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Performance of Lambs Born to Energy- and Protein-Supplemented Ewes During Gestation and Lactation

N. C. Silva[1,*], S. L. S. Cabral Filho[2], I. O. M. A. Freitas[2], F. C. Rocha[2], A. M. Azevedo[3], V. R. Rocha Júnior[4], & M. D. Ribeiro[1]

[1] Universidade Federal de Goiás, Departamento de Zootecnia, Campus II Samambaia, Rodovia Goiânia - Nova Veneza, Km 8, 74690-900 Goiânia, GO, Brazil
[2] Universidade Federal de Brasília, Faculdade de Agronomia e Medicina Veterinária (FAV), Rural Core, Campus Darcy Ribeiro, Asa Norte, 70910-900 Brasília, DF, Brazil
[3] Universidade Federal de Minas Gerais, Instituto de Ciências Agrarias, University Avenue, 1000, 39.404-547 Montes Claros, MG, Brazil
[4] Universidade Estadual de Montes Claros, Departamento de Ciências Agrárias, Reinaldo Viana Avenue, 2630, Bico da Pedra neighborhood, 39440-000 Janaúba, MG, Brazil
*Corresponding author: neytoncarlos@yahoo.com.br

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ABSTRACT

Maternal nutrition and supplementation during gestation and lactation improve fetal development, survival, and growth of lambs. The aim of this study was to evaluate the effects of energy and protein supplementation in the diet before, during, and in the final third of gestation and lactation, within a synchronized breeding system, on the health and performance of lambs. Forty Santa Inês × Dorper ewes, averaging ten months of age and two previous births, were distributed in a completely randomized design with four treatments: mineral supplementation only, supplementation initiated before breeding, supplementation starting after pregnancy confirmation, and supplementation restricted to late gestation. Supplements were provided daily at 4 pm at 1% of body weight (BW), adjusted every 15 days when ewes were weighed. The experimental period lasted 60 days, with 10 days of adaptation. Variance analysis was performed in subdivided plots, and the means were compared using Tukey's test at 5% probability. Concentrated supplementation altered ($p<0.0072$) birth weight 3.79 kg, colostrum intake, daily gain ($p<0.0028$) 242.39 g/animal/day, morphometric measurements, eggs per gram of feces, protein and energy metabolic profiles, and cortisol levels in lambs. There was no difference in lambing rate, litter type, sex, fecal score, or mineral metabolism. However, supplementation begun 20 days before breeding and after pregnancy confirmation improved anthelmintic response 272.49 EPG and produced heavier lambs at weaning 20.78 kg. Maternal energy and protein supplementation, especially when started before breeding or after pregnancy confirmation, improves lamb growth, metabolic status, and parasitic resistance.

***Keywords:** colostrum; nutrition; production; reproduction; sheep*

INTRODUCTION

Maternal nutrition is a key determinant of fetal development, neonatal viability, and postnatal performance in sheep production systems. In tropical and semi-intensive conditions, nutritional fluctuations during gestation and lactation frequently limit productive efficiency, compromising birth weight, colostrum yield, immune competence, and lamb growth. These effects are particularly relevant in hair sheep breeds raised under pasture-based systems, where nutritional supply often fails to meet the increasing metabolic demands of late gestation and early lactation.

Previous studies have demonstrated that nutritional restriction during gestation induces fetal programming effects that persist after birth, negatively affecting growth rate, metabolic profile, and slaughter age, even when adequate nutrition is provided postnatally (Klein *et al.*, 2021; Brondani *et al.*, 2020). Supplementation strategies during late gestation have been associated with improvements in birth weight, colostrum production, and early lamb growth, highlighting the importance of targeted nutritional management during this critical period (Andrade *et al.*, 2024; Campos *et al.*, 2019). However, most available studies focus on supplementation restricted to a single gestational phase, with limited information regarding the comparative effects of supplementation initiated before breeding, after pregnancy confirmation, or exclusively during late gestation, especially under synchronized breeding systems.

Furthermore, emerging evidence suggests that maternal nutrition may influence not only productive traits but also parasitological resistance, metabolic adaptation, and stress responses in lambs, yet these outcomes remain poorly integrated in experimental designs (Asadi *et al.*, 2024; Melo *et al.*, 2023). This knowledge gap limits the development of precise nutritional strategies that can optimize lamb performance while reducing health-related challenges in early life.

In this context, the present study introduces a comparative evaluation of energy and protein supplementation at distinct physiological stages: before breeding, after pregnancy confirmation, and during the final third of gestation, within a synchronized reproduction system. The novelty of this approach lies in the integrated assessment of growth performance, colostrum intake, metabolic profile, parasitological burden, and cortisol response in lambs.

Therefore, the objective of this study was to evaluate the effects of different maternal supplementation strategies before and during gestation and lactation on the health, performance, and physiological responses of lambs born to Santa Inês × Dorper ewes.

MATERIALS AND METHODS

Local, Animals, and Treatments

The experiment was carried out in an experimental livestock production area located in Brasília, Federal District, Brazil (15°56′–15°59′ S; 47°55′–47°58′ W), from January to November 2019. All experimental procedures involving animals were conducted in accordance with ethical standards and approved by the Animal Experimentation Ethics Committee of the Federal University of Brasília (protocol no. 10/2019).

Forty crossbred Santa Inês × Dorper ewes, approximately ten months old and with two previous parturitions, were selected and allocated to a completely randomized design. Animals were grouped according to age, body weight, and body condition score, forming four experimental treatments with ten replicates each. Throughout the experimental period, ewes grazed *Brachiaria brizantha* cv. Marandu pasture in a 5 ha area subdivided into seven paddocks, allowing collective grazing and minimizing pasture-related variation among treatments. Protein–energy supplementation was formulated according to NRC (2007) recommendations and offered daily at 16:00 h at a rate equivalent to 1% of body weight. Supplement quantities were adjusted every 15 days following individual weighing of the ewes. During supplementation, animals were temporarily housed in individual stalls to ensure complete intake, after which they were returned to collective pens with unrestricted access to water. Metabolizable energy requirements (2.777 ME (kcal/kg)) were recalculated biweekly based on ewe body weight, gestational stage, and number of fetuses, following ARC (1980) guidelines. Due to the stage of pregnancy,

Table 1. Centesimal and nutritional composition of concentrate, pasture (*Brachiaria brizantha* cv. Marandu), and sugarcane in the natural matter (%) supplied to ewes during gestation and lactation

Ingredients (%)	Gestation				Lactation	
	T1	T2	T3	T4	T1	T2; T3; T4
Soybean meal	–	56.37	56.37	36.04	–	45.59
Corn	–	40.24	40.24	61.53	–	50.61
Urea	–	–			–	0.50
Calcitic limestone	–	–	–	0.59	–	–
Vitamin mineral supplement[1]	+	3.39	3.39	1.84	–	3.30
Protein mineral supplement[2]	–	–	–	–	+	–
Nutrient contents	Gestation				Lactation	
	T2; T3; T4				T1	T2; T3; T4
	Supplement	Pasture	Supplement	Pasture	Sugarcane	Supplement
DM (% NM)	96.61	93.05	92.75	94.79	95.81	89.72
CP (% DM)	29.98	9.55	23.14	4.70	2.86	25.55
NDF (% DM)	18.33	75.56	25.75	71.11	38.74	18.18
ADF (% DM)	4.98	35.25	5.61	38.84	23.25	4.85
EE (% DM)	2.74	4.60	2.65	2.85	2.10	3.66
MM (% DM)	7.33	8.96	5.61	6.22	2.96	13.21
CHOt (% DM)	59.91	80.54	68.58	86.20	92.35	57.56
NFC (% DM)	41.58	4.98	42.83	15.09	53.61	39.38
NDIN (% DM)	13.15	3.55	21.94	2.82	1.95	16.87
ADIN (% DM)	6.68	1.26	9.37	0.96	0.98	9.61
TDN (% DM)	58.42		68.43		69.11	

Note: T1 = mineral salt; T2 = supplementation started 20 days before the estrus synchronization protocol (ES); T3 = supplementation from confirmation of pregnancy (60 days after ES); T4 = supplementation in the final third of gestation (90 days after ES). NM – natural matter; DM – dry matter; CP – crude protein; NDF – neutral detergent fiber; ADF – acid detergent fiber; EE – ether extract; MM – mineral matter; CHOt – total carbohydrates; NFC – non-fibrous carbohydrates; NDIN – neutral detergent insoluble nitrogen; ADIN – acid detergent insoluble nitrogen; TDN – total digestible nutrients. (1) Composition of vitamin mineral premix: calcium (max.) 150 g, calcium (min.) 130 g, phosphorus (min.) 65 g, sodium (min.) 130 g, fluorine (max.) 650 mg, sulfur (min.) 12 g, magnesium (min.) 10 g, iron (min.) 1,000 mg, manganese (min.) 3,000 mg, cobalt (min.) 80 mg, zinc (min.) 5,000 mg, iodine (min.) 60 mg, selenium (min.) 10 mg, vitamin A (min.) 50000 IU, vitamin E (min.) 312 IU. (2) Protein mineral supplement: calcium 85 g, cobalt 20 mg, copper 400 mg, sulfur 6 g, fluorine 243 mg, phosphorus 18 g, iodine 50 mg, manganese 500 mg, crude protein 300 g, NPN (protein eq.) 255 g, selenium 10 mg, sodium 39 g, zinc 800 mg.

the needs for metabolizable energy and proteins were different (Table 1).

The experimental treatments consisted of: T1, mineral supplementation only; T2, protein–energy supplementation initiated 20 days prior to estrus synchronization; T3, supplementation initiated after pregnancy confirmation (approximately 60 days after synchronization); and T4, supplementation restricted to the final third of gestation (approximately 90 days after synchronization). To reduce handling stress, ewes were visually identified by treatment using colored markings. Reproductive synchronization was achieved through the male effect using a Dorper ram, with natural mating conducted at a ratio of one male per 50 females. Mating activity was monitored using marking paint applied to the ram's chest. Pregnancy diagnosis was performed approximately 55 days after mating by transrectal ultrasonography using an Aloka 500 device equipped with a 5-MHz linear probe.

Following parturition, ewes and their offspring were housed in maternity pens equipped with feeders and waterers. Diets during lactation were based on sugarcane and a concentrate mixture containing corn, soybean meal, urea, mineral supplement, and protein mineral salt, formulated to meet the nutritional requirements of lactating ewes according to NRC (2007). The experimental period lasted 60 days, including a 10-day adaptation phase. Feed allowances permitted approximately 15% refusals, which were weighed daily. Representative samples of diets and refusals were collected weekly and stored at −20 °C for subsequent chemical analyses. Leftovers were sampled at a proportion of 35% of the total daily amount supplied to each animal.

The lambs remained confined in facilities with a cemented floor and containing a bed of hay, with water ad libitum, from birth to weaning (at 60 days). A complete mesh feed (20% CP and 80% TDN), formulated for a daily weight gain of 0.300 kg per day (NRC, 2007), was offered *ad libitum* in collective creep feeding from the 10th day onwards. According to the treatments, the ewes were maintained in collective stalls with their respective lambs, with a buoy drinking trough. The dimensions of the collective creep feedings were 1.4 m high, 1.5 m wide, and 4.0 m long. The protein-energy supplement was provided in the morning, and the lambs had free access to *creep feeding* throughout the day.

Variables at Birth

The following characteristics were observed: lamb weight at birth (LWB), according to the maternal supplementation phase, type of lambing (single or twin), sex (male or female), the birth rate of the total herd (BR), and the number of lambs at birth per lambed ewe (OFFSP).

Colostrum Intake

Colostrum was ingested in the first 12 h after birth and determined by the indirect method of double weighing. The lambs were weighed individually on a digital scale before breastfeeding, and every two hours after twin births, and were immediately placed on the breast and allowed to feed until satiety, when they were weighed again. The difference between the lamb's weight before and after suckling corresponds to the amount of colostrum produced by the ewe.

Weight Gain

Weight characteristics (average daily gain (ADG, g/animal/day), total gain (TG, kg/animal), and final weight (FW)) were used for performance analyses. The animals were weighed after lambing to obtain the initial live weight (ILW) and final live weight (FLW) 60 days after weaning.

Body Morphometry Measurements

The body development of lambs was evaluated through morphometric measurements taken using measuring tapes with the animals on flat ground and upright. The measurements of withers height, body length, and thoracic perimeter were taken. The withers height was measured between the highest point of the interscapular region and the ground. Body length was measured from the withers to the caudal part of the ischial tuberosity. The thoracic perimeter was measured on the external circumference of the thoracic cavity, next to the axillae.

Eggs Per Gram of Feces Count

The eggs per gram of feces (EPG) count was determined at 30 and 60 days from feces samples collected directly from the rectum for counting, culture, and identification of larvae (Eduarda *et al.*, 2025).

Fecal Ccore

The fecal score of lambs was evaluated at 30 and 60 days, as described by Sacoman *et al.* (2016), with visual observation and using photos, with which a photographic pattern was developed to classify the feces from 1 – normal to 6 – pasty.

Metabolic Profile

The metabolic profile of lambs was evaluated using blood samples collected at different times: 0 h (before colostrum intake) 12, 24, and 48 h, counted from colostrum intake. These periods are important for evaluating the transfer of passive immunity via colostrum through the apparent efficiency of absorption into the bloodstream before bowel closure at 48 hours. Blood samples were obtained through jugular venipuncture, using a vacuum tube (Vacutainer®) and specialized needles for vacuum tubes. Two types of duly identified vacuum tubes were used: one with sodium fluoride and the other without it. The samples remained after collection at rest until complete coagulation, followed by centrifugation for 10 minutes at 2,500 rpm. The serum was then transferred to microtubes with a capacity of 0.5 mL for storage at −20 °C. Biochemical analyses were performed using diagnostic kits with enzymatic methodological principles from Labtest Diagnóstica® S.A., BioClin®, and Randox®, following their respective protocols. The reading was performed in an automated COBAS MIRA PLUS (Roche®, Germany) equipment, specific for biochemical analysis. Protein (total protein, albumin, globulin, and urea), energy (glucose and β-hydroxybutyrate – BHB), and mineral metabolites (magnesium – Mg^{2+}, calcium – Ca^{2+}, and phosphorus – P^{-}) were evaluated.

Physiological Response of Animals to the Weaning Challenge

The physiological response of lambs to weaning was studied by analyzing plasma cortisol levels. Blood samples were taken at the following times: 20 minutes before the separation of the ewe from its lamb(s) (−20 min), immediately before separation (0 min), and 1, 6, 12, 24, and 48 h after separation. Blood samples (9 mL) were collected via jugular vein puncture in a heparinized vacuum tube. After being collected, the samples were immediately centrifuged for 17 minutes at 4 °C and 3000 rpm. Plasma was pipetted and placed in 2.5 mL plastic tubes and stored at −20 °C until analysis. The analyses were carried out using an appropriate immunoenzymatic kit for cortisol quantification in blood plasma (Assay Design®, Michigan, USA) and the ELISA equipment (Multiscan MS, Labsystem®). Readings were taken at a wavelength of 450 nm.

Bromatological Analysis

Samples of the supplied diet, leftovers, and feces were pre-dried in a forced-air ventilation oven at 55 °C and ground in a Willey mill with a 1-mm diameter opening sieve to determine the dry matter according to the AOAC (2017) (DM; method number 930.15), mineral matter (MM; method number 942.05), crude protein (CP; method number 984.13), and ether extract (EE; method number 920.39). The fibrous fractions, that is, neutral detergent fiber (NDF) and acid detergent fiber (ADF), were determined using the methodology proposed by Van Soest *et al.* (1991). Neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were determined following the recommendations by Licitra *et al.* (1996). The percentage of total carbohydrates (TC) was calculated using the equation proposed by Sniffen *et al.* (1992), while the non-fibrous carbohydrates (NFC) were determined using the equation recommended by Geraseev *et al.* (2023).

Statistical Analysis

The data were subjected to analysis of variance and comparison of means using Tukey's test at a 5% significance level. For this, the easyanova package (Arnhold, 2013) of the R software (R Core Team, 2024) was used. For data collected only once over time, a completely randomized design was considered based on the statistical model $y_{ij} = \mu + t_i + e_{ij}$, where: y_{ij} is the observed value in the plot that received treatment i in replication j; μ is the overall mean; t_i is the effect of treatment i; and e_{ij} is the experimental error in the plot that received treatment i in replication j. For variables whose data were collected at different times, a split-plot design over time was considered in a completely randomized design. For this, the model $y_{ijk} = \mu + a_i + d_{ij} + b_j + ab_{ij} + e_{ijk}$ was considered, where: y_{ijk} is the observed value in the subplot that received treatment i, at time j and replication k; μ is the effect of the overall mean; a_i is the effect of treatment i; d_{ij} is the effect of the experimental error associated with the plot that received treatment i in replication j; b_j is the effect of time j; ab_{ij} is the interaction between treatment i and time j; and e_{ijk} is the experimental error in the subplot that received treatment i, at time j and repetition k. Both models were considered to be fixed in nature.

RESULTS

Lambs born from experimental treatments were: T1 (n=12), T2 (n=12), T3 (n=12) T4 (n=13). Lamb birth weight differed among treatments (p=0.0072). Lambs from ewes supplemented after pregnancy confirmation (T3) were heaviest at birth (4.26 kg), exceeding those from mineral-only supplementation (T1 = 3.62 kg) and late-gestation supplementation (T4 = 3.50 kg). No differences were observed for type of lambing, sex ratio, or birth rate (100 % across treatments) (Table 2).

Maternal supplementation influenced colostrum intake (p<0.05). At birth (0 h), lambs from T2 ewes had the highest intake (0.213 kg), while at 12 h postpartum, intake was greatest in T4 lambs (0.201 kg), indicating stage-specific effects of supplementation on early colostrum availability (Table 3).

Lamb performance was strongly affected by supplementation strategy (p<0.0028). Lambs from T2 ewes showed the highest average daily gain (293.07 g/day), total gain (17.58 kg), and final weight at weaning (21.37 kg). In contrast, lambs from mineral-only ewes (T1) had the lowest gains (ADG = 178.14 g/day; FW = 14.31 kg), demonstrating the negative impact of the absence of energy–protein supplementation (Table 4).

Supplementation affected withers height gain (p=0.0099) and body length gain (p=0.001). Lambs from T2 ewes showed superior skeletal growth (WHG = 13.93 cm; BLG = 25.71 cm). Thoracic perimeter gain was not influenced by treatments (p=0.97) (Table 4).

Eggs per gram of feces (EPG) were significantly reduced by supplementation (p<0.0065). At 30 days, T2 and T3 lambs had markedly lower EPG counts (33–42) compared with T1 (352). This effect persisted at 60 days, confirming improved parasitic resistance in lambs from supplemented ewes. Fecal scores were unaffected (Table 5).

Protein metabolites varied significantly over time with a cubic pattern (p<0.05), peaking at 24 h postpartum. Lambs from T2 ewes showed the highest concentrations of total protein (72.15 g/L), albumin (29.67 g/L), globulin (42.47 g/L), and urea (49.73 mg/100 mL), indicating superior passive transfer and metabolic status compared with T1 lambs (Table 6).

Table 2. Reproductive performance of lambs with according to supplementation strategies during gestation

Variables	Treatments				CV (%)	p
	T1	T2	T3	T4		
LWB (kg)	3.62^{ab}	3.78^{ab}	4.26^{a}	3.50^{b}	17.38	0.0072
TL (Single/Twin)	8/2	8/2	8/2	8/2	-	1
S (Male/Female)	6/6	7/5	6/6	5/7	-	0.9532
BR (%)	100	100	100	100	-	-

Note: Means followed by different letters on the row differ from each other by Tukey's test (p<0.05). Lamb weight at birth (LWB) in kg, while type of lambing (TL), sex (S), birth rate (BR), and coefficient of variation (CV) expressed in %. T1 = mineral salt; T2 = supplementation started 20 days before the estrus synchronization (ES) protocol; T3 = supplementation from confirmation of pregnancy (60 days after ES); T4 = supplementation in the final third of gestation (90 days after ES).

Table 3. Colostrum intake in lambs produced with different supplementation strategies during ewe gestation

Variables	Treatments				CV (%)
	T1	T2	T3	T4	
CI 0 h (kg)	0.148^{b}	0.213^{a}	0.160^{b}	0.189^{b}	14.28
CI 12 h (kg)	0.126^{b}	0.152^{b}	0.180^{a}	0.201^{c}	12.80

Note: Means followed by different letters on the row differ from each other by Tukey's test (p<0.05). Colostrum intake (CI) at 0 and 12 hours (h) and coefficient of variation (CV) expressed in kg and %, respectively. T1 = mineral salt; T2 = supplementation started 20 days before the estrus synchronization (ES) protocol; T3 = supplementation from confirmation of pregnancy (60 days after ES); T4 = supplementation in the final third of gestation (90 days after ES).

Table 4. Weight gain and body morphometric measurements of lambs from ewes fed different supplementation strategies during gestation and lactation

Variables	Treatments				CV (%)	P
	T1	T2	T3	T4		
ILW (kg)	3.62^{ab}	3.78^{ab}	4.26^{a}	3.50^{b}	17.38	0.0381
ADG (g/animal/day)	178.14^{c}	293.07^{a}	265.61^{ab}	232.74^{b}	20.48	0.0028
TG (kg/animal)	10.68^{c}	17.58^{a}	15.93^{ab}	13.96^{b}	20.48	0.0028
FW (kg)	14.31^{c}	21.37^{a}	20.20^{ab}	17.47^{bc}	18.53	0.0013
WHG (cm)	10.05^{b}	13.93^{a}	12.50^{ab}	12.59^{ab}	22.04	0.0099
BLG (cm)	19.33^{c}	25.71^{a}	24.45^{ab}	20.50^{bc}	16.56	0.001
TPG (cm)	6.24^{a}	7.67^{a}	5.87^{a}	6.15^{a}	26.91	0.9719

Note: Means followed by different letters on the row differ from each other by Tukey's test (p<0.05). Initial live weight (ILW), average daily gain (ADG), total gain (TG), final weight (FW), withers height gain (WHG), body length gain (BLG), thoracic perimeter gain (TPG), and coefficient of variation (CV) expressed in kg, g/animal/day, kg/animal, cm, and %. T1 = mineral salt; T2 = supplementation started 20 days before the estrus synchronization (ES) protocol; T3 = supplementation from confirmation of pregnancy (60 days after ES); T4 = supplementation in the final third of gestation (90 days after ES).

Table 5. Mean values of eggs per gram (EPG) of feces and the fecal score of lambs from ewes fed different supplementation strategies during gestation and lactation

Collection (days)	Treatments				CV (%)	p
	T1	T2	T3	T4		
30	351.66^{c}	33.33^{a}	41.66^{a}	258.33^{b}	23,67	0,007
60	863.75^{c}	469.16^{a}	545.83^{a}	133.33^{b}	20,08	0,003
FS (30)	2.25^{a}	2.5^{a}	2.33^{a}	2.5^{a}	9,24	0,075
FS (60)	3.75^{a}	3.0^{a}	3.16^{a}	3.16^{a}	10,37	0,086

None: Means followed by different letters on the row differ from each other by Tukey's test (p<0.05). Fecal score at 30 and 60 days (FS) and coefficient of variation (CV) expressed in %. T1 = mineral salt; T2 = supplementation started 20 days before the estrus synchronization (ES) protocol; T3 = supplementation from confirmation of pregnancy (60 days after ES); T4 = supplementation in the final third of gestation (90 days after ES).

Glucose levels increased from birth to 48 h (p<0.05) and were consistently higher in T2 lambs (up to 73.49 mg/100 mL). β-hydroxybutyrate levels differed among treatments (p<0.05), with lower values in lambs from T3 ewes, but all values remained within physiological ranges (Table 6).

Calcium, phosphorus, and magnesium concentrations increased over time (p<0.05) but remained within normal reference ranges for sheep. No clinically relevant deficiencies or excesses were observed among treatments (Table 6).

Cortisol concentrations did not differ among treatments (p>0.05), indicating similar stress responses at weaning. However, cortisol peaked at 12 h post-weaning (26.84 ng/mL; p<0.05), reflecting a transient physiological response rather than treatment-related stress (Table 7).

Table 6. Means for serum levels of metabolites in lambs from ewes fed different supplementation strategies during gestation

Variables	Treatments																	
	0 h				12 h				24 h				48 h					
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	RV*	CV (%)
Protein metabolism																		
Total protein (g/L)	40.90^{c}	46.01^{a}	44.47^{ab}	42.41^{bc}	43.11^{c}	52.10^{a}	48.39^{b}	45.02^{c}	54.94^{d}	72.15^{a}	62.12^{b}	58.69^{c}	52.24^{d}	70.89^{a}	61.88^{b}	57.33^{c}	60-79	4.69
Albumin (g/L)	24.07^{c}	27.08^{a}	26.18^{ab}	24.96^{bc}	25.37^{c}	30.67^{a}	28.49^{b}	26.50^{c}	22.59^{d}	29.67^{a}	25.54^{b}	24.13^{c}	21.48^{d}	29.15^{a}	25.44^{b}	23.57^{c}	26-42	5.14
Globulin (g/L)	16.81^{c}	18.92^{a}	18.28^{ab}	17.44^{bc}	17.52^{c}	21.42^{a}	19.90^{b}	18.51^{c}	32.34^{d}	42.47^{a}	36.56^{b}	34.55^{c}	30.75^{d}	41.76^{a}	36.42^{b}	33.75^{c}	35-57	4.41
Urea (mg/100 mL)	30.47^{d}	40.71^{a}	37.47^{b}	34.34^{c}	32.88^{d}	45.86^{a}	40.68^{b}	37.73^{c}	37.59^{d}	49.73^{a}	44.74^{b}	41.73^{c}	26.74^{d}	35.23^{a}	32.97^{b}	28.17^{c}	17-43	6.04
Energy metabolism																		
Glucose (mg/100 mL)	48.29^{d}	60.62^{a}	57.63^{b}	53.39^{c}	52.51^{d}	64.66^{a}	61.17^{b}	55.45^{c}	60.74^{c}	68.73^{a}	64.81^{b}	61.24^{c}	65.20^{c}	73.49^{a}	69.27^{b}	66.20^{c}	50-80	5.27
β-hydroxybutyrate (mmol/L)	0.20^{b}	0.22^{b}	0.14^{c}	0.31^{a}	0.16^{b}	0.17^{b}	0.12^{c}	0.25^{a}	0.13^{b}	0.15^{ab}	0.09^{c}	0.17^{a}	0.19^{b}	0.20^{ab}	0.14^{c}	0.23^{a}	0-10	11.59
Mineral metabolism																		
Calcium (mg/100 mL)	12.0^{ab}	12.4^{a}	11.9^{c}	11.7^{bc}	12.4^{a}	12.9^{a}	12.1^{a}	12.1^{a}	13.7^{a}	13.6^{a}	12.9^{b}	13.1^{a}	14.0^{a}	14.1^{a}	13.6^{b}	13.8^{b}	11.5-12.8	5.44
Phosphorus (mg/100 mL)	5.09^{b}	5.74^{a}	4.97^{b}	5.19^{b}	5.43^{c}	6.08^{a}	5.60^{bc}	5.86^{ab}	6.01^{b}	6.30^{a}	6.01^{b}	6.10^{ab}	6.40^{b}	7.04^{a}	6.40^{b}	6.65^{b}	5.0-7.3	4.61
Magnesium (mg/100 mL)	2.23^{c}	2.40^{a}	2.3^{b}	2.19^{d}	2.27^{c}	2.87^{a}	2.49^{b}	2.30^{c}	2.29^{d}	2.92^{a}	2.58^{b}	2.39^{c}	2.40^{d}	2.97^{a}	2.70^{b}	2.52^{c}	2.2-2.8	1.37

Note: Means followed by different letters on the row differ from each other by Tukey's test (p<0.05). *Reference value for sheep (Kaneko *et al*., 1997) and coefficient of variation (CV) expressed in %. T1 = mineral salt; T2 = supplementation started 20 days before the estrus synchronization (ES) protocol; T3 = supplementation from confirmation of pregnancy (60 days after ES); T4 = supplementation in the final third of gestation (90 days after ES).

Table 7. Cortisol levels in lambs submitted to the weaning challenge from ewes fed different supplementation strategies during gestation and lactation

Collection	Treatments				RV*	CV (%)	p
	T1	T2	T3	T4			
−20 min	19.26^{a}	20.25^{a}	19.96^{a}	17.17^{a}	6.0 to 20 ng/mL	11.65	0.00064
0 min	20.64^{a}	19.73^{a}	20.75^{a}	17.31^{a}			
1 h	24.66^{a}	23.46^{a}	22.80^{a}	19.50^{a}			
6 h	26.15^{a}	23.50^{a}	24.57^{a}	22.69^{a}			
12 h	31.42^{a}	25.40^{b}	26.56^{ab}	24.01^{b}			
24 h	28.35^{a}	24.18^{ab}	24.09^{ab}	21.80^{b}			
48 h	24.90^{a}	22.63^{a}	23.08^{a}	21.75^{a}			

Note: Means followed by different letters on the row differ from each other by Tukey's test (p<0.05). Collections: 20 minutes before separation of the ewe from its lamb(s) (−20 min), immediately before separation (0 min), and 1, 6, 12, 24, and 48 h after separation. *Reference value for sheep (Kaneko *et al*., 2008) and coefficient of variation (CV) expressed in %. T1 = mineral salt; T2 = supplementation started 20 days before the estrus synchronization (ES) protocol; T3 = supplementation from confirmation of pregnancy (60 days after ES); T4 = supplementation in the final third of gestation (90 days after ES).

DISCUSSION

The present results demonstrate that the timing of maternal energy and protein supplementation exerts a decisive influence on lamb performance, even when ewes exhibit adequate body condition scores. Supplementation initiated before breeding and after pregnancy confirmation resulted in superior birth and weaning weights, indicating that early nutritional modulation enhances fetal development and postnatal growth. Recent studies emphasize that nutritional adequacy before and during early gestation optimizes placental development and nutrient partitioning to the fetus, which may explain the superior outcomes observed in these treatments (Klein *et al*., 2021; Andrade *et al*., 2024).

The greater birth weight observed in lambs from ewes supplemented after pregnancy confirmation aligns with recent evidence showing that nutritional interventions during early to mid-gestation improve fetal growth without altering reproductive indices such as birth rate or litter size (Andrade *et al*., 2024; Brougham *et al*., 2024). These findings reinforce that maternal supplementation primarily improves offspring quality rather than reproductive efficiency, which remained unchanged across treatments in the present study.

Differences in colostrum intake among treatments highlight the biological relevance of maternal energy supply during gestation. Recent investigations have demonstrated that dietary energy density is a major determinant of colostrum yield and immunoglobulin concentration, directly influencing neonatal immunity and survival (Campos *et al*., 2019; Higano *et al*., 2025). In the current study, higher colostrum intake in lambs from early-supplemented ewes suggests improved mammary gland function and enhanced passive transfer of immunity.

From a metabolic perspective, supplementation with starch-rich concentrates likely increased ruminal propionate production, the primary gluconeogenic precursor in sheep. Enhanced hepatic gluconeogenesis increases glucose availability for lactose synthesis in the mammary gland, thereby improving colostrum quality and neonatal glycemia. Similar metabolic adaptations have been reported in recent studies evaluating maternal energy supplementation and neonatal metabolic profiles (Asadi *et al.,* 2024; Andrade *et al.,* 2024). The higher plasma glucose concentrations observed in lambs from supplemented ewes corroborate this mechanism.

The superior average daily gain and weaning weight observed in lambs from ewes supplemented before breeding and after pregnancy confirmation suggest that early nutritional strategies mitigate the adverse effects of fetal programming. Klein *et al*. (2021) highlighted that nutritional restriction during gestation induces long-term metabolic adaptations that compromise growth efficiency. Therefore, early supplementation likely prevented such programming effects, allowing lambs to express their genetic growth potential throughout lactation.

Enhanced morphometric development, particularly withers height and body length, further supports the role of early maternal supplementation in promoting skeletal growth. Recent studies have associated improved linear growth with better carcass traits and productive efficiency in lambs, emphasizing the importance of adequate prenatal nutrition for structural development (Silva *et al.,* 2019; Andrade *et al.,* 2024).

The significant reduction in eggs per gram of feces in lambs from supplemented ewes underscores the biological relevance of maternal nutrition on parasitological resistance. Recent evidence indicates that improved prenatal and early postnatal nutrition enhances immune competence, reducing parasite establishment and fecal egg output (Asadi *et al.,* 2024; Melo *et al.,* 2023). The lower EPG values observed in this study suggest that improved protein and energy status supported immune mechanisms involved in parasite control.

Improvements in protein and energy metabolic profiles, particularly higher concentrations of total protein, albumin, globulin, and glucose, reflect enhanced passive immunity transfer and metabolic stability in lambs from supplemented ewes. Contemporary studies highlight metabolic profiling as a reliable indicator of neonatal health and productive potential, linking adequate maternal nutrition to improved offspring resilience and performance (Klein *et al.,* 2021; Andrade *et al.,* 2024).

Cortisol responses to weaning did not differ among treatments, indicating that supplementation strategies did not exacerbate stress. The transient cortisol peak observed approximately 12 hours after weaning reflects a normal physiological response, consistent with recent findings demonstrating that nutritional adequacy attenuates excessive hypothalamic–pituitary–adrenal axis activation during management challenges (Melo *et al.,* 2023; Titto *et al.,* 2025).

Overall, the superior outcomes associated with supplementation initiated before breeding and after pregnancy confirmation highlight the importance of early and continuous maternal nutritional strategies. By improving fetal development, metabolic adaptation, and parasitological resistance, these strategies contribute to enhanced lamb health and productivity, in agreement with recent literature emphasizing precision nutrition during critical reproductive stages.

CONCLUSION

Supplementation strategies during gestation and lactation positively influenced birth weight, colostrum intake, weight gain, and morphometric measurements, EPG, metabolic profile, and cortisol levels of lambs. However, supplementation starting 20 days before the breeding season and after pregnancy was confirmed provided a higher anthelmintic effect and heavier lambs at weaning. Furthermore, there is a need for new research aimed at clarifying the effect of this management on the carcass characteristics of feedlot lambs.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used Grammarly to refine sentence structure and Elicit to assist literature searches. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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