lipids. The activity of this enzyme is greatly increased in the mammary gland during lactation, but at the same time, it is markedly decreased in the adipose tissue just before parturition, remaining low throughout lactation (Havel, 1987; Nazifi et al., 2002). As a result, during lactation, a large proportion of the dietary fat is diverted to the mammary gland for milk synthesis and, to a lesser extent, to adipose tissue for storage (Del Prado et al., 1993). Accordingly, it could be inferred that supplementation of a rumen-protected fat of palm-oil modifies metabolic profile of ewes by a manner supporting milk production. On the other hand, the contribution of a large portion of the energy in molasses-supplemented ewes was directed to maintain body weight rather than milk production. Similarly, Liu et al. (2009) found that propylene glycol supplementation to dairy cows contributed more efficiently to the body weight deposit rather than milk production. These findings could be explained by the facts related to the responsiveness of different tissues to insulin effects and the manner of glucose uptake during the postpartum period. In lactating animals, peripheral tissue responses to insulin remain severely attenuated during early lactation but recover as the animal progresses through lactation (Bell and Bauman, 1997). At the same time, glucose uptake into the mammary gland is slightly affected by insulin, and the mammary gland does not express significant insulin-dependent glucose transport (Nielsen et al., 2001). Together, these physiological events may outweigh the competition between the peripheral tissues and the mammary gland in favor of peripheral tissues, resulting in an improvement in body weight rather than milk production.

In this study, milk composition was not greatly affected by the source of energy, however molasses supplementation increased the percentages of solids-not-fat and lactose as well as protein yield. These findings are in accordance with those obtained by Chopping et al. (1976). It is interesting as some investigators contend that lactose drives milk production by its osmoregulatory property (Rigout et al., 2003) which may explain the moderate increase in milk yield obtained in this group. The

increased protein yield obtained following molasses feeding could be explained by the ability of serum insulin to increase amino acid uptake by the mammary gland (McGuire et al., 1995).

Results of the present study showed that the percentage of ewes had ovulated during the treatment period and mean number of corpora lutea/ewe (ovulation activity) increased due to fat supplementation (Table 4). Thus, again, the protected-fat was more efficient in improving an important reproductive event of lactating ewes. It has been reported that body fat does not regulate luteinizing hormone-releasing hormone (LHRH) secretion, but it is possible that LHRH release is regulated by the available oxidizable metabolites such as glucose and stratified fatty acids (Schillo, 1992). In the present study, protected-fat supplementation increased serum triglycerides that may provide fatty acids for oxidation as a source of energy. Indeed, protection of unsaturated fatty acids with calcium salts relatively prevents bio-hydrogenation, and allows unsaturated fatty acids to remain intact, increasing availability of unsaturated fatty acids to different organs including reproductive organs. Protected-fat used in this study had 36% oleic acid. Previous studies showed that supplementation of rumen bypass fatty acids originated from palm oil to ewes increases the number of high-quality oocytes, because both oleic acid (262 g/kg) and palmitic acid (247 g/kg) are the major fatty acids contributing to ovine zona pellucida structure (Zeron et al., 2002; Ashworth et al., 2010). Also, Tripathi et al. (2015) stated that oleic acid improved oocyte development in vitro. Thus, it could be suggested that the fatty acids content of the fat product used in this study had direct effects on the quality of ovarian follicles, improving their survival or responses to luteinizing hormone.

Collectively, results of the present study indicated that rumen-protected fat supplementation modulated the metabolic profile of postpartum ewes by a manner supporting milk production, and may also provide specific fatty acids that are important for the quality of the ovulatory follicle and thus ovulation activity.

### Conclusion

The present study suggested that protected-fat in the form of palm oil could be recommended as an effective energy source for enhancing important physiological functions of lactating ewes such as milk production and ovulation.

### **Conflict of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the manuscript.

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