

## RESEARCH ARTICLE

# Post-lambing maternal effects in hair ewes fed omega-6 polyunsaturated fatty acids in the late gestation

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## ABSTRACT

Soybean oil (SBO) is rich in omega-6 polyunsaturated fatty acids and its dietary supplementation during late gestation to ewes improves colostrum production and lamb pre-weaning growth; however, little is known about the long-term maternal effects after lambing. So, aim was to determine the effects of dietary inclusion level of SBO (0 [control], 30 and 60 g kg<sup>-1</sup> dry matter) during late pregnancy on udder size, milk secretion, and dam metabolism during the first 60 d post-lambing. Thirty Katahdin x Pelibuey multiparous ewes (100 d of gestation) were blocked and randomly assigned within each block to three treatments. After lambing, ewes were fed the same lactation diet until 60 d post-lambing and study variables were measured on this period every 10 d. Solids non-fat in milk from control ewes were lower at day 10 and greater at day 20 compared to milk from SBO-fed ewes, although opposite effects were observed for lactose content on those days. Udder volume, depth and width increased linearly as the SBO level increased. Body status, physiological variables, milk production and serum analyte concentrations were unaffected by SBO. It was concluded that feeding hair ewes in late gestation with diets including SBO improves udder size, and solids non-fat and lactose content in milk, without affecting dam metabolism, during the post-lambing period.

**Keywords:** Energy metabolism; Hair-breed sheep; Lactose; Thermography; Udder morphometry

## INTRODUCTION

Pregnant ewes during late gestation decrease their feed intake by diminishing the ruminal capacity while their nutrient demands increase for fetal growth, udder development and lactogenesis process (Vicente-Pérez et al., 2015). In consequence, high mobilization of body reserves in this phase of pregnancy is observed, which leads to a negative energy balance (NEB) at lambing and during the first weeks post-lambing (Macías-Cruz et al., 2013). This nutritional status post-lambing in mothers causes metabolic alterations that could lead to a poor milk yield, as well as milk with low nutritional value for the offspring (Collier et al., 2017). Therefore, nutritional strategies should be applied during the last third of pregnancy to prevent NEB and to increase milk yield and quality after parturition in ewes.

The feeding of pregnant ewes with diets containing a larger amount of polyunsaturated fatty acids (PUFA)

could be an appropriate strategy to improve their post-lambing performance. The PUFA are essential fatty acids for ruminants which are positively involved in the fetal development and the expression of maternal genes associated with energy metabolism (Clarke, 2001; Coleman et al., 2018). So, feeding ewes with PUFA during late gestation could be beneficial to exert long-lasting post-lambing effect on maternal metabolism, udder development, and milk yield and quality. Notably, those post-lambing effects have not been evaluated by feeding ewes with some source of fat rich in omega-6 during late pregnancy in the long term.

In the short-term, the dietary inclusion of soybean oil (SBO; fat rich in omega-6 PUFA) during the last third of pregnancy has showed to improve udder size pre-lambing and colostrum yield within the first 24 h post-lambing, without affecting its chemical composition (Macías-Cruz et al., 2017; Mejía-Vázquez et al., 2017). In Holstein cows, the dietary

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supplementation of calcium salts with SBO during the last three weeks of pregnancy proved to be effective throughout the entire lactation by improving milk yield, body condition, daily lactose and protein yield, and fat metabolism (i.e. lower serum non-esterified fatty acid [NEFA] variables; Jolazadeh et al., 2019). Ewes fed diets rich in omega-3 PUFA during the pre-lambing showed an increase for content of lactose and total solids in milk (Nudda et al., 2015; Nickles et al., 2019). Then, we hypothesized that SBO inclusion, as source of omega-6 PUFA in the pre-partum diet of hair sheep ewes, might improve milk yield and composition by promoting long-term effects on udder growth and dam metabolism in the post-lambing period. Therefore, the objective was to determine the effects of dietary inclusion level of SBO during late gestation on udder growth, milk yield and composition, and dam metabolism during the first 60 d post-lambing in hair-breed ewes.

## MATERIALS AND METHODS

### Study location, experimental design and managements

The field phase was carried out at the Sheep Experimental Unit, while both handling of blood samples and analysis of serum analytes were conducted in the Animal Physiology Laboratory; both locations belong to the Instituto de Ciencias Agrícolas of the Universidad Autónoma de Baja California, located in northwestern México (32.8° N, 114.6° W). It should be noted that experimental procedures were in agreements with the Mexican Standard for production, care and use of laboratory animals (NOM-062-ZOO-1999). Likewise, accepted guidelines by the Federation Animal Science Society (FASS, 2010) for the experimental development with animals were followed.

Thirty Katahdin x Pelibuey crossbred multiparous ewes with 90 d of gestation and body condition score (BCS) of  $3.02 \pm 0.04$  units (Russel et al., 1969) were used. Between days 90 and 99 of gestation, all ewes were adapted to the dietary inclusion of SBO by applying increasing levels of 10 g of oil per ewe every two days. On day 100 of gestation and before the morning feeding, ewes were individually weighed (body weight [BW] =  $50.8 \pm 0.38$  kg) to be assigned under a randomized complete block design (blocking factor = initial BW) to one of three treatments ( $n = 10$ ). Treatments were diets with similar metabolizable energy (ME = 2.4 Mcal/kg dry matter [DM]) and crude protein (CP = 120 g/kg DM) content, which were formulated with different levels of SBO: 0 (control), 30 or 60 g kg<sup>-1</sup> DM. Experimental diets met the ME and CP requirements suggested for pregnant ewes carrying two products in late gestation (NRC, 2007; Table 1). Dietary treatments were offered from day 100 of gestation until the lambing day. After lambing, all ewes were fed the same

**Table 1: Ingredients and chemical composition of isoenergetic and isoprotein diets formulated with different inclusion levels of soybean oil**

Items <sup>a</sup>	Soybean oil levels <sup>b</sup> (g/kg DM)		
	0	30	60
Ingredients (g/kg DM)			
Soybean oil <sup>b</sup>	0	30	60
Wheat Straw	305	345	430
Alfalfa hay	160	200	170
Ground wheat	425	300	200
Soybean meal	45	65	100
Premix of vitamins and minerals	5	5	5
Cane molasses	45	40	20
Limestone	6	6	6
Calcium phosphorus	6	6	6
White salt	3	3	3
Chemical composition			
Dry matter (g/kg)	930	936	934
Organic matter (g/kg DM)	865	852	854
Crude protein (g/kg DM)	128	122	131
Neutral detergent fibre (g/kg DM)	435	471	491
Acid detergent fibre (g/kg DM)	239	305	282
Ash (g/kg DM)	99	116	97
DE (MJ/kg DM) <sup>c</sup>	12.1	11.8	12.1
ME (MJ/kg DM) <sup>c</sup>	10.0	9.7	10.0
Fatty acids (g/100 g fat) <sup>b</sup>			
C18:0	0.65	1.8	2.9
C18:1, cis 9	8.5	11.3	16.8
C18:2, n-6	9.2	26.3	45.1
C18:3, n-3	1.6	3.9	6.3
Monounsaturated fatty acid	8.5	11.3	16.8
Polyunsaturated fatty acid	10.8	30.2	61.9

<sup>a</sup> DM = Dry matter, DE = Digestible energy, ME = Metabolizable energy.

<sup>b</sup> Dietary treatments containing 0, 30 or 60 g of levels of soybean oil kg<sup>-1</sup> DM

lactation diet within 60 d post-lambing. This lactation diet was formulated to 2.4 Mcal of ME kg<sup>-1</sup> DM and 160 g of CP kg<sup>-1</sup> DM, according to the required nutritional profile (NRC, 2007). The following amounts (g/kg DM) of each ingredient were used to formulate the lactation diet: 155 g chopped alfalfa hay, 440 g chopped sudan hay, 255 g ground wheat, 138 g soybean meal, 5 g limestone, 5 g calcium phosphorus and 2 g while salt. All ewes were always fed *ad libitum* serving twice a day (0700 and 1700 h), with unlimited access to lean and fresh water. The health status of females was continuously monitored by a veterinarian.

All diets were mixed every week to offer fresh feed to ewes, as well as to collect diet samples to perform proximal (AOAC, 1990), fiber analysis (Van Soest et al., 1991) and ME calculation (NRC, 1985). The gas chromatography technique was used to determine the fatty acid profile in the experimental diets, specifically Long-chain fatty acid.

Regarding animal housing, 16 ewes ( $n = 4$ /treatment) were enclosed within 1.6 m<sup>2</sup> individual corrals during

the pre-lambing period (d 100 of pregnancy to lambing) to adjust daily feed intake. The remaining ewes were enclosed in common corrals per treatment (i.e.  $n = 6$ ; area = 2.0 m<sup>2</sup>/ewe). From parturition to weaning day (d 60), the ewes with their respective offspring were distributed in two common corrals of 36 m<sup>2</sup> each.

In addition, all study variables (i.e. BW, BCS, physiological variables, udder development, milk production and composition, and blood analyte concentrations) were evaluated in dams during the post-lambing period at 10-d intervals, starting on day 10 and ending on day 60. No variables were evaluated in lambs.

### Body status and physiological variables

Body state of ewes was measured by recording BW and BCS before the morning feeding on their respective sampling days. The body condition was measured using a 5-point scale (Russel et al., 1969). Physiological variables such as rectal temperature (RT), respiratory rate (RR) and surface temperatures (i.e. eye, ear, nostril, loin, flank, rump, shoulder, leg and belly) were measured in the morning (0600 h) and afternoon (1700 h) on each sampling day. Methodologies and equipment used to measure the physiological variables were according to those described by Macías-Cruz et al. (2018).

### Udder morphometry and volume

The evaluation of udder morphometry was done by measuring with a flexible tape different traits in udder (i.e. maximum circumference [UMC], depth [UD] and width) and teats (i.e. circumference, depth, width and distance between teats), as suggested by Ünal et al. (2008). Additionally, the udder volume (UV) was estimated using some data of udder traits in the following formula (Ayadi et al. 2011):  $UV = \pi \times R^2 \times UD$ , where  $R^2 = UMC^2 / 4\pi$ .

### Milk production and composition

A manual milking protocol was conducted on each sampling day to determine milk production per day (Bencini et al., 1992). The milk collected from each ewe was deposited in 100-mL graduated tubes to measure volume, and then was weighed. Given that the milk collected represented the 6-h production, both volume and weight were multiplied by four to calculate 24-h production (daily milk production). Additionally, a milk sample per ewe was analyzed for chemical composition (density and percentages of fat, solids non-fat [SNF], protein and lactose) immediately after the evaluation of milk production. The equipment used was a portable Lactichcek™ (Page & Pedersen International, Ltd, Hopkinton, MA, USA). Milk components were expressed as g/d. Finally, energy in milk (EM, KJ/g) was estimated with formula (Milis, 2008):  $EM = (39 \times \text{Fat}) + (18.2 \times \text{SNF}) + 52$ .

### Blood analytes

Individual blood samples were taken from jugular vein in 10-mL vacutainer tubes in fasted ewes. All the handling of samples, as well as methodologies and equipment used to determine the blood analytes were described by Macías-Cruz et al. (2018). In general, serum concentrations of glucose, cholesterol, triglycerides, urea, total protein, sodium, potassium and chlorine were measured.

### Statistical analysis

Data from all study variables were analyzed initially to demonstrate normality with the PROC UNIVARIATE statement, and then subjected to an analysis of variance under completely randomized block design with repeated measurements over time using the PROC MIXED statement. Several variance-covariance structure were tested to fit the model of each variable based in the criteria of lower values for AIC and BIC. The model of most of the variables had a better fit with unstructured covariance structure. Means were analyzed using the option LSMEAN/PDIFF when interaction was significant at  $P \leq 0.05$ . Also, analysis of orthogonal polynomials was applied to treatment means when there was no interaction effect ( $P > 0.05$ ). All statistical procedures are statements of the Statistical Analysis Software (SAS Institute 2004).

## RESULTS

With exception of SNF and lactose content in milk, the treatment  $\times$  time interaction did not affect body status (Table 2), physiological variables (Table 2), serum levels of metabolites and electrolytes (Table 3), udder morphometry (Table 4), and milk production and composition (Table 5). Additionally, only udder depth, width and volume changed ( $P \leq 0.05$ ) with increasing levels of SBO in the diet. These udder measurements linearly increased as SBO level increased from 0 to 60 g/kg DM.

Both SNF and lactose percentage in milk changed ( $P < 0.05$ ) on days 10 and 20, but not on days 30, 40, 50 and 60 post-lambing, as a consequence of the dietary inclusion of SBO (Fig. 1). The SNF percentage in milk from SBO-fed ewes was greater ( $P \leq 0.04$ ) on day 10, but lower ( $P \leq 0.02$ ) on day 20 than in milk from control ewes. However, lactose content showed to be lower ( $P \leq 0.04$ ) on day 10 and greater ( $P \leq 0.01$ ) on day 20 in milk from SBO-fed ewes than in milk from control ewes. The milk of ewes fed 30 or 60 g of SBO/kg DM had similar ( $P = 0.48$ ) SNF and lactose percentages on days 10 and 20 post-lambing.

**Table 2: Body state and physiological variables (mean±s.e.) during the post-lambing period of ewes supplemented with soybean oil (SBO) in the last third of gestation**

Items <sup>a</sup>	SBO level <sup>b</sup> (g/kg DM)				P-Values			Treatment effects <sup>c</sup>
	0	30	60	S.E.	SBO	Day	SBO x Day	
BW (kg)	47.4	47.1	46.7	1.7	0.96	<0.01	0.90	n.s.
BCS (units)	3.0	3.1	2.9	0.1	0.14	<0.01	0.44	n.s.
Physiological variables AM								
RR (rpm)	68.9	70.8	67.8	2.4	0.69	<0.01	0.63	n.s.
RT (°C)	39.1	39.1	39.0	0.1	0.67	<0.01	0.11	n.s.
T <sub>ear</sub> (°C)	33.4	33.8	33.2	0.5	0.69	<0.01	0.26	n.s.
T <sub>nostril</sub> (°C)	30.8	31.6	31.4	0.6	0.60	<0.01	0.50	n.s.
T <sub>loin</sub> (°C)	28.6	28.7	28.9	0.8	0.96	<0.01	0.63	n.s.
T <sub>flank</sub> (°C)	30.1	30.8	29.9	0.7	0.64	<0.01	0.80	n.s.
T <sub>rump</sub> (°C)	27.9	28.7	27.8	1.0	0.75	<0.01	0.62	n.s.
T <sub>shoulder</sub> (°C)	30.9	30.7	30.2	0.7	0.73	<0.01	0.68	n.s.
T <sub>leg</sub> (°C)	29.3	29.9	29.4	0.7	0.81	<0.01	0.66	n.s.
T <sub>belly</sub> (°C)	31.3	31.6	31.0	0.6	0.74	<0.01	0.75	n.s.
Physiological variables PM								
RR (rpm)	106.2	107.9	113.4	3.1	0.17	<0.01	0.67	n.s.
RT (°C)	39.4	39.5	39.5	0.1	0.88	<0.01	0.46	n.s.
T <sub>ear</sub> (°C)	37.2	37.3	37.5	0.3	0.62	<0.01	0.24	n.s.
T <sub>nostril</sub> (°C)	37.1	37.1	37.2	0.4	0.96	<0.01	0.50	n.s.
T <sub>loin</sub> (°C)	36.4	36.6	36.6	0.6	0.94	<0.01	0.11	n.s.
T <sub>flank</sub> (°C)	36.4	36.3	36.5	0.4	0.97	<0.01	0.14	n.s.
T <sub>rump</sub> (°C)	36.4	36.5	36.1	0.6	0.92	<0.01	0.13	n.s.
T <sub>shoulder</sub> (°C)	36.7	36.9	36.9	0.4	0.90	<0.01	0.51	n.s.
T <sub>leg</sub> (°C)	36.4	36.5	36.4	0.4	0.99	<0.01	0.19	n.s.
T <sub>belly</sub> (°C)	36.6	36.7	36.8	0.3	0.93	<0.01	0.51	n.s.

<sup>a</sup> BW= Body weight, BCS= Body condition score, RR= Respiration rate, RT= Rectal temperature, T= Body surface temperature. <sup>b</sup> Dietary treatments containing 0, 30 or 60 g of levels of soybean oil kg<sup>-1</sup> dry matter (DM). <sup>c</sup> n.s.= Nor linear or quadratic effect was significant ( $P > 0.05$ )

**Table 3: Serum concentrations of metabolites and electrolytes (mean±s.e.) during the post-lambing period of ewes supplemented with soybean oil (SBO) in the last third of gestation**

Items	SBO level <sup>a</sup> (g/DM)				P-Values			Treatment effects <sup>b</sup>
	0	30	60	S.E.	SBO	Day	SBO x Day	
Metabolites (mg/dL)								
Glucose	54.1	53.5	55.2	2.2	0.86	<0.01	0.82	n.s.
Cholesterol	59.7	55.8	53.2	4.1	0.47	<0.01	0.11	n.s.
Triglycerides	30.4	31.7	31.1	1.4	0.78	<0.01	0.81	n.s.
Urea	45.2	43.9	44.7	1.1	0.69	<0.01	0.12	n.s.
Total protein	7.0	7.3	7.2	0.1	0.19	0.07	0.13	n.s.
Electrolytes (mmol/L)								
Sodium	133.8	134.8	133.9	0.7	0.52	<0.01	0.43	n.s.
Chloride	111.3	112.8	111.8	0.4	0.09	<0.01	0.13	n.s.
Potassium	5.2	5.3	5.2	0.1	0.84	<0.01	0.19	n.s.

<sup>a</sup> Dietary treatments containing 0, 30 or 60 g of levels of soybean oil kg<sup>-1</sup> dry matter (DM). <sup>b</sup> n.s.= Nor linear or quadratic effect was significant ( $P > 0.05$ )

## DISCUSSION

The PUFA are essential fatty acids for ruminants as they may not synthesize them naturally by lack of specific enzymes to generate double bonds (Simpson et al., 2017). In pregnant and lactating ewes, these fatty acids have a key role to improve fetal programming, lambing ease, colostrum and milk production, udder health, and offspring development (Nudda et al., 2015; Macías-Cruz et al., 2017; Nickles et al., 2019). So, PUFA must be

exogenously administered. This study was designed to test the hypothesis regarding that dietary inclusion of omega-6 PUFA during the last third of pregnancy has long-term postpartum effects on udder size, milk secretion and dam metabolism in ewes. However, results supported very little this hypothesis, since only udder size reflected long-lasting effect (beneficial) by the pre-lambing inclusion of SBO in the diet of ewes. Lactose and SNF content were also altered (beneficial) due to SBO, but only during early lactation. Previous studies have reported greater udder



**Table 4: Udder development (mean±s.e.) during the post-lambing period of ewes supplemented with soybean oil (SBO) in the last third of gestation**

Items	SBO level <sup>a</sup> (g/kg DM)				P-Values			Treatment effects <sup>b</sup>
	0	30	60	s.e.	SBO	Day	SBO x Day	
Udder								
Circumference (cm)	44.5	44.8	45.7	1.1	0.71	<0.01	0.13	n.s.
Depth (cm)	16.7	17.4	18.9	0.8	0.05	<0.01	0.12	L
Width (cm)	13.6	14.6	15.1	0.3	<0.01	<0.01	0.39	L
Volume (L)	2.7	2.9	3.4	0.2	0.04	<0.01	0.31	L
Teats								
Circumference (cm)	5.2	5.3	5.4	0.2	0.77	<0.01	0.18	n.s.
Depth (cm)	3.1	3.2	3.3	0.2	0.75	<0.01	0.14	n.s.
Width (cm)	2.2	2.2	2.2	0.1	0.77	<0.01	0.18	n.s.
Distance/teats	13.3	12.9	13.1	0.5	0.85	0.02	0.55	n.s.

<sup>a</sup> Dietary treatments containing 0, 30 or 60 g of levels of soybean oil kg<sup>-1</sup> dry matter (DM). <sup>b</sup> L= Linear effect was significant at  $P \leq 0.05$ , and n.s.= Nor linear or quadratic effect was significant ( $P>0.05$ )

**Table 5: Milk production and composition (mean±s.e.) during the post-lambing period of ewes supplemented with soybean oil (SBO) in the last third of gestation**

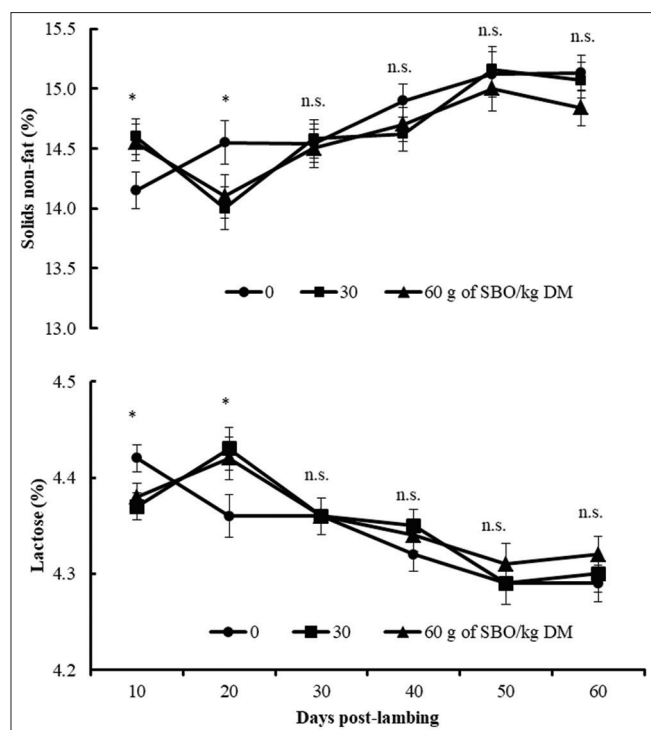
Items	SBO level <sup>a</sup> (g/kg DM)				P-Values			Treatment effects <sup>b</sup>
	0	30	60	s.e.	SBO	Day	SBO x Day	
Production								
Weight (g)	989	884	999	83	0.54	<0.01	0.18	n.s.
Volume (mL)	967	867	980	81	0.55	<0.01	0.16	n.s.
Composition								
Fat (%)	7.6	7.3	7.8	0.2	0.42	<0.01	0.46	n.s.
Solids Non-fat (%)	14.7	14.6	14.6	0.1	0.51	<0.01	0.01	n.s.
Crude protein (%)	5.8	5.8	5.7	0.1	0.33	<0.01	0.71	n.s.
Lactose (%)	4.3	4.3	4.4	0.01	0.50	<0.01	0.02	n.s.
Density (g/cm³)	31.0	31.3	30.2	0.4	0.12	<0.01	0.11	n.s.
Energy (KJ/g)	5715	5592	5750	103	0.52	<0.01	0.66	n.s.
Composition (g/d)								
Fat	76.0	64.5	77.6	6.8	0.33	<0.01	0.41	n.s.
Non-fat solids	144.6	129.7	145.1	12.1	0.57	<0.01	0.11	n.s.
Crude protein	57.2	52.1	56.4	4.9	0.71	<0.01	0.12	n.s.
Lactose	43.1	38.5	43.6	3.6	0.65	<0.01	0.22	n.s.

<sup>a</sup> Dietary treatments containing 0, 30 or 60 g of levels of soybean oil kg<sup>-1</sup> dry matter (DM). <sup>b</sup> n.s.= Nor linear or quadratic effect was significant ( $P>0.05$ )

**Fig 1.** Katahdin x Pelibuey crossbred ewes used in the experiment and theirs lambs in the post-partum period.

growth pre-lambing and colostrum production during the first 24 h post-lambing due to feeding SBO in the last third of pregnancy of hair breed ewes (Macías-Cruz et al., 2017; Mejía Vázquez et al., 2017). Therefore, all these findings suggest that dietary administration of SBO as source of omega-6 PUFA during late gestation has mainly short-term post-lambing effects on sheep dams.

Physiological variables (i.e. RT, RR and body thermography) were unaffected by SBO, which suggests that the post-lambing metabolism of lactating hair ewes does not change with the nutritional strategy of including omega-6 in the pre-lambing diet. Any alteration of metabolic activity causes variations on the endogenous production of body heat, and consequently, on physiological variables (Montanholi et al., 2008; 2010). This metabolic fact suggested by the physiological response was confirmed with those results



**Fig 2.** Solids non-fat and lactose contents (mean  $\pm$  s.e.) in milk collected during the post-lambing period of hair ewes fed different soybean oil (SBO) levels during the last third of gestation. Differences among treatments in each post-lambing day are indicated with asterisk (\* $P < 0.05$ ) or n.s. when there were differences ( $P > 0.05$ ). DM= Dry matter.

of blood metabolite concentrations recorded during the pre-weaning period in dams. Thus, our ewes did not show differences at level of energy, protein and water metabolism through the 60 d post-lambing as a long-term effect attributed to the SBO addition in the late gestation diet. In coincidence with our findings, Coleman et al. (2018) reported that feeding with omega-3 PUFA during the last 50 d of gestation did not modify ewe metabolic profile (i.e. plasma levels of glucose, non-esterified fatty acids [NEFA] and ghrelin) during the 60-d post-lambing period. Similarly, Holstein cows have showed minimal effects in their blood metabolite levels associated to energy and protein metabolism during the first months post-lambing by the dietary supplementation of omega-3 and/or 6 fatty acids in late gestation (Salehi et al., 2016; Jolazadeh et al., 2019). Therefore, our results on post-lambing metabolism are confirming those findings previously published for cows. They also confirm, together with those results from Coleman et al. (2018), that dietary inclusion of omega-3 and 6 PUFAs in late pregnancy did not alter dam postpartum metabolism in sheep.

Interestingly, SBO-fed ewes during pre-lambing showed better udder size without any modification on milk production and dam metabolism during the 60-d post-lambing period. A maximum udder size was found with

the inclusion level of 60 g SBO/kg DM. Previously, we revealed that udder size before and at lambing increased significantly by feeding hair ewes with 60 g of SBO/kg DM during the last third of pregnancy (Macías-Cruz et al., 2017). This fact was attributed to a stimulatory effect of PUFA on activation receptors for mammogenic hormones, which in turn promoted greater growth of mammary epithelial tissue (McFadden et al., 1990). However, the largest post-lambing udder size due to SBO is likely a long-lasting effect, which persisted from the better udder development observed in the pre-partum in this same treatment (60 g). It is not possible to associate this finding with a greater milk production, neither with an increase in the formation of mammary tissue as both development and growth of the gland occurs mainly during the pregnancy (Neville et al., 2002). Previous studies have not reported results on this topic.

On the other hand, milk yield and body status of dams during the 60-d post-lambing period were unaffected by pre-lambing diet. This could be because the dietary inclusion of SBO in late gestation did not cause long-term effects on dam metabolic parameters (Collier et al., 2017; Coleman et al., 2018), as previously mentioned. According to these results, studies conducted in sheep (Capper et al., 2007; Coleman et al., 2018) and dairy cattle (Garcia et al., 2016) indicate that there were no changes in dam BW and BCS or milk yield during the post-lambing period when sources of PUFA were supplemented in late pregnancy. However, Sarda ewes showed an increase in milk yield during early lactation by consuming linseed (rich in omega-3 PUFA) 8 weeks before lambing (Nudda et al., 2015). The authors associated this beneficial effect with a greater differentiation of udder secretory cells around parturition, although udder development was not measured in that study. In the current study, we found larger udder size due to SBO in the pre-lambing diet, which could suggest greater amount of secretory cells; however, there were no changes in milk production. So our findings suggest that milk production is independent of udder development when ewes are fed PUFA.

Moreover, in addition to the fact that the available information is scarce, feeding with omega-3 PUFA during late gestation has generated controversial effects on milk composition in sheep. While there are studies indicating no change (Coleman et al., 2018), others have reported variations in some components, mainly an increase in lactose content between the second and third week post-parturition (Nudda et al., 2015; Nickles et al., 2019). The latter partially coincides with our results, where dietary inclusion of SBO (rich in omega-6) before lambing increased lactose and SNF percentage in milk during early lactation of hair breed ewes. After day 20 post-lambing,

no long-term effect was found due to the pre-lambing feeding on milk components. Previous studies reported similar effects on milk lactose using omega-3, so it can be inferred that any dietary source of PUFA offered during late gestation improves the lactose content of milk in early lactating ewes. There is no a clear explanation for this finding, however, Nickles et al. (2019) recently suggested that dietary supplementation of PUFA during pregnancy increases the lactose percentage in milk because they might have modified in the mammary gland the gene expression of lactose synthase; this enzyme is a limiting factor for lactose synthesis in milk (Kuhn et al., 1980).

## CONCLUSIONS

In conclusion, including SBO as source of omega-6 PUFA in the pre-lambing diet of hair breed ewes is a nutritional strategy increasing the udder size, as well as lactose and SNF content in milk in the post-lambing period, particularly during the early lactation. These beneficial effects of omega-6 PUFA seem to be a direct effect of them, as those fatty acids did not modify maternal metabolism and milk production during the first 60 d post-lambing. The use of this nutritional strategy is recommended in combination with any other that increases milk production. The optimal inclusion level of SBO in the pre-lambing diet is 60 g/kg DM, since the udder growth is maximum to this level without negatively affecting body state and milk secretion. In future studies, it could be evaluated whether extending the dietary inclusion of SBO in the early lactation period could cause more powerful beneficial effects on postpartum maternal development in hair breed ewes.

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### Authors' contributions

Ulises Macías-Cruz and Leonel Avendaño Reyes developed the research project, sought financial support, designed the experiment, analyzed data and wrote the first draft of the manuscript. Ricardo Vicente Pérez and Abelardo Correa Calderón directed the field and laboratory phase, as well as they collected and organized the data in database for statistical analysis. Carlos Luna Palomera analyzed blood metabolites concentrations, helped to prepare tables and also to interpret results. Cesar A. Meza Herrera and Miguel Mellado helped in the statistical analysis, interpretation

of results and revision of the manuscript. All authors contributed to the final revision of the manuscript.

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