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Research article

Chromium propionate or calcium salts of palm oil in the diets of ewes in late pregnancy and lactation and the effects on the offspring

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ABSTRACT: Ruminants in late gestation and lactation have high energy requirements, which can be effectively met with diets that include chromium and protected fat. This study aimed to compare the effects of chromium propionate and rumen-protected fat supplementation in the diet of ewes in late gestation and lactation and to evaluate their impact on the performance, metabolism, carcass, and meat quality of the offspring. The study population consisted of 25 mixed-breed ewes, aged 3 ± 1 years, with a body weight (BW) of 57 ± 10 kg, and a single gestation. All ewes in the study gave birth to males. The experimental design was a randomized block design with three dietary treatments: a control diet (CTL; n = 8) consisting of corn as the primary energy source; a chromium propionate treatment (CRPR; n = 9) consisting of the CTL diet plus 0.5 mg of chromium propionate per kg of dry matter (DM); and a calcium-salts from palm oil treatment (FAT; n = 8) - CTL diet plus calcium salts from palm oil. The ewes were fed the diets for 50 days of gestation and 70 days of lactation. Following weaning, the lambs were confined for of 60 days and subsequently slaughtered. The means were compared using Tukey's test with a statistical probability of 5 %. The maternal diets of CRPR and FAT resulted in lambs with higher BW at weaning and slaughter, greater chest and leg width, and higher aspartate aminotransferase (AST) and insulin values, as well as lower cholesterol and low-density lipoprotein (LDL) levels. The FAT treatment reduced cooking losses and alterations in lambs' sarcomere length (SL) and muscle fiber area. It can be concluded that the inclusion of CRPR and FAT in the diet of pregnant and lactating ewes has beneficial effects on the offspring.
Keywords: performance, cholesterol, insulin, sarcomere, fiber muscle

Introduction

It has been established that maternal nutrition during pregnancy can influence fetal development (Reynolds et al., 2019). In certain circumstances, foraging alone is insufficient to fulfill the nutritional requirements for pregnancy. Therefore, alternative feeds are imperative, particularly during the final third of an ewe's pregnancy and the initial stages of lactation, when the animal's nutritional requirements are heightened (NRC, 2007). Therefore, research has been conducted to improve maternal nutrition and evaluating its effects on the offspring's performance, metabolism, carcass, and meat quality (Piaggio et al., 2018; Ramírez-Zamudio et al., 2022). The quantity of nutrients in the ewe's diet can influence fetal development (McGovern et al., 2015a, b). However, the type of feed utilized in the diet can also be a significant factor in this process (Brochine et al., 2023), both directly affecting fetal development and subsequently impacting the adult phase of the animal and the entire production system (Santos et al., 2022b).

The objective of providing ewes with calcium salts of palm oil (FAT, rumen-protect fat) during the months of pregnancy and lactation is to furnish the animals with the requisite energy to achieve a reduction in negative energy balance. The administration of rumen-protect fat has been demonstrated to mitigate weight loss and improve body condition scores in ewes during pregnancy and lactation (Santos et al., 2022a). Furthermore, this intervention has been shown to exert an influence on fetal programming, resulting in alterations to progeny characteristics such as body weight (BW), meat quality, and carcass quality parameters (Brochine et al., 2023; Miranda et al., 2023; Ramírez-Zamudio et al., 2022). Chromium propionate (CRPR) has been demonstrated to enhance glucose utilization and mitigate the deleterious effects of stress, thereby promoting enhanced cellular energy and functionality. The administration of chromium in the diet has been demonstrated to enhance maintenance, reproduction, growth, and immunity (Vincent, 2000, 2010). The administration of chromium to the diet of pregnant and lactating dams has been shown to improve energy metabolism, thereby conferring benefits to the developing fetus and adult offspring. These benefits encompass reproductive characteristics, slaughter weight, and the proportion of commercial cuts in the carcass, among other livestock factors (Brochine et al., 2023; Santos et al., 2022a).

The objective of the experiment was to compare the effects of CRPR and FAT on the nutrition of ewes in late gestation and lactation and evaluate their impact on growth performance, metabolism, carcass and meat quality in the offspring. The hypothesis is that dietary supplementation will have a beneficial effect on the overall performance of the lamb.

Materials and Methods

Study site, animals, and treatments

The experiment was conducted at the Sheep Farm of the Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, municipality of Pirassununga, São Paulo state, Brazil. The coordinates of the experimental area are 21°59′ S, 47°26′ W, and the altitude is 635 m. In accordance with the Köppen classification (Köppen & Geiger, 1928), the region's climate is categorized as Cwa, a mesothermal climate with a predominant occurrence of summer rainfall, dry winters, and hot summers, exhibiting an average annual temperature of 22 °C and an average annual rainfall of approximately 1,363 mm. The Animal Experimentation Ethics Committee of the Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, approved the experimental protocol under the number 3657171218 on Mar 1, 2019.

The initial cohort study comprised 72 ewes of Santa Inês and Dorper crossbreeds, which had their estrous cycle synchronized with hormonal protocols and were naturally mated with two contemporary Dorper breed sires. Pregnancy diagnosis was conducted via ultrasound 40 days post-breeding season. The initial flock yielded data from 25 ewes that had single male births. The ewes were aged 3 ± 1 years and had an average BW of 57 ± 10 kg.

The ewes were randomly assigned to one of the three dietary treatments, as follows: CTL (n = 8) (control) that used corn as energy source; CRPR (n = 9) that used CTL diet plus 0.5 mg of chromium propionate per kg of dry matter (DM); and FAT (n = 8) that used CTL diet with partial substitution of corn by 3 % (DM basis) of calcium salts of palm oil (Table 1). The ewes were given the experimental diet for the final 50 days of gestation and the subsequent 70 days of lactation. The feed was provided twice daily, at 7h00 and 16h00, with the daily CTL amount offered and the leftovers.

The feed samples were subjected to chemical analysis in accordance with the procedures established by AOAC (2000) for determining the following parameters: DM (ID 930.15), organic matter (ID 942.05), crude protein (as 6.25 × N - ID 954.01), acid detergent fiber (ADF) (ID 973.18), and neutral detergent fiber (NDF) (Mertens, 2002). The Small Ruminant Nutrition System (Cannas et al., 2022) was employed to estimate the metabolizable energy (Mcal).

At 135 ± 7 days of gestation and 60 ± 7 days of lactation, the ewes were weighed and evaluated for body condition using a scale of 1 to 5, as described by Kenyon and Blair (2014). The lambs remained with their mothers from birth until weaning, after which they were immediately transferred to the feedlot, where they remained for a further 60 days and were slaughtered at 130 ± 10 days of age.

Table 1 – Feed composition and chemistry analysis of experimental diets of ewes and lamb.

	Ewe			
	CTL	CRPR	FAT	Lamb
Ingredients, % DM				
Hay				20
Corn silage	50	50	58	
Corn grain	37	37	27	54
Soybean meal	11	11	12	24
Chromium propionate, mg Cr[1]		0.5		
Rumen Protected Fat			3	
Mineral[2]	1	1	1	1
Limestone	1	1		
Calcium chloride				1
Nutrition composition, kg kg^{-1} DM				
Crude Protein	0.111	0.111	0.111	0.166
Ether Extract	0.024	0.024	0.046	0.032
Metabolizable Energy, Mcal kg^{-1}	2.460	2.460	2.470	2.818
ADF	0.245	0.245	0.275	0.123
NDF	0.404	0.404	0.448	0.233
Ash	0.067	0.069	0.064	0.040
Chromium propionate, mg kg^{-2}	0.155	0.660	0.164	0.110
Calcium, g kg^{-1}	7.500	7.500	7.700	4.500
Phosphor, g kg^{-1}	3.200	3.200	3.100	3.100

[1]KemTrace 0.4 % Cr; Kemin Agrifoods South America. [2]Mineral composition: Ca = 140 g, P = 65 g, Mg = 10 g, S = 12 g, Na = 130 g, Co = 80 mg, Fe = 1000 mg, I = 60 mg, Mn = 3000 mg, Se = 10 mg, Zn = 5000 mg, F = 650 mg, Vitamin A = 50000 UI, Vitamin E = 312 UI. CTL = corn as energy feed; CRPR = CTL plus 0.5 mg of chromium propionate per kg of DM; FAT = CTL plus inclusion of calcium salts of palm oil. DM = dry matter; ADF = acid detergent fiber; NDF = neutral detergent fiber. Table presented in Brochine et al. (2023). Both works had the same team of researchers and the same line of research.

Feedlot and lamb performance

The lambs were weighed 24 h after birth on a portable electronic scale with a maximum capacity of 50 kg and a precision of 20 g (Walmur, Ref. 4434). The animals were weighed at weaning and slaughter using an electronic scale with a maximum capacity of 300 kg and precision of 100 g (Coimma, Ref. ICS-300). The average daily gain was calculated as the difference between the final and initial weights divided by the feedlot period.

In the feedlot, the nutritional regimen was identical for all the lambs to assess only the maternal nutritional differences (Table 1). The total ration was provided twice daily, at 7h00 and 16h00 with daily control of the amount offered and the leftover, individually, allowing for the calculation of DM intake.

In vivo measurements were taken one day preceding slaughter with the animals standing on a flat surface. The body length was determined, corresponding to the distance between the cervico-thoracic joint and the base of the tail. The forelimb height was also measured, which is the distance between the withers and the distal end of the forelimb. The thoracic perimeter was then calculated, which indicates the perimeter based on the

sternum and withers, passing the tape measure behind the shoulder. Finally, the croup width was determined, which is the maximum distance between the trochanters of the femurs.

Metabolic profile and animal performance

At the conclusion of the feedlot phase, blood samples were collected from all lambs via venepuncture of the jugular vein into sterile tubes for each test. Biochemical parameters were analyzed in blood collected in vacuum tubes with a 10 mL clot activator (except for glucose analysis, in which 4 mL fluoride vacuum tubes were used). The samples were then centrifuged for 20 min at 261.80 rad s^{-1} to obtain a serum blood aliquot, which was stored in 1.5 mL Eppendorf tubes at a temperature of −20 °C until analysis. The variables under examination were kits from the Labtest Company. The number code test and methodology were as follows: glucose (133-1, GOD – Trinder), triglyceride (87-2/100, Colorimetric - Trinder reaction), cholesterol (76-2, Colorimetric - Trinder), urea (104-4, UV Enzyme), creatinine (96, Colorimetric - Alkaline picrate - Jaffé), creatine kinase (CK, 117-2/30, UV - IFCC), aspartate aminotransferase (AST, 109), gamma-glutamyl transferase (GGT, 105), total protein (99, Colorimetric - Biuret), albumin (19, Colorimetric - Bromocresol Green), globulin (mathematical calculation - PT = albumin + globulins), calcium (Colorimetric - CPC - cresolphthalein, 90-2/60) and phosphorus (12-200, Colorimetric-Phosphomolybdate).

Serum insulin levels were determined using an Insulin Kit - Accubind EIA Kit, Code. 2425-300 (Monobind Inc.), employing the ELISA technique and reading with the Labsystems Multiskan MS equipment (Thermo Fisher Scientific Inc.).

Carcass

The animals were slaughtered immediately following the feedlot period. The procedure commenced with a stunning, followed by bleeding, skinning, evisceration, inspection, and cleaning. Carcass weight, pH, and temperature measurements were taken 30 min and 24 h after slaughter. Evaluations of pH, temperature, loin eye area (LEA), and fat thickness were conducted between the 11^{th} and 13^{th} ribs of the left carcass. The carcasses were stored in a refrigerated room at a temperature of 4 °C for 24 h.

The carcass's pH and temperature were measured using a portable digital pH thermometer with a penetration electrode, calibrated to pH 4 and 7 (AKSO, model pH In). Subcutaneous fat thickness was quantified with a digital caliper (Western brand). The LEA was delineated by outlining the muscle and measuring regions A (medial-lateral direction) and B (ventral-dorsal direction), and the area was calculated using the equation $(A/2 \times B/2) \times 3.1416$.

Carcass yield was calculated as carcass weight divided by slaughter weight and multiplied by 100. Omental and mesenteric fat were removed and weighed on a counter scale with a maximum capacity of 15 kg and a variation of 5 g (Toledo do Brasil, Prix 3 model). At the deboning stage, the *Longissimus dorsi* muscle (LD) was collected and stored in a vacuum-packed −80 °C freezer for subsequent analysis. Additionally, 2 g of this muscle were frozen rapidly in liquid nitrogen for histology analysis.

Meat quality analyses

Three steaks from the LD, each 2.5 cm thick, were used to determine cooking loss (CL). Initially, the samples were weighed and cooked in a preheated electric oven. At 36 °C, the samples were turned over and maintained in the oven until the internal temperature reached 72 °C (temperature control was conducted using a digital thermometer). Subsequently, the steaks were placed to cool at room temperature and were weighed once more. The CL was calculated by subtracting the post-cooking weight from the pre-cooking weight.

The shear force (SF) procedure was conducted by the guidelines established by AMSA (2015). The same samples utilized for CL assessment were employed in SF testing. Between five and eight cylinders, each 1.25 cm in diameter and cut parallel to the muscle fibers, were extracted from the steaks. The SF was measured using a Warner-Bratzler machine (G-R Manufacturing Co.) equipped with a Warner-Bratzler 'V' slot blade accessory (3.0 mm thickness and 60° triangular apertures) and a load cell of 500 N (basic force gauge) and a speed of 20 cm min^{-1}. The result for each steak is the mean of the cylinder measurements, expressed in Newtons (N).

The myofibrillar fragmentation index (MFI) was determined in accordance with the procedures described by Culler et al. (1978), with the following adaptations: 1 g of muscle sample was homogenized in 10 volumes of the MFI buffer. The protein concentration was determined by the biuret method, as described by Gregor et al. (1977). The protein concentration was adjusted to ensure the same protein concentration of 0.5 mg mL^{-1}. The myofibrillar suspension was diluted and stirred, and absorbance was immediately read in a spectrophotometer (Unico, model 1205) at a wavelength of 540 nm. The index was calculated according to the methodology described by Culler et al. (1978).

The sarcomere length (SL) was determined using five sub-samples representing distinct anatomic positions (lateral, medial, intermediate, ventral, and dorsal) of the LD muscle obtained from each steak. Approximately 0.5 g of muscle tissue was homogenized from each sub-sample with 5 % glutaraldehyde in 0.1 MNaHPO4 buffer, with the pH adjusted to 7.2. Subsequently, the homogenate was incubated for 4 h and transferred to a fixation solution (0.2 M sucrose in buffer 0.1 M NaHPO4 with pH 7.2). The sarcomeres were measured with 100×

amplification on a Nikon Eclipse 80i light microscope (Nikon). Five subsamples were taken from each sample, with 10 to 20 sarcomere measurements made from each subsample. The average value obtained for each subsample represents the SL value of each animal and is expressed in micrometers (µm).

The analysis for collagen determination, including total and soluble collagen, will be conducted in accordance with the methodology outlined by Brown et al. (2001). The collagen content and its fractions were evaluated by quantifying the amino acid hydroxyproline following hydrolysis of the material. The results were calculated using a response curve. The results are expressed in the hydroxyproline values obtained from the absorbance readings on a spectrophotometer at 570 nm. The analysis of soluble collagen was conducted by subjecting the samples to a 75-min hating process at 80 °C in a water bath, followed by a 10-min centrifugation at 418.88 rad s^{-1} at 20 °C. The conversion factor employed for both analytical procedures was 7.14 times the hydroxyproline concentration.

To measure the area of muscle fibers, the samples were wrapped in neutral talc and frozen in liquid nitrogen for transportation, also in nitrogen. Slices (10 µm) were prepared using a cryostat. The samples were fixed with Baker's calcium formalin and then stained with a solution containing hematoxylin, eosin, alcohol and xylene. The Hematoxylin-Eosin technique was employed to evaluate muscle morphology. The photographs of the slides were taken using a biological microscope (Biovideo) with 40× amplification, and the fiber area was measured using the ImageJ program.

Statistical analysis

The statistical design was a random complete block design, and the treatments were considered fixed effects bythe model:

$$Y_{ijk} = \mu + T_i + B_k + e_{ij}$$

where Y_{ijk} is the dependent variable, (i = treatment and j = repetition), µ is the overall mean, T_i is the fixed effect of the diet (i = 1 – 3), B is the block (ewe age) and e_{ij} is the residual error.

The data were subjected to tests to ascertain their normality: Shapiro-Wilk and Levene's. They were then analyzed using PROC GLM and the Tukey's test at a 5 % significance level (Statistical Analysis System, version 9.4).

Results

The BW and body condition score of the ewes at the conclusion of the pregnancy and lactation period demonstrated no statistically significant alteration because of the distinct dietary regimens ($p > 0.05$, Table 2). The mean lamb birth weight was also similar between ewe diets ($p = 0.6870$, Table 2). However, at the time of weaning, which occurred 70 days after birth, the lambs from the CRPR and FAT treatments exhibited greater initial weight at the beginning of the feedlot period ($p = 0.0202$) This difference persisted until slaughter, as indicated by the final feedlot weight, which was recorded at the age of 130 days ($p = 0.0354$). Maternal nutrition did not have a statistically significant effect (p

Table 2 – Performance, weight, and measurements of lambs born to ewes fed chromium propionate or calcium salts of palm oil during final period of gestation and lactation.

		CTL	CRPR	FAT	STD Error	Mean	*p*-value
Ewe							
BW at gestation, kg		77.53	76.08	76.17	1.68	76.35	0.9385
BW at lactation, kg		65.07	62.91	69.29	1.62	65.48	0.3932
BCS at gestation, 1-5 scale		3.86	3.93	3.86	0.08	3.83	0.9482
BCS at lactation, 1-5 scale		3.27	3.55	3.78	0.10	3.49	0.2413
Lamb performance							
Birth weight, kg		4.60	4.60	4.40	0.01	4.53	0.6870
BW at feedlot,	Initial, kg	22.40 b	27.12 a	26.70 a	1.01	25.01	0.0202
	Final	43.16 b	46.92 a	46.27 a	1.90	45.45	0.0354
DMI, % BW		4.36	4.13	4.28	0.14	4.26	0.4405
ADG, kg d^{-1}		0.345	0.330	0.326	0.03	0.334	0.7032
Feed efficiency, ADG kg DMI kg^{-1}		0.241	0.216	0.208	0.03	0.222	0.0686
Lamb Body measurements							
Withers height, cm		61.83	59.46	62.36	0.60	60.78	0.1121
Body length, cm		60.56	63.19	63.37	0.97	62.03	0.5187
Thorax width, cm		92.8 b	99.34 a	100.32 a	1.35	97.14	0.0357
Leg width, cm		6.06 b	6.69 a	6.95 a	0.16	6.48	0.0372

CTL = corn as energy feed; CRPR = CTL plus 0.5 mg of chromium propionate per kg of DM; FAT = CTL plus inclusion of calcium salts of palm oil; STD Error = standard error. BW = body weight; BCS = body condition score; DMI = dry matter intake; ADG = average daily gain. Lowercase letters indicate a statistical difference between the means using the Tukey's test at ≤ 5 % probability.

> 0.05) on the characteristics of the offspring, including DM consumption, average daily weight gain, and feed efficiency, during the feedlot period. When measuring the body dimensions, it was observed that the lambs from the CRPR and FAT diets exhibited greater thorax width (p = 0.0357) and leg width (p = 0.0372) than the lambs from the CTL group. However, no significant difference was noted in withers height and body length between the treatments (p > 0.05).

The maternal diet did not have a statistically significant effect (p > 0.05, Table 3) the concentrations of glucose, GGT, CK, urea, creatinine, total protein, albumin, globulin, triglycerides, high-density lipoprotein, very low-density lipoprotein (LDL), calcium, and phosphorus. However, lambs in the CRPR and FAT treatments exhibited elevated concentrations of insulin (p = 0.0320) and AST (p = 0.0593) and diminished concentrations of cholesterol (p = 0.0532) and LDL (p = 0.0274) in comparison to the CTL group.

The maternal diet did not significantly alter the offspring's hot and cold carcass yield, visceral fat, carcass fat thickness, pH, or carcass temperature (p > 0.05, Table 4). The hot and cold carcass yields were 48.29 and 46.75 %, respectively. The mean visceral fat weight was 1.57 kg, while the mean subcutaneous fat thickness was 1.87 mm. The pH was recorded at 6.50 at 30 min and 5.59 at 24 h post-slaughter. The mean meat temperature was 34.73 and 8.44 °C at 30 min and 24 h post-slaughter, respectively.

Table 3 – Blood parameters of lambs born to ewes fed chromium propionate or calcium salts of palm oil during the final period of gestation and lactation.

	CTL	CRPR	FAT	STD Error	Mean	*p*-value
Enzyme						
AST, U L^{-1}	74.88 b	82.88 a	84.22 a	2.12	81.04	0.0593
GGT, U L^{-1}	75.34	81.22	83.03	3.78	78.43	0.6375
CK, U L^{-1}	444.50	291	216.33	73.33	281.65	0.4698
Energy metabolic						
Glucose, mg dL^{-1}	85.69	82.15	92.98	2.38	85.90	0.2659
Insulin, ng mL^{-1}	0.37 b	0.61 a	0.68 a	0.05	0.52	0.0320
Creatinine, mg dL^{-1}	0.75	0.68	0.65	0.01	0.70	0.1380
Triglycerides, mg dL^{-1}	55.16	56.84	61.93	4.22	58.25	0.8775
Cholesterol, mg dL^{-1}	93.71 a	72.80 b	69.08 b	4.15	77.82	0.0532
LDL, mg dL^{-1}	22.60 a	12.62 b	9.61 b	1.82	14.47	0.0274
HDL, mg dL^{-1}	60.01	48.84	47.01	2.84	51.69	0.2541
VLDL, mg dL^{-1}	11.08	11.33	12.45	0.83	11.65	0.8645
Protein metabolic						
Urea, mg dL^{-1}	27.68	27.75	28.71	1.64	28.83	0.9471
Protein, g dL^{-1}	5.34	4.98	5.18	0.07	5.12	0.2781
Albumin, g dL^{-1}	3.00	2.87	2.84	0.04	2.87	0.2089
Globulin, g dL^{-1}	2.33	2.11	2.34	0.05	2.24	0.3052
Mineral						
Calcium, mg dL^{-1}	11.34	11.24	12.14	0.18	11.45	0.2081
Phosphorus, mg dL^{-1}	9.86	11.07	9.55	0.33	10.48	0.2027

CTL = corn as energy feed; CRPR = CTL plus 0.5 mg of chromium propionate per kg of DM; FAT = CTL plus inclusion of calcium salts of palm oil; STD Error = standard error. AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; CK = creatine kinase; LDL = low-density lipoprotein; HDL = high-density lipoprotein; VLDL = very low-density lipoprotein. Lowercase letters indicate a statistical difference between the means using the Tukey's test at ≤ 5 % probability.

Table 4 – Carcass characteristics of lambs born to ewes fed chromium propionate or calcium salts of palm oil during the final period of gestation and lactation.

	CTL	CRPR	FAT	STD Error	Mean	*p*-value
Hot carcass yield, %	47.31	48.63	48.68	0.55	48.29	0.6936
Cold carcass yield, %	45.67	47.15	46.98	0.57	46.75	0.6994
Visceral fat, kg	1.31	1.81	1.39	0.12	1.57	0.2769
Subcutaneous fat thickness, mm	2.04	1.80	2.14	0.10	1.87	0.6523
pH 30 min	6.60	6.48	6.64	0.06	6.50	0.7893
Temperature 0 h, °C	33.80	35.25	35.00	0.46	34.73	0.2723
pH 24 h	5.62	5.55	5.57	0.02	5.59	0.5986
Temperature 24 h, °C	9.18	7.95	8.88	0.23	8.44	0.2737

CTL = corn as energy feed; CRPR = CTL plus 0.5 mg of chromium propionate per kg of DM; FAT = CTL plus inclusion of calcium salts of palm oil; STD Error = standard error.

The maternal diet did not result in any statistically significant alterations in the LEA, which exhibited a mean of 19.7 cm^2 (p > 0.05, Table 5). The CL was significantly lower in the FAT gourd compared to the CTL and CRPR treatments (p = 0.0008). The maternal diet did not affect the total collagen content (average 13.29 mg g^{-1}), soluble collagen (6.29 mg g^{-1}), and insoluble collagen (7 mg g^{-1}), nor did it impact SF and MFI (p > 0.05). The FAT treatment produced of lambs with shorter SL (p = 0.0551) and smaller muscle fiber area (p = 0.0498) compared to those from the CTL and CRPR treatments.

Discussion

The maternal diet did not affect the birth weight of the lambs. However, at weaning and slaughter, the CRPR and FAT lambs exhibited higher BW than the CTL lambs, suggesting the presence of muscle hyperplasia. This may be attributed to the alteration of the fetal diet at the end of gestation. The benefit of supplementation is not always apparent at the time of birth but may be observed subsequently (Miranda et al., 2023). The increased energy provided by the maternal diet supported enhanced fetal muscle development throughout gestation, which is evident postnatally as higher slaughter weights and improved leg development. The supplementation of CRPR allowed for the more efficacious utilization of dietary energy for pregnant and lactating females (Leiva et al., 2017). Conversely, FAT, a high-energy feed, supplies the necessary energy for fetal development (Brandão et al., 2020). The implementation of these nutritional strategies (CRPR and FAT) in the diet of ewes in late gestation and lactation was beneficial for the offspring's growth (Brochine et al., 2023; Santos et al., 2022a).

Table 5 – Meat characteristics of lambs born to ewes fed chromium propionate or calcium salts of palm oil during the final period of gestation and lactation.

	CTL	CRPR	FAT	STD Error	Mean	*p*-value
LEA, cm^2	17.60	20.14	19.04	0.90	19.70	0.6495
CL, %	34.26 a	34.97 a	29.67 b	0.73	33.06	0.0008
Collagen total, mg g^{-1}	10.69	12.78	16.60	1.15	13.29	0.2539
Collagen soluble, mg g^{-1}	5.18	5.79	7.69	0.56	6.29	0.1890
Collagen insoluble, mg g^{-1}	5.50	6.98	8.90	0.86	7.00	0.4567
SF, N	57.56	66.48	64.23	2.84	62.07	0.4236
MFI	67.07	70.52	67.60	3.33	69.66	0.8756
SL, µm	1.76 a	1.73 a	1.68 b	0.02	1.73	0.0551
Fiber area, $µm^2$	1623.17 a	1634.03 a	1464.24 b	180.81	1573.00	0.0498

CTL = corn as energy feed; CRPR = CTL plus 0.5 mg of chromium propionate per kg of DM; FAT = CTL plus inclusion of calcium salts of palm oil; STD Error = standard error. LEA = loin eye area; CL = cooking loss; SF = shear force; MFI = myofibrillar fragmentation index; SL = sarcomere length. Lowercase letters indicate a statistical difference between the means using the Tukey's test at ≤ 5 % probability.

Fetal programming has been demonstrated to exert a significant influence on the adult phase of the animal (Sartori et al., 2020). This study observed the effect until the lamb reached slaughter weight, with heavier animals in CRPR and FAT. The supplementation of CRPR in the diet of ewes and its effects up to adulthood are well established and have been shown to have a positive economic impact. This is because heavier animals were slaughtered, more meat was produced, and more financial income (Santos et al., 2022b).

The width of the thorax has a positive correlation with the weight of the animal, which is why the CRPR and FAT lambs exhibited higher values for this measurement. In sheep allometric growth measurements, the leg is an early region in the Santa Inês breed. Consequently, when measuring leg width, this measurement was greater for the heavier animals (Furusho-Garcia et al., 2006). The prime cuts of the carcass are made in the thorax, exemplified by the French rack. Similar to the leg, it has a high commercial value. Supplementing chromium or incorporating protected fat into the ewe's diet resulted in offspring with increased carcass weight and greater weight of prime cuts, including loin and leg.

Adequate trace mineral supplementation during pregnancy is imperative to facilitate fetal development and survival and promote infant health during the early postnatal nursing period (Van Saun, 2023). The observed blood values for all assessed parameters fall within the reference range (Weiss and Wardrop, 2010).

The circulating insulin levels of lambs on supplemented diets (CRPR and FAT) were higher compared to the CTL group. Lambs born to ewes fed a high-energy diet during gestation and lactation exhibited elevated levels of circulating insulin (Brochine et al., 2023). These results are corroborated in this study. The presence of energy-rich nutrients in the diet raises levels of propionic acid, which subsequently increases blood glucose and circulating insulin levels. Furthermore, pancreas's size and insulin circulation in the fetus may be reduced because of nutritional restriction during gestation (Limesand et al., 2005, 2006). Insulin performs various functions, including the promotion of growth by activating receptors for insulin-like growth factors (Igwebuike, 2010). Despite the similarity in birth weights observed across all animals, those derived from ewes that received supplementation with either CRPR or FAT, which exhibited elevated insulin levels, demonstrated greater weight at both weaning and the conclusion of the feedlot period.

The concentration of AST was higher and the serum cholesterol level was lower in lambs born to ewes fed CRPR and FAT than in lambs in the CTL group. However, no alterations in GGT levels or the proteins/urea levels were observed in these animals. Moreover, the lambs from supplemented ewes exhibited elevated insulin levels. An elevated AST concentration may indicate fatty liver or abnormalities in bile salt synthesis, particularly when accompanied by reduced cholesterol levels and diminished albumin values (Gross, 2023). However, these observations were not evident in the experiment. Moreover, an association has been established between fatty liver and elevated GGT levels, which indicate heightened oxidative stress within hepatocytes (reference). Additionally, there is a correlation between fatty liver and insulin resistance, which can lead to hyperglycemia. However, these conditions were not observed in the present study. In contrast, the animals in the CRPR and FAT groups demonstrated reduced LDL levels, primarily attributed to their lower cholesterol levels. One of the primary functions of LDL is to facilitate the transport of cholesterol and triglycerides from the liver to the cells of the body's tissues (Tobert, 2022). An alteration in LDL levels associated with liver transaminases like AST may be indicative of an impact on lipoprotein metabolism caused by the early stages of liver disease (Jiang et al., 2014). Conversely, despite the majority of LDL clearance being carried out by the liver, the observed differences may be related to extrahepatic LDL clearance, whereby LDL receptors and lipoprotein lipase activities are upregulated by insulin.

The maternal nutrition regimen did not impact the parameters evaluated, including carcass yield, visceral fat, subcutaneous fat, meat pH levels, and temperature.

The results of this experiment for carcass yield and fat deposition are consistent with the expected breed standard and the nutritional regimen administered in the feedlot (Gallo et al., 2019, 2014). The initial and final pH and temperature values indicate that the sheep meat is within the established parameters (Hopkins and Fogarty, 1998). The supplementation of chromium or protected fat to the ewe during pregnancy did not result in any discernible impact on carcass quality parameters, particularly when the ewe was not observed on a restricted diet and was then supplemented (Reynolds et al., 2019). In our study, the ewe did not undergo any form of dietary restriction.

In a study of beef cattle with protein supplementation in the late stages of pregnancy, Maresca et al. (2019) found no difference in carcass yield of the offspring. However, they did observe a reduction in water loss from the meat in animals fed the protein diet. The reduction in meat water loss was associated with higher fat content, which is linked to meat pH, marbling, and subcutaneous fat thickness. However, these parameters remained unaffected by the maternal diet.

In meat with normal parameters, approximately one-third of the loss of water-holding capacity can be attributed to a decline in pH. Furthermore, the onset of rigor mortis also affects water retention. The reduction in adenosine triphosphate (ATP) and the protein interactions associated with rigor mortis are responsible for the formation of a thick network of contractile proteins. Specific ions, particularly divalent cations such as calcium and magnesium, can bind to the negatively charged relative groups of proteins, resulting in the closer proximity of protein chains and preventing the hydrophilic groups from binding water. The lack of space for the five water molecules in the protein structure is referred to as the steric effect of water retention. muscle proteins produce electrical effects in direct proportion to the degradation of ATP in *post-mortem* conditions (Devine at al., 2014; Honikel, 2014). This can be quantified by the sarcomere length and the muscle fiber's size.

The SL was observed to be shorter in the group that received protected fat in their maternal diet, and the area of the muscle fiber was also reduced. This suggests that the development of muscle fibers is influenced by the maternal diet with protected fat from the end of gestation to the slaughter age. Despite the observed difference in muscle fiber morphology, no difference in SF and MFI was evident. In contrast to the findings in the present study, Miranda et al. (2023) reported that the inclusion of protected fat derived from soybean oil increased the expression of the MyoG gene. However, the lipid profile of the oil used may result in distinct metabolic reactions and potentially different consequences in offspring (Ladeira et al., 2018). Cattle that were administered protected fat supplementation during the final stage of pregnancy exhibited offspring with no alterations in loin eye size, collagen levels, or SF (Miranda et al., 2023).

The supplementation of CRPR and FAT in ewes' diets in late pregnancy and lactation has been demonstrated to affect the performance and meat quality of the offspring. The two dietary regimens resulted in heavier lambs at the time of slaughter, thereby increasing meat production. Furthermore, the more energetic diet of the ewes allowed for greater leg and thorax growth in the offspring, resulting in heavier carcasses. The supplemented ewes produced lambs with modified insulin and cholesterol levels, suggesting shifts in these metabolites linked to muscle growth and hormone synthesis. The maternal diet containing FAT decreased muscle fiber size but did not affect the LEA until slaughter. No significant difference was observed between the diets used to supplement the ewe.

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Authors' Contributions

Conceptualization: Gallo SB, Delgado EF. Data curation: Gallo SB, Oliveira GM, Oliveira MC, Santos FF, Brochine L, Silva MM, Negrão JA, Delgado EF. **Formal analysis:** Gallo SB, Oliveira GM, Oliveira MC, Santos FF, Brochine L, Negrão JA, Delgado EF. **Funding acquisition: Gallo SB, Delgado EF. Investigation:** Gallo SB, Oliveira GM, Oliveira MC, Santos FF, Brochine L. **Methodology: Gallo SB, Delgado EF. Project administration:** Gallo SB. **Resources: Gallo SB, Delgado EF. Supervision: Gallo SB, Delgado EF. Writing-original draft: Gallo SB, Delgado EF,** Oliveira GM. **Writing-review & editing: Gallo SB, Delgado EF,** Silva MM.

Conflict of interest

The authors declare no conflict of interest.

Data availability statement

All the data from the experiment is presented in this article.

Declaration of use of AI Technologies

The authors did not use AI in writing this article.

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