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Effect of supplementation strategies for ewes during gestation

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ABSTRACT - This study aimed to evaluate the productive and reproductive performance and the nutritional metabolic condition of ewes supplemented at different stages of gestation in a synchronized reproduction system. Fourty Santa Inês × Dorper crossbreed ewes, pregnant with an average age of ten months and two previous births, were distributed in a completely randomized design, with four treatments: mineral salt, supplementation started 20 days before the estrus synchronization (ES) protocol, supplementation from confirmation of pregnancy (60 days after ES), and supplementation in the final third of gestation (90 days after ES). The adoption of supplementation strategies during the beginning, middle, or end of pregnancy changed the intake and digestibility of dry matter (g/day), with a reduction of 10.53 and 9.14%, respectively, for the mineral salt treatment, in addition to organic matter, crude protein, neutral detergent fiber, acid detergent fiber, non-fibrous carbohydrates, ether extract, and total digestible nutrients, as well as protein, energy, and mineral profiles. There was no difference in the number of ovulations and reproductive and productive performance using supplementation alternatives. Supplementation strategies during the gestation stages influence the intake and digestibility of nutrients, as well as the animal metabolic profiles. However, it does not change reproductive and productive performance.

Keywords: nutrition, performance, reproduction, sheep

1. Introduction

Adequate supplementation for pregnant ewes is a nutritional strategy that impacts the sheep production system, such as obtaining healthy lambs (Silva et al., 2019). However, attention must be paid to the metabolic and physiological adaptations that such a period requires, like increased nutritional requirements of females.

Oliveira et al. (2014), evaluating the nutritional metabolic condition of sheep in different stages of pregnancy supplemented with 1.2 kg of concentrate/animal/day, observed that in none of the stages of pregnancy there were severe deficiencies in the protein and energy supply of the diet, with the need for calcium adequacy and phosphorus towards the end of pregnancy. Therefore, knowing the metabolic profile and its relationship with nutritional status can be an important alternative for sheep farmers to improve performance indices.

Nutritional techniques, such as flushing, are strategies used to increase fertility. Flushing consists of the increase in nutritional intake, mainly with energy feeds, before mating, aiming to increase the ovulation rate (Macedo Junior et al., 2018).

The final third of pregnancy corresponds to the period in which 70% of the fetus is developed, and is therefore critical for ewe and lamb health. The energy demand is destined to maintain the mother and develop the fetus in a more delicate way than in other phases of pregnancy. At this time, the low availability of nutrients and energy can negatively compromise the animal's physiology after birth, being defined as fetal programming. Klein et al. (2021) reported that the effects of prenatal restriction have an impact on the animal's performance even when subjected to correct nutritional management after birth, resulting in lower weight gain and late age at slaughter, justifying supplementation of sheep in the final third of pregnancy.

In a study carried out by Brondani et al. (2020) ewes that received food supplementation throughout the gestational period had higher body weight, differing from the other experimental treatments. This result indicates that the nutritional supply promoted by supplementation met the physiological needs of the animals, which did not need to overload the body for its physiological maintenance.

Thus, this study aimed to evaluate the productive and reproductive performance and the nutritional metabolic condition of ewes supplemented at different stages of gestation in a synchronized reproduction system.

2. Material and Methods

The experiment was conducted in an experimental area of animal production in Brasília, DF, Brazil (15°56' to 15°59' S and 47°55' to 47°58' W) from February to July 2019. The research on animals was conducted according to the institutional committee on animal use (protocol 10/2019).

2.1. Animals, feed, and management

Forty crossbred Santa Inês × Dorper pregnant ewes with an average age of ten months and two previous births were distributed in a randomized design, according to age, weight, and body score, with four treatments and 10 replications. The ewes were maintained on a pasture with *Brachiaria brizantha* cv. Marandu, in an area of 5 ha. The area was divided into seven paddocks, where the animals grazed together to remove the effect of pasture. Supplements formulated according to NRC (2007) were provided daily at 16:00 h in an amount equivalent to 1% of body weight (BW), and this value was adjusted every 15 days when the ewes were weighed. The animals were confined in stalls, where they received supplementation according to the treatment. After intake, they were kept in the same pen with water *ad libitum*, where they spent the night. Due to the stage of pregnancy, the needs for metabolizable energy and proteins were different (Table 1).

The treatments consisted of only mineral salt specific for sheep, only supplementation started 20 days before the estrus synchronization (ES) protocol, only supplementation from confirmation of pregnancy (60 days after ES), and only supplementation in the final third of gestation (90 days after ES). Ewes were identified with paint on the lumbar region according to their treatment aiming to generate less stress. The ewes were synchronized with the presence of a Dorper sire, causing the effect of male with release of pheromones to stimulate estrus at the start of the breeding season. Oil and powder paint were used in the breast region of the sire to control which sheep were covered. The type of mounting was natural, which consists of releasing the sire into the batch of females. A ratio of one male to up to fifty females is recommended. Gestation was diagnosed about 55 days after mating using an Aloka 500 ultrasound device (Aloka Co. Ltd., Japan) coupled to a 5 MHz linear transducer positioned in the rectal region.

Table 1 - Centesimal and nutritional composition of concentrate and pasture (*Brachiaria brizantha* cv. Marandu) in terms of natural matter (%) supplied to pregnant ewes

Item (%)	Treatment[1]			
	T1	T2	T3	T4
Soybean meal	–	56.37	56.37	36.04
Corn	–	40.24	40.24	61.53
Calcitic limestone	–	–	–	0.59
Vitamin-mineral supplement[2]	+	3.39	3.39	1.84
Nutrient	T2; T3		T4	
	Supplement	Pasture	Supplement	Pasture
DM (% NM)	96.61	93.05	92.75	94.79
CP (% DM)	29.98	9.55	23.14	4.70
NDF (% DM)	18.33	75.56	25.75	71.11
ADF (% DM)	4.98	35.25	5.61	38.84
EE (% DM)	2.74	4.60	2.65	2.85
MM (% DM)	7.33	8.96	5.61	6.22
CHOt (% DM)	59.91	80.54	68.58	86.20
NFC (% DM)	41.58	4.98	42.83	15.09
NDIN (% DM)	13.15	3.55	21.94	2.82
ADIN (% DM)	6.68	1.26	9.37	0.96
TDN (% DM)	58.42		68.43	

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] 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).

[2] 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.), 1000 mg; manganese (min.), 3000 mg; cobalt (min.), 80 mg; zinc (min.), 5000 mg; iodine (min.), 60 mg; selenium (min.), 10 mg; vitamin A (min.), 50,000 IU; vitamin E (min.), 312 IU.

2.2. Reproductive performance

Reproductive performance was evaluated using measurements of pregnancy rates ((number of pregnant ewes/number of mated ewes) × 100), parturition rates ((number of lambed ewes/number of mated ewes) × 100), and birth rates ((number of lambs born/number of mated ewes) × 100).

The ewes in estrus had the number of ovulations determined by transrectal ultrasonography, using a CHISSON 500 device with a 5 MHz linear transducer, which was attached to a rigid extension (3 cm in diameter and 16 cm in length) that allows its manipulation via the rectum. Ovulation was determined by the disappearance of the dominant follicles present on the surface of the ovaries in the final third of estrus or heat, between 24 and 30 h after the beginning of estrus, being the phase of the estrous cycle in which the female accepts mating, letting itself be mounted by the male (Saunders et al., 2012).

2.3. Weight variation and body score condition

Performance was evaluated by weighing all dams every 15 days to obtain the average daily weight gain (ADG, g/animal/day), calculated by the difference in animal weight between weighings divided by the number of days between each weighing. Total weight gain (kg/animal) was obtained by the difference between the initial live weight and the day of birth.

Body condition score (BCS) was determined by palpations of the spine after the last rib, above the kidney region, according to the methodology described by Osório and Osório (2005), assigning values from 1 to 5, with 1 corresponding to animals excessively thin animals and 5 to excessively fat animals, also considering intermediate values in variations of 0.25.

2.4. Produced biological mass

The produced biological mass corresponds to the total weight of lambs produced by the female, originating from simple or twin dishes relative to the dam's body weight (Geraseev et al., 2006).

2.5. Nutrient intake and digestibility

Total dry matter (DM) intake was obtained by the sum of supplement dry matter intake (SDMI) and the estimated forage dry matter intake (FDMI), estimated through fecal excretion and the use of markers. Nutrient intake was obtained by the sum of SDMI and FDMI, multiplied by the respective nutrient concentrations. Supplement intake was determined in each gestational period, that is, beginning (50 and 60 days), middle (80 and 90 days), and end (130 and 140 days), by the ratio between the amount of DM supplied and the DM of leftovers. Consumption in metabolic weight corresponds to live weight raised to the power of 0.75 ($LW^{0.75}$).

The estimate of fecal excretion per animal and experimental period was obtained using an external marker of titanium dioxide (TiO_2), given orally administered in capsule form to the animals in daily doses of 5 g during nine days. Fecal samples were taken directly from the rectum of the animals on the 6th, 7th, 8th, and 9th day of each experimental period to determine the TiO_2 concentration. The samples were placed in identified plastic bags and frozen at −10 °C for further analysis. Titanium concentration was determined following the methodology described by Detmann et al. (2012), in which the fecal samples were digested in sulfuric acid, and the reading was performed in a UV spectrophotometer at 410 nm, using a calibration curve with known titanium concentration.

Fecal dry matter excretion was calculated according to Burns et al. (1994), based on the ratio between the amount of indicator provided and its concentration in the feces: fecal excretion (g DM/day) = [Amount of indicator provided (g/day)/Indicator concentration in the feces (%)] × 100. Then, FDMI was estimated using the indigestible neutral detergent fiber (iNDF) as an internal indicator (Casali et al., 2008) and applying the equation proposed by Detmann et al. (2012): FDMI = [(FE × CIF) – IS]/CIFO, in which FE is the fecal excretion (kg/day), CIF is the concentration of the indicator in the feces (kg/kg), IS is the indicator present in the supplement (kg/day), and CIFO is the concentration of the indicator in the forage (kg/kg).

Nutrient digestibility (ND) was obtained by the following formula: [ND = (ingested DM × % Nutrient) – (excreted DM × % Nutrient) × 100] / (ingested DM × % Nutrient).

The total digestible nutrients (TDN) content was calculated considering the intake and fecal excretion of nutrients, using the following equation: TDN (%) = DCP + (DEE × 2.25) + FDND + DNFC, in which DCP is the digestible crude protein, DEE is the digestible ether extract, DNDF is the digestible neutral detergent fiber, and DNFC is the digestible non-fiber carbohydrates (NRC, 2001).

2.6. Metabolic profiles

A single blood collection was performed in the morning in each ewe and at different stages of gestation to allow environmental comfort for the animals. Blood samples were obtained through jugular venipuncture, using a vacuum tube (Vacutainer®) and specialized needles. Two types of duly identified vacuum tubes were used: one containing sodium fluoride for energy analysis and another without it for obtaining serum. The collections strictly followed the regime of intervals predetermined by the experimental treatments, the initial phase of gestation is between 50 and 60 days, the middle between 80 and 90 days, and the final between 130 and 150 days. After collection, the samples remained at rest until complete coagulation, followed by centrifugation for 10 min at 1400 rcf (g) and transference of the serum 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 the companies Labtest Diagnóstica® S.A., BioClin®, and Randox®, following their respective protocols. Reading was performed in an automated COBAS MIRA PLUS (Roche®, Germany) equipment, specific for biochemical analysis. The evaluated metabolites were protein (total protein, albumin, globulin, and urea), energy (glucose and βhydroxybutyrate (BHB)), and mineral (magnesium (Mg^{2+}), calcium (Ca^{2+}), and phosphorus (P^{-})).

2.7. Chemical analysis

Samples of the supplied diet, leftovers, and feces were partially 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 DM according to the AOAC (2005) (method 930.15), mineral matter (MM; method 942.05), crude protein (CP; method 984.13), and ether extract (EE; method 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 and acid detergent insoluble nitrogen were determined following the recommendations by Licitra et al. (1996). The percentage of total carbohydrates was calculated using the equation proposed by Sniffen et al. (1992), while the non-fibrous carbohydrates (NFC) were determined using the equation recommended by Weiss (1993).

2.8. Statistical analysis

Data were subjected to analysis of variance, considering the type of diet (feeding strategy) of ewes and gestational stages as sources of variation. The statistical model used was:

$$Y_{ijk} = \mu + T_i + B_j + \varepsilon_{ij} + D_k + TD_{ik} + \varepsilon_{ijk},$$

in which μ = general average; T_i = effect of treatment (i = 1, 2, 3, 4); B_j = effect of the score range (j = 1, 2, 3, 4, 5); ε_{ij} = experimental error associated with the plot, considered independent and identically distributed from a zero mean normal and variance σ^2; D_k = effect of time (k = 20 days before the estrus synchronization (ES) protocol, supplementation from confirmation of pregnancy (60 days after ES), and supplementation in the final third of gestation (90 days after ES); TD_{ik} = interaction between treatment and time; and ε_{ijk} = experimental error associated with the subplot, considered independent and identically distributed from a zero mean normal and variance σ^2. The comparison between the effects of the type of feeding management and gestation was performed using Tukey's test at 0.05 probability, using the statistical procedures PROC GLM of SAS (Statistical Analysis System, version 9.2.). The Kruskal-Wallis non-parametric test was used for the variable BCS, with slicing or not of the interaction according to the significance.

3. Results

Supplementation strategies affected DM and organic matter (OM) intake by the animals (Table 2). Dry matter intake ranged from 2,056 to 1,965 g/day and OM intake from 1,881 to 1,813 g/day, being higher ($P<0.05$) in the beginning and middle of gestation for animals that received supplementation from 20 days before the estrus synchronization ES protocol. This behavior was already expected since these animals had higher TDMI (forage + concentrate) than the other treatments. There was an effect ($P<0.05$) of supplementation on DM and OM intake in the final third of gestation, with higher averages for the group that received supplementation after pregnancy confirmation, that is, 2,126 and 1,997 g/day, respectively.

There was an increase ($P<0.05$) in CP intake (g/day, $g/kg^{0.75}$, and % BW) with the supplementation due to the effect on DM and OM intake (Table 2) and the difference in the isonitrogenous profile of the diets offered (Table 1).

A significant increase ($P<0.05$) in the average NDF intake was observed for the control group (Table 2), which can be explained by the pasture quality during the experiment (Table 1) and DM intake (Table 2). However, there were no differences among treatments in early gestation. The higher intake of roughage in the treatment that received mineral supplementation during gestation increased ($P<0.05$) ADF intake among periods (Table 2), with an average of 641.0 g/day. However, no differences in the early stage of gestation were observed.

Table 2 - Nutrient intake as a function of supplementation strategy for ewes at different stages of gestation

Variable	Beginning				Middle				End				CV (%)
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	
DMI													
g/day	1,584b	2,056a	1,425b	1,623b	1,947ab	1,965a	1,690b	1,728ab	1,774b	1,830b	2,126a	1,869ab	12.06
g/kg$^{0.75}$	41.97b	53.91a	38.45b	42.83b	48.09a	45.32a	41.28a	41.62a	42.37ab	39.97b	49.68a	43.36ab	13.57
%BW	3.148b	4.043a	2.884b	3.212b	3.607a	3.399a	3.096a	3.121a	3.178ab	2.998b	3.726a	3.252ab	13.57
OMI													
g/day	1,442b	1,881a	1,297b	1,477b	1,794ab	1,813a	1,560b	1,592ab	1.664b	1,719b	1,997a	1,756b	11.82
g/kg$^{0.75}$	38.21b	49.32a	35.00b	38.99b	44.30a	41.82a	38.11a	38.34a	39.74ab	37.56b	46.67a	40.74ab	13.18
%BW	2.865b	3.699a	2.625b	2.924b	3.323a	3.136a	2.858a	2.875a	2.980ab	2.817b	3.500a	3.056ab	13.18
CPI													
g/day	119.8b	285.5a	107.8b	122.8b	186.1c	306.2a	279.9b	165.1c	83.5b	188.7a	202.6a	190.5a	9.98
g/kg$^{0.75}$	3.17b	7.47a	2.90b	3.24b	4.59b	7.07a	6.86a	3.97b	1.99b	4.14a	4.73a	4.42a	12.44
%BW	0.238b	0.560a	0.218b	0.243b	0.344b	0.530a	0.514a	0.298b	0.149b	0.311a	0.355a	0.331a	12.44
NDFI													
g/day	1,197a	1,222a	1,077a	1,226a	1,443a	1,132b	0.929c	1,280ab	1,262a	1,049b	1,259a	1,077ab	14.84
g/kg$^{0.75}$	31.72a	32.08a	29.05a	32.36a	35.63a	26.07bc	22.62c	30.83ab	30.13a	22.84b	29.43a	24.98ab	15.65
%BW	2.379a	2.406a	2.179a	2.427a	2.672a	1.955bc	1.697c	2.312ab	2.260a	1.713b	2.207a	1.873ab	15.65
ADFI													
g/day	558.69a	549.62a	502.67a	572.39a	675.05a	509.10b	410.86c	599.5ab	689.26a	526.03b	640.97a	541.34b	14.83
g/kg$^{0.75}$	14.80a	14.43a	13.55a	15.10a	16.66a	11.71a	9.98a	14.42a	16.46a	11.43c	14.97ab	12.55bc	15.46
%BW	1.110a	1.082a	1.016a	1.132a	1.250a	0.878b	0.749b	1.081a	1.234a	0.857c	1.122ab	0.941bc	15.46
NFCI													
g/day	52.16b	289.70a	46.93b	53.44b	97.06b	310.13a	296.42a	86.13b	267.87c	430.6b	475.35a	436.6b	8.22
g/kg$^{0.75}$	1.38b	7.56a	1.26b	1.41b	2.39b	7.18a	7.28a	2.07b	6.39b	9.44a	11.11a	10.12a	15.94
%BW	0.103b	0.567a	0.094b	0.105b	0.179b	0.538a	0.546a	0.155b	0.479b	0.708a	0.833a	0.759a	15.94
EEI													
g/day	73.03b	83.99a	67.63b	74.82ab	68.12a	64.38ab	55.14b	60.45ab	50.69b	51.19b	60.81a	52.31ab	13.30
g/kg$^{0.75}$	1.93ab	2.20a	1.83b	1.97ab	1.68a	1.48ab	1.34b	1.45ab	1.21a	1.11a	1.42a	1.21a	15.13
%BW	0.145ab	0.165a	0.137b	0.148ab	0.126a	0.111ab	0.100b	0.109ab	0.090a	0.083a	0.106a	0.091a	15.14
TDNI (kg)	1.001b	1.418a	0.891b	1.058b	1.244b	1.447a	1.152b	0.835c	1.146b	1.249b	1.493a	1.299b	13.21

DMI - dry matter intake; OMI - organic matter intake; CPI - crude protein intake; NDFI - neutral detergent fiber intake; ADFI - acid detergent fiber intake; NFCI - non-fibrous carbohydrates intake; EEI - ether extract intake; TDNI - total digestible nutrient intake; CV - coefficient of variation.
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).
Means followed by different letters in the row differ from each other by Tukey's test ($P<0.05$).

The average NFC intake was influenced (P<0.05) by diet, with increasing effect. This result can be justified by the increase in NFC concentration in diets with the supplement (Table 1), as roughage results in an increase in the fiber content of the diet and a reduction in non-fiber carbohydrates.

There was an effect (P<0.05) on EE intake (g/day, g/kg$^{0.75}$, and % BW) with the supplementation strategy due to the presence of an effect on DM intake (Table 2) and the heterogeneous profile of the offered diets (Table 1).

Total digestible nutrients intake varied (P<0.05) along the gestation stages (Table 2), as verified for DM and OM intake.

Dry matter (DMD) and OM digestibility (OMD) was influenced (P<0.05) by the treatments in the gestational stages (Table 3). However, ewes supplemented 20 days before the ES protocol showed higher coefficients in the initial period. In contrast, the middle of gestation showed lower DMD for the group supplemented 90 days after ES, with no effect on OMD. Ewes from the control treatment in the final third had a lower OMD (66.73%) but with no effect on DMD (67.96%).

A higher effect (P<0.05) of the supplementation was observed on the CP digestibility coefficient. Possibly, it reflects the lack of similarity in the CP contents of the diets (Table 1).

There was an effect (P<0.05) on NDF digestibility among treatments (Table 3), with an average of 67.13%. Despite this, no significant difference was observed among treatments in the initial and final stages of gestation, possibly reflecting the quality of roughage fiber during the study. However, in the middle of gestation, ewes supplemented in the final third had lower NDFD (40.47%).

No significant difference (P>0.05) was observed in the initial and final stages of gestation. In the middle of gestation, ewes supplemented with mineral salt had a higher ADF digestibility (69.24%).

The NFC digestibility was influenced (P<0.05) by supplementation strategies during gestation (Table 3). However, animals supplemented 20 days before the ES protocol had higher coefficients at the initial stage (85.91%). Despite this, groups supplemented in the final third of gestation had lower and higher NFC digestibility in the middle and end of gestation, with averages of 69.10 and 89.71%, respectively.

An effect (P<0.05) of the supplementation addition on the EE digestibility coefficient was observed (Table 3), with averages of 45.12, 29.02, and 58.16%, respectively, in the gestational periods. Possibly, it reflects the lack of similarity in the EE contents of diets. Furthermore, ewes supplemented 20 days before and 90 days after ES had higher digestibility in the initial stage and in the middle of the period; however, only the second treatment had the greatest value at the end of gestation.

Supplementation did not affect (P>0.05) %TDN in the initial and final thirds of gestation, with averages of 62.25 and 68.24%, respectively. However, there was an increase (P<0.05) in the middle of gestation, with an average of 71.81% TDN (Table 3) as a function of supplementation.

The number of ovulations between the ovaries and the total corpus luteum did not differ (P>0.05) between ewes subjected or not to supplementation strategies (Table 4), with average counts of 0.7, 0.8, and 1.65, respectively.

Pregnancy, parturition, and birth variables (Table 4) were not influenced (P>0.05) by supplementation in pre-breeding, with means of 100, 100, and 1.25%, respectively. The absence of the effect of supplementation, offered from 20 days before the estrus synchronization ES protocol, indicates that the nutritional condition of ewes was adequate for all treatments to guarantee good reproductive performance.

The supplementation strategy did not affect (P>0.05) the final live body weight, daily weight gain, and total weight gain of ewes despite the observed changes in nutrient intake (Table 5).

No effect (P>0.05) of treatments was observed on the produced biological mass (Table 5), with an average of 0.073 kg. Body condition score (Table 5) was not affected by supplementation (P>0.05), with an average of 2.93. Thus, the similarities in the final BCS observed in this study occurred because the supplementation did not reduce the daily, total, and final weight gains (Table 5).

Table 3 - Digestibility coefficient of nutrients as a function of the supplementation strategy for ewes at different stages of gestation

Variable	Beginning				Middle				End				CV (%)
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	
DMD (%)	63.32b	71.85a	61.71b	64.93b	64.01a	67.00a	66.53a	59.14b	63.99a	67.92a	70.23a	69.73a	4.77
OMD (%)	64.73b	72.82a	62.97b	65.89b	65.92a	68.84a	68.38a	61.12a	66.73b	70.27ab	72.60a	72.23a	4.45
CPD (%)	47.19b	77.96a	44.77b	49.85b	56.76b	74.15a	77.00a	50.90c	23.29b	70.66a	68.98a	68.24a	6.48
NDFD (%)	63.09a	60.88a	60.66a	61.69a	70.85a	67.93b	67.06b	40.47c	67.47a	65.31a	68.61a	66.86a	7.05
ADFD (%)	69.23a	68.98a	68.56a	70.00a	69.24a	64.71ab	63.88b	63.88b	69.16a	64.44a	69.42a	67.20a	6.72
NFCD (%)	61.74b	85.91a	61.82b	60.58b	78.24a	85.07a	80.12a	69.10b	79.04b	82.11ab	86.62ab	89.71a	8.04
EED (%)	43.62b	48.35a	39.97b	48.54a	26.83b	27.74ab	24.06b	37.63a	54.96b	70.39a	57.58b	49.74b	15.19
TDND (%)	63.55a	69.09a	62.55a	65.68a	63.84b	74.57a	69.06ab	52.39c	64.54a	68.48a	70.33a	69.61a	12.06

DMD - dry matter digestibility; OMD - organic matter digestibility; CPD - crude protein digestibility; NDFD - neutral detergent fiber digestibility; ADFD - acid detergent fiber digestibility; NFCD - non-fibrous carbohydrates digestibility; EED - ether extract digestibility; TDND - total digestible nutrient digestibility; CV - coefficient of variation.
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).
Means followed by different letters in the row differ from each other by Tukey's test ($P<0.05$).

Table 4 - Number of ovulations and reproductive performance according to supplementation strategies

Item	Treatment[1]				P-value
	T1	T2	T3	T4	
Left ovary	0.9a	0.6a	0.9a	0.7a	0.8633
Right ovary	0.8a	0.8a	1.2a	0.7a	0.0988
Total corpus luteum	1.7a	1.4a	2.1a	1.4a	0.2708
Pregnancy (%)	100	100	100	100	-
Parturition (%)	100	100	100	100	-
Birth (%)	1.20a	1.20a	1.20a	1.4a	0.4949

[1] 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).
Means followed by different letters in the row differ from each other by Tukey's test (P<0.05).

Table 5 - Live weight gain, produced biological mass, and body condition score (BCS) according to supplementation strategies

Item	Treatment[1]				CV (%)	P-value
	T1	T2	T3	T4		
FLW (kg)	65.51a	71.65a	69.72a	66.70a	13.52	0.1735
ADG (g/animal/day)	98.71a	130.42a	134.29a	102.75a	28.10	0.0899
TLWG (kg/animal)	14.81a	19.57a	20.14a	15.41a	28.09	0.0892
BM (kg)	0.072a	0.069a	0.076a	0.078a	20.61	0.0017
Final BCS	2.5a	3.50a	3.0a	2.75a	10.47	0.1276

FLW - final live weight; ADG - average daily gain; TLWG - total live weight gain; BM - biological mass; CV - coefficient of variation (CV).
[1] 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).
Means followed by different letters in the row differ from each other by Tukey's test (P<0.05).

An increase was observed among the evaluated groups, with a difference (P<0.05) for protein metabolites (Table 6), which remained within the reference limits, except for urea in the final third of gestation, which showed increased levels for the group that received supplementation started 20 days before the ES protocol.

Mean globulins were different (P<0.05) between times and treatments, averaging 34.77, 37.80, and 37.75 g/L (Table 6). The values are within the normality range for sheep (from 35 to 57 g/L). However, a higher serum concentration of globulin was observed in the initial and final stages of gestation for animals supplemented from 20 days before the ES protocol (Table 6).

Glucose means were different among treatments (P<0.05), showing a numerically increasing concentration with the advancement of gestation, especially in the final third, in which animals supplemented 20 days before the ES protocol had an average of 55.42 mg/100 mL, which is within the minimum normal value for sheep, that is, 50 to 80 mg/dL (Table 6).

The variation of BHB was significant (P<0.05) in the three stages of gestation (Table 6), with increasing levels. It also remained close to the reference standards for sheep (<0.6 mmol/L).

A significant difference (P<0.05) was observed with an increase and decrease in serum calcium levels (Table 6), but animals supplemented with mineral salt were within the reference values, with an average of 11.62 mg/100 mL, showing that the balance of this mineral was adequate for the gestational period.

The serological concentration of phosphorus increased during the beginning and middle of gestation, with an average of 6.23 mg/100 mL, but a significant decrease (P<0.05) was observed in the final third of pregnancy (5.53 mg/100 mL) (Table 6).

Serum magnesium levels remained within the reference standards (Table 6), with an increasing effect. However, they were statistically different (P<0.05) among groups, with the highest concentrations observed for animals that received supplementation from 20 days before the estrus synchronization ES protocol.

Table 6 - Metabolic profile of ewes at different stages of gestation according to supplementation strategy

Variable	Beginning				Middle				End				RV[1]	CV (%)
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4		
Protein metabolism														
Total proteins (g/L)	60.92b	69.89a	61.74b	63.12b	62.40b	72.54a	70.77a	64.10b	66.55c	75.89a	72.22b	66.39c	60-79	3.18
Albumin (g/L)	27.78b	31.87a	28.15b	28.78b	27.42b	31.88a	31.10a	28.17b	30.77c	35.10a	33.40b	30.71c	26-42	3.18
Globulin (g/L)	33.14b	38.02a	33.59b	34.34b	34.97b	40.65a	39.66a	35.92b	35.76c	40.78a	38.81b	35.68c	35-57	3.35
Urea (mg/100 mL)	24.65b	36.12a	26.53b	26.14b	25.86c	38.46a	31.89b	27.30c	29.25c	46.00a	36.41b	32.03c	17-43	8.10
Energy metabolism														
Glucose (mg/100 mL)	33.87c	45.13a	37.85b	35.19bc	36.19c	48.28a	40.15b	38.04bc	40.98c	55.42a	47.87b	32.27d	50-80	4.99
B-hydroxybutyrate (mmol/L)	0.13d	0.22c	0.76a	0.58b	0.12d	0.22c	0.74a	0.58b	0.16d	0.26c	0.78a	0.62b	<0.6	8.34
Mineral metabolism														
Calcium (mg/100 mL)	11.69a	10.58b	9.68c	8.71d	11.79a	10.70b	9.80c	8.81d	11.40a	10.30b	9.40c	8.45d	11.5-12.8	6.15
Phosphorus (mg/100 mL)	6.21b	7.04a	5.82b	5.08c	6.40b	7.20a	6.50b	5.59c	5.10c	6.30a	5.70b	5.04c	5.0-7.3	6.09
Magnesium (mg/100 mL)	2.21b	2.55a	2.19bc	2.11c	2.21b	2.55a	2.20bc	2.11c	2.22b	2.57a	2.21b	2.12c	2.2-2.8	5.35

CV - coefficient of variation.
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).
[1] Reference values for sheep (Kaneko et al., 1997).
Means followed by different letters on the row differ from each other by Tukey's test (P<0.05).

4. Discussion

The supplement increased the supply of nutrients to the animals, which increases the CP and NFC intake in the different periods of gestation, thus leading to higher DM and OM intake for the supplemented groups.

Sousa et al. (2018), evaluating the impact of supplementation levels of 0.5 to 1.5% concentrated based on body weight in the reproductive phases (gestation and lactation) of Santa Inês hair sheep, observed that sheep fed 1.5% of BW had higher dry matter intake (DMI). This behavior was already expected since these animals had higher TDMI (forage + concentrate) than the other treatments.

According to Silva et al. (2019), intake becomes limited by the physiological demand for energy if energy density is high or fiber concentration is low compared with the requirements. This finding is probably related to the energy density of the diet, which is lower based on roughage, with a consequent lower amount of corn, starch, and energy supply, which justifies the increase in intake to meet energy requirements (Table 2).

The fact that the ewe is in the final third of gestation, a stage in which the gravid uterus is compressing the gastrointestinal tract, may have significantly affected its intake. In addition, supplements increase energy density, which, in turn, may also affect intake. Geraseev et al. (2023) stated that satiety is due to the caloric density of the diet. This fact was not observed for animals supplemented in the final third of gestation, which had higher DMI and OM intake, even when large amounts of soluble carbohydrates were used in the diets.

According to NRC (1985), DMI for ewes with 60 kg of live weight in the last four weeks of gestation should be 1.6 kg or 2.8% of live weight. In the study, the average DMI was 1.89 kg, or 3.28% of live weight, showing that the results observed in the present study are within the recommended by this committee.

Similarly, Macedo Junior et al. (2012) observed an NDFI of 34.37 g/kg$^{0.75}$ when evaluating the influence of different roughage and concentrate ratios (10, 20, 30, and 40% of roughage) in the diet of sheep on intake, glycemia, and apparent digestibility in pregnant females. Intake in the present study was 28.97 g/kg$^{0.75}$, showing that the results are within the range recommended for animals on pasture.

The DMD and OMD of animals that received supplementation 20 days before ES may have occurred due to the higher energy intake provided to animals in this treatment, which possibly resulted in an adequate balance of carbohydrates and nitrogenous compounds available in the rumen, which contributed to the development of cellulolytic microorganisms, responsible for the degradation of cell wall components, thus resulting in increased digestibility. Sousa et al. (2018) verified that an increase in the energy value of diets improved the digestibility coefficients of DM, OM, total carbohydrates, and NFC of Santa Inês ewes supplemented (0.5 and 1.5% live weight) on pasture pre-and postpartum.

The reduction of NFC affects the development of the rumen microbiota (Zhang et al., 2020). Geraseev et al. (2023), when evaluating the effects of including sunflower bran on performance, nutrient digestibility, and respirometric parameters in Dorper × Santa Inês sheep, observed a decrease in the *in vivo* digestibility of DM, NFC, and NDF with the inclusion of bran, which may be associated with the reduction of NFC in diets and roughage quality.

Flushing is one of the nutritional management tools with an impact on reproduction and is perhaps the most widespread among technicians and producers. This tool provides supplementation with a high nutritional level during a short period, which includes before and during the breeding season (Macedo Junior et al., 2018). However, significant increases in the ovulation rate through flushing will only be obtained if ewes are subjected to a period of supplementation equivalent to one estrous cycle before the beginning of the breeding season, with no effect when ewes present satisfactory body condition (Yıldırır et al., 2022). Thus, ewes could have better reproductive performance throughout the experimental period by receiving supplementation starting 20 days before the ES protocol until lambing, however, without effect because the sheep have satisfactory nutritional and body conditions.

According to Saunders et al. (2012), the minimum body condition for Santa Inês sheep to manifest their reproductive capacity is 2.5. Thus, this could probably have been the reason for the lack of difference in the number of ovulations, as ewes already started the experiment with a BCS of 3.7, above the established threshold of 2.5.

Khaiseb et al. (2022) evaluated the interactions between nutrition and the ram effect on the control of ovarian function in Merino sheep. They observed that supplementation before the introduction of rams did not affect the ovulation rate in ram-induced ovulation, with an average of 1.66, that is, close to that work with average counts of 1.65 follicles.

Yıldırır et al. (2022) found that ewes with body scores higher than or equal to 2.5 had better reproductive performance than animals with a lower body condition. Thus, the importance of using BCS as a herd nutritional monitoring tool is evident (González-Maldonado et al., 2023).

According to NRC (2007), ewes at the end of gestation have a CP requirement of 193 g/day. In the present study, ewes had an average protein intake of 186.60 g/day, which is close to the recommended intake for the three stages of gestation. Furthermore, the supplementation strategy increased CP intake (g/day, g/kg$^{0.75}$, and % BW) (Table 2) and CP digestibility coefficient (Table 3). It is possibly a reflection of the lack of similarity in CP contents of the diets (Table 1) and, consequently, changed the serum levels of total protein (Table 6). The concentration of total proteins in the blood reflects the protein nutritional status reliably (Castillo et al., 2016).

When evaluating the nutritional metabolic condition of sheep at different stages of pregnancy, Oliveira et al. (2014) observed that the concentrations of total proteins, albumin, and globulins did not differ from each other. In our study, the protein concentration was close to the lower limit reported by Kaneko et al. (1997).

The highest urea concentrations, with averages ranging from 38.46 to 46 mg/100 mL in the middle and final third of gestation (Table 6) for animals supplemented from 20 days before the ES protocol, are related to the higher protein intake. The higher the dietary protein intake, the higher the blood urea level, and, on the other hand, blood urea levels reach low values when protein intake is insufficient (Barbosa et al., 2023).

Serological concentrations of glucose differed statistically, showing a numerically increasing concentration with the advancement of gestation and below the minimum normal value for sheep, which is 50 to 80 mg/dL. Nutrient requirements, especially energy, increase in the last six weeks of gestation due to fetal growth, in which the fetus reaches up to 80% of its final size and weight (McCarty et al., 2020). Oliveira et al. (2014) found glucose values between 38.58 and 44.29 mg/100 mL at different gestational stages of pregnant Santa Inês ewes reared in a semi-intensive system. These results are below the reference values defined by Kaneko et al. (2008) but are within the range defined in this study, with an average of 40.93 mg/mL. Song et al. (2018) found lower values of plasma glucose in sheep subjected to a diet with reduced energy intake.

The variation in BHB can be attributed to the genetic and adaptive characteristics of these animals, related to the rearing system and feed. The ewes had good BCS (3 to 3.5), thus allowing body energy reserves. These characteristics are extremely important to control metabolic disorders such as toxemia (Mendes et al., 2021).

Serum calcium levels were always below the reference values for ewes that received supplementation. Oliveira et al. (2014), working with sheep in different thirds of gestation (beginning, middle, and end), found calcium values of 9.39, 9.22, and 8.86, respectively, and observed a decrease in this mineral in the final third of gestation, which was attributed to the fetal growth and milk synthesis. In this research, the lowest value of this mineral was observed at the end of pregnancy (9.88 mg/100 mL).

Phosphorus serum concentration decreased during the final third of gestation, being different from the concentration of animals in early gestation. It may indicate a phosphorus deficiency in the soil and, consequently, the forage in the region, considering the phosphorus requirement of ewes in the final third of gestation. Oliveira et al. (2014) worked with ewes at different stages of gestation (initial, medium, and final) and found phosphorus values of 7.15, 7.42, and 5.88 mg/100 mL, respectively. The low values were attributed to phosphorus deficiency in the soil and, consequently, in the pasture ingested by the animals. Feijó et al. (2014) observed mean values of 5.33 mg/dL for non-pregnant females. Santos et al. (2014) observed that phosphorus levels decreased seven days before parturition, maintaining this behavior up to 15 days after parturition. Therefore, the balance of this mineral in the present study was adequate for the gestational period, being within the reference standards (Table 6).

Hypomagnesemia, a nutritional disease, usually caused by low magnesium intake in the diet, can lead to tetany, hyperexcitability, retained placenta, abnormalities of ruminal digestion, and decreased milk production (Moreira et al., 2019). Normally, serological alterations are due to a deficiency of this mineral in the soil and, therefore, in the pasture. However, the magnesium balance in the present study is within the reference standards (Table 6).

Nutritional restriction in the prenatal phase has negative effects during the postnatal life of the animal, causing an increase in the age at slaughter of lambs, a reduction in the quality of the final product, a delay in the age at puberty of lambs, and low reproductive efficiency of the herd (Siqueira et al., 2020). Therefore, it is essential to meet the female's nutritional requirements during the different phases of the gestational period, due to the impact on the productive and reproductive performance of her offspring.

5. Conclusions

Supplementation strategies during the gestation stages influence the intake and digestibility of nutrients, as well as the animal metabolic profiles. However, they do not change reproductive and productive performance.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Silva, N. C. and Ribeiro, M. D. **Data curation:** Silva, N. C. and Cabral Filho, S. L. S. **Formal analysis:** Cabral Filho, S. L. S.; Geraseev, L. C. and Silva, B. D. M. **Funding acquisition:** Silva, N. C and Ribeiro, M. D. **Investigation:** Silva, N. C.; Ferreira, M. S.; Fonseca, A. A. and Silva, B. D. M. **Methodology:** Silva, N. C.; Cabral Filho, S. L. S.; Silva, B. D. M. and Ribeiro, M. D. **Project administration:** Silva, N. C.; Cabral Filho, S. L. S.; Silva, C. J. and Ribeiro, M. D. **Resources:** Silva, N. C.; Cabral Filho, S. L. S.; Ferreira, M. S.; Silva, C. J.; Fonseca, A. A.; Geraseev, L. C.; Silva, B. D. M. and Ribeiro, M. D. **Supervision:** Silva, N. C.; Cabral Filho, S. L. S. and Silva, C. J. **Validation:** Silva, N. C. **Visualization:** Ribeiro, M. D. **Writing – original draft:** Silva, N. C. **Writing – review & editing:** Silva, N. C.

References

AOAC - Association of Official Analytical Chemists. 2005. Official methods of analysis of AOAC International. 18th ed. AOAC International, Gaithersburg.

Barbosa, L. K. G.; Silva, N. C.; Oliveira, N. J. F.; Passetti, R. A. C. and Geraseev, L. C. 2023. Supplementation of banana by-products hay does not alter serum biochemistry of lambs. Tropical Animal Science Journal 46:330-336. https://doi.org/10.5398/tasj.2023.46.3.330

Brondani, W. C.; Silveira, F. A.; Lemes, J. S.; Evangelho, L. A. and Ferreira O. G. L. 2020. Suplementação gestacional na produção de lã de ovelhas e cordeiros. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 72:977-984. https://doi.org/10.1590/1678-4162-11208

Burns, J. C.; Pond, K. R. and Fisher, D. S. 1994. Measurament of forage intake. p.494-532. In: Forage quality, evaluation and utilization. Fahey Jr., G. C., ed. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison. https://doi.org/10.2134/1994.foragequality.c12

Casali, A. O.; Detmann, E.; Valadares Filho, S. C.; Pereira, J. C.; Henriques, L. T.; Freitas, S. G. and Paulino, M. F. 2008. Influência do tempo de incubação e do tamanho de partículas sobre os teores de compostos indigestíveis em alimentos e fezes bovinas obtidos por procedimentos *in situ*. Revista Brasileira de Zootecnia 37:335-342. https://doi.org/10.1590/S1516-35982008000200021

Castillo, C.; Abuelo, A. and Hernández, J. 2016. Usefulness of metabolic profiling in the assessment of the flock's health status and productive performance. Small Ruminant Research 142:28-30. https://doi.org/10.1016/j.smallrumres.2016.02.019

Detmann, E.; Souza, M. A.; Valadares Filho, S. C.; Queiroz, A. C.; Berchielli, T. T.; Saliba, E. O. S.; Cabral, L. S.; Pina, D. S.; Ladeira, M. M. and Azevedo, J. A. G. 2012. Métodos para análise de alimentos. INCT - Ciência Animal. Suprema, Visconde do Rio Branco.

Feijó, J. O.; Pzoerazli, D.; Silva, L. G. C.; Aragão, R. B.; Martins, C. F.; Pereira, R. A.; Ferreira, M. B.; Del Pino, F. A. B.; Rabassa, V. R. and Corrêa, M. N. 2014. Clinical and biochemical parameters evaluation of pregnant and non-pregnant ewes from pantaneiro genetic group. Brazilian Journal of Veterinary Research and Animal Science 51:111-117.

Geraseev, L. C.; Perez, J. R. O.; Carvalho, P. A.; Oliveira, R. P.; Quintão, F. A. and Lima, A. L. 2006. Efeitos das restrições pré e pós-natal sobre o crescimento e o desempenho de cordeiros Santa Inês do nascimento ao desmame. Revista Brasileira de Zootecnia 35:245-251. https://doi.org/10.1590/S1516-35982006000100031

Geraseev, L. C.; Silva, N. C.; Chaves, A. S.; Costa, D. S.; Ornelas, L. T. C.; Crocomo, L. F. and Moreira, S. J. M. 2023. Use of sunflower meal as a protein source in diets of growing lambs. Revista Brasileira de Zootecnia 52:e20220144. https://doi.org/10.37496/rbz5220220144

González-Maldonado, J.; Tejeda-Sartorius, O.; Ramírez-Ramírez, A. I. and Gallegos-Sánchez, J. 2023. Reproductive performance of hair sheep under different body conditions and feeding levels. Pesquisa Agropecuária Brasileira 58:e03092. https://doi.org/10.1590/S1678-3921.pab2023.v58.03092

Kaneko, J. J.; Harvey, J. W. and Bruss, M. L. 1997. Clinical biochemistry of domestic animals. 5th ed. Academic Press, San Diego.

Kaneko, J. J.; Harvey, J. W. and Bruss, M. L. 2008. Clinical biochemistry of domestic animals. 6th ed. Academic Press, San Diego.

Khaiseb, P. C.; Hawken, P. A. R. and Martin, G. B. 2022. Interactions between nutrition and the "ram effect" in the control of ovarian function in the merino ewe. Animals 12:362. https://doi.org/10.3390/ani12030362

Klein, J. L.; Adams, S. M.; Alves Filho, D. C.; Brondani, I. L.; Pizutti, L. A. D.; Antunes, D. P.; Poletto, V.; Karsten, M. S.; Bem, P. H. T. and Mello, D. A. S. 2021. Fetal programming and the consequences on progeny development - a review. Research, Society and Development 10:e557101220766. https://doi.org/10.33448/rsd-v10i12.20766

Licitra, G.; Hernandez, T. M. and Van Soest P. J. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology 57:347-358. https://doi.org/10.1016/0377-8401(95)00837-3

Macedo Junior, G. L.; Perez, J. R. O.; Paula, O. J.; Almeida, T. R. V.; Assis, R. M. and França, P. M. 2012. Consumo, digestibilidade e curva glicêmica de ovelhas em final de gestação recebendo diferentes relações volumoso:concentrado. Ciência Animal Brasileira 13:180-188. https://doi.org/10.5216/cab.v13i2.12454

Macedo Junior, G. L.; Rodrigues, V. J. C.; Cruz, W. F. G.; Ferreira, D. A. and Andrade, M. E. B. 2018. Perfil metabólico, produtivo e reprodutivo de ovelhas recebendo flushing de diferentes fontes energéticas. Veterinária Notícias 24:12-29.

McCarty, K. J.; Washburn, J. L.; Taylor, R. K. and Long, N. M. 2020. The effects of early-or mid-gestation nutrient restriction on bovine fetal pancreatic development. Domestic Animal Endocrinology 70:106377. https://doi.org/10.1016/j.domaniend.2019.07.005

Mendes, J. P.; Valentim, J. K. and Barbeira, S. S. 2021. Toxemia da prenhes em ovinos. Ensaios e Ciência 25:166-171. https://doi.org/10.17921/1415-6938.2021v25n2p166-171

Moreira, R. T.; Assis, L. C.; Lima, E. M. M.; Facury Filho, E. J. and Borges, J. R. J. 2019. Perfil metabólico durante o periparto de ovelhas da raça Santa Inês com gestação simples e múltipla. Ciência Animal Brasileira 20:1-15. https://doi.org/10.1590/1089-6891v20e-34181

NRC - National Research Council. 1985. Ruminant nitrogen usage. National Academies Press, Washington, D.C.

NRC - National Research Council. 2001. Nutrient of requirements of dairy cattle. 7th rev. ed. National Academies Press, Washington, D.C.

NRC - National Research Council. 2007. Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. National Academies Press, Washington, DC.

Oliveira, R. P. M.; Maduro, A. H. P.; Lima, E. S. and Oliveira, F. F. 2014. Perfil metabólico de ovelhas Santa Inês em diferentes fases de gestação criadas em sistema semi-intensivo no estado do Amazonas. Ciência Animal Brasileira 15:81-86. https://doi.org/10.5216/cab.v15i1.15720

Osório, J. C. S. and Osório, M. T. M. 2005. Produção de carne ovina: Técnicas de avaliação "in vivo" e na carcaça. Universidade Federal de Pelotas, Pelotas.

Santos, F. M. S. C.; Soares, P. C.; Mesquita, E. P.; Oliveira Filho, E. F.; Guido, S. I.; Alves, K. H. G.; Bartolomeu, C. C. and Amorim, M. J. A. A. L. 2014. Perfil bioquímico em ovelhas da raça Morada Nova nos períodos de gestação, parto e pós parto. Ciência Veterinária nos Trópicos 17:24-29.

Saunders, G. A.; Alves, N. G.; Pérez, J. R. O.; Souza, J. C.; Moura, A. M.; Muniz, J. A.; Lima, R. R. and Lazarin, G. B. 2012. Efeito do nível nutricional antes e após a ovulação sobre a taxa de gestação e a prolificidade em ovelhas Santa Inês. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 64:1085-1093. https://doi.org/10.1590/S0102-09352012000500001

Silva, N. C.; Gaspar, R. C.; Chaves, A. S.; Geraseev, L. C.; Athayde, A. L. M. and Crocomo, L. F. 2019. Morphometric measurements of sheep fed with increasing levels of sunflower meal. Acta Scientiarum. Animal Sciences 41:e42891. https://doi.org/10.4025/actascianimsci.v41i1.42891

Siqueira, M. T. S.; Jesus, T. A. V.; Silva, A. L.; Araújo, M. J. P.; Sousa, L. F. and Macedo Júnior, G. L. 2020. Suplementação nutricional para ovelhas em final de gestação: parâmetros nutricionais e metabólicos. Caderno de Ciências Agrárias 12:1-9. https://doi.org/10.35699/2447-6218.2020.24109

Sniffen, C. J.; O'Connor, J. D.; Van Soest, P. J.; Fox, D. G. and Russell, J. B. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. Journal of Animal Science 70:3562-3577. https://doi.org/10.2527/1992.70113562x

Song, S.; Wu, J.; Zhao, S.; Casper, D. P.; Zhang, L.; He, B.; Lang, X.; Wang, C.; Gong, X.; Wang, F. and Liu, L. 2018. The effect of periodic energy restriction on growth performance, serum biochemical indices, and meat quality in sheep. Journal of Animal Science 10:4251-4263. https://doi.org/10.1093/jas/sky299

Sousa, S. V.; Araújo, M. J.; Silva, T. P. D.; Marques, C. A. T.; Torreão, J. N. C.; Bezerra, L. R.; Lima, I. S. S. and Gottardi, F. P. 2018. Dietary supplementation for Santa Inês hair ewes on pastures in pre and postpartum periods: dry matter intake, digestibility, milk production and mineral metabolism. Tropical Animal Health and Production 50:1903-1912. https://doi.org/10.1007/s11250-018-1643-2

Van Soest, P. J.; Robertson, J. B. and Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74:3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Weiss, W. P. 1993. Predicting energy values of feeds. Journal of Dairy Science 76:1802-1811. https://doi.org/10.3168/jds.S0022-0302(93)77512-8

Yıldırır, M.; Çakır, D. U. and Yurtman, I. Y. 2022. Effects of restricted nutrition and flushing on reproductive performance and metabolic profiles in sheep. Livestock Science 258:104870. https://doi.org/10.1016/j.livsci.2022.104870

Zhang, J.; Zheng, N.; Shen, W.; Zhao, S. and Wang, J. 2020. Synchrony degree of dietary energy and nitrogen release influences microbial community, fermentation, and protein synthesis in a rumen simulation system. Microorganisms 8:231. https://doi.org/10.3390/microorganisms8020231