The impact of fetal programming in ewe nutrition with chromium propionate or calcium salts of palm oil on the meat quality and bone of the progeny

Luciano Brochine
University of São Paulo
Fernanda Ferreira Santos
University of São Paulo
Flávia Mallaco Moreira
University of São Paulo
Paulo Roberto Leme
University of São Paulo
Luis Orlindo Tedeschi
Texas A&M University
Sarita Bonagurio Gallo (saritabgallo@usp.br)
University of São Paulo

Research Article

Keywords: Femur, Ruminant, Nutrition, Lamb, Sheep, Viscera

Posted Date: May 5th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1605147/v1

Abstract

This study aimed to evaluate the inclusion of chromium propionate or calcium salts of palm oil in ewes' diet in the final third of gestation and lactation on the effects of progeny performance, carcass characteristics, non-carcass components, and bone density. Forty-three ewe, Santa Inês and Dorper breeds, 3 ± 1 years old and weighing 57 ± 10 kg were used. The ewes were distributed in causal blocks in three treatments, CTL treatment (n = 15) with starch from corn; CR (n = 15) diet CTL plus chromium propionate; PF (n = 13) diet CTL plus calcium salts of palm oil. After weaning, 23 male lambs from these ewes were confined in individual stalls, with the same diet for 60 days, slaughtered. The data were analyzed using PROC GLM and means evaluated using the Tukey at 5%. Maternal diet did not alter the DMI, FE, and ADWG. Therefore, weaning and slaughter weights were higher for Cr and PF groups than CTL ($P < 0.05$). Carcass yield was also higher for these treatments ($P < 0.05$), but loin eye area and fat thickness were not influenced ($P > 0.05$) by diet regimen. The spleen and the respiratory tract were smaller for PF and larger for CTL ($P < 0.05$). The leg weight was about half of the carcass weight for the CR. The perimeter and depth of the shank for the CR and PF lambs were higher, indicating an effect of maternal nutrition in this commercial cut. The CR group had a smaller epiphysis measurement and femur length than the CTL group. We concluded that the fetal programming effect in ewes fed with Cr propionate and Ca salts of palm oil benefited the progeny by increasing their body weight, better carcass yield, and a higher proportion of prime cuts.

1. Introduction

Ewes in gestation and lactating have an increased nutritional requirement due to fetal growth, colostrum, and milk synthesis (NRC, 2007) and poor nutrition can result in metabolic diseases such as pregnancy toxemia [2]. However, maternal nutrition may affect lamb’s body weight [3], development of the liver, lung, and pancreas [4], meat quality, and weight of premium cuts [5], and changes in bone tissue [6]. The fetal programming study is investigating the effect of maternal nutrition on offspring. The correct energetic support to the ewe at the end of gestation can result in greater growth of muscle fibers [7] and better quality meat of the progeny. Therefore, it is important to study feeds that promote better fetal growth and post-birth development.

Corn starch is the most common feed used for energy supplementation in ruminants [8]. However, the use of rumen-protected fat in nutrition should increase the energetic support of the diet, increase milk production [9], reduce the risk of ruminal acidosis because of excess starch, and reduce the negative energy balance at the end of pregnancy and early lactation, avoiding metabolic problems such as toxemia (Simões et al., 2021).

Nutritionists have used chromium in animal nutrition as an insulin receptor enhancer to increase the use of blood sugar in cells [12]. In ruminants, the use of chromium in an energy-rich diet aims to increase the energy utilization of this diet and, thus, promote greater productive and reproductive performance [13]. Therefore, Cr supplementation affects glucose and lipid metabolism, with changes in blood profiles such as cortisol, glucagon, insulin, antioxidant defense, and immune system, especially in stressful situations, as occurs at the end of the ewes' gestation. The action of Cr on carbohydrate and lipid metabolism may explain the improvement in performing ruminants in pregnancy, lactation, growth, and fattening [14], but as pointed out by Tedeschi and Fox (2020). Cr has been used in mineral supplementation to enhance stressed ruminants' nutritional and health status, but unstressed animals have reported inconsistent results.

The study hypothesized that alternative energy sources in the diet of pregnant ewes are positive in the generation of progeny. We had aimed to evaluate the inclusion of Cr propionate and Ca salts of palm oil in the diet of ewes at the end of pregnancy and during lactation and to evaluate its effects on the fetal programming of lambs.

2. Material And Methods

2.1 Ewes

The experimental procedures were performed at the Faculty of Animal Sciences and Food Engineering of the University of Sao Paulo (FZEA/USP). Forty-three pregnant ewe, Santa Ines and Dorper breed, 3 ± 1 years old weighing 57 ± 10 kg, single gestating were randomly selected (out of a sixty animals in the herd). The ewes were distributed in completely randomized block in three treatments according to the feed studied, CTL (n = 15) with starch from corn; CR (n = 15) diet CTL plus 0.5 mg of Cr propionate; PF (n = 13) diet CTL plus Ca salts of palm oil (Table 1). The animal was fed twice a day (800 and 1600 h), and feed consumption was controlled daily. The ewes received the experimental diet for 120 days (from 100 days of gestation until 70 days of lactation) when weaning of the lambs was done.

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

	Ewe			
	CTL	Cr	PF	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		0.5		
Rumen Protected Fat			3	
Mineral [1]	1	1	1	1
Limestone	1	1		
Calcium chloride				1
Nutrition composition, kg/kg DM				
Crude Protein	0.111	0.111	0.111	0.166
EE	0.024	0.024	0.046	0.032
ME, Mcal/kg	2.46	2.46	2.47	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	0.66	0.164	0.110
Calcium, g/kg	7.5	7.5	7.7	4.5
Phosphor, g/kg	3.2	3.2	3.1	3.1

Abbreviations: CTL = with starch from corn; Cr = diet CTL plus chromium propionate; PF = diet CTL plus calcium salts of palm oil; EE = ether extract; ME = metabolizable energy; ADF = acid detergent fiber; NDF = neutral detergent fiber.

[1]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= 3.000 mg, Se= 10 mg, Zn= 5.000 mg, F= 650 mg, Vitamin A= 50.000 U.I., Vitamin E =312 U.I.

[2] KemTrace 0.4% Cr; Kemin Agrifoods South America.

The samples of the diet offered and orts were chemically analyzed according to AOAC (2000) for DM: dry matter - ID 930.15; OM: organic matter - ID 942.05; CP: crude protein - as 6.25 × N - ID 954.01; ADF - acid detergent fiber - ID 973.18) and NDF- neutral detergent fiber by Mertens et al. (2002). The Small Ruminant Nutrition System [18] was used for estimating the metabolizable energy (ME, Mcal).

The ewes, with 135 ± 3 days of gestation, were weighed on an electronic scale and the body condition score (BCS) was evaluated using a scale from 1 to 5, with 1 being extremely thin and 5 being extremely fat, according to the methodology of [19].

Blood samples were collected to analyze non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), insulin, and glucose. The procedure for blood collection was by venipuncture of the jugular vein in specific sterile tubes suitable for each test. The determination of serum insulin and glucose levels was performed with Kit Insulin (Monobind, Accubind EIA Kit, Code. 2425 – 300) and glucose by the kit with Ref. 133-1; by the ELISA technique and reading through the Lasystems Multiskan MS equipment. NEFA and BHB were quantified by colorimetric enzymatic methodology in an Automatic Biochemical Analyzer brand RX Daytona® - Randox Laboratories, United Kingdom, using a commercial kit branded Randox®, reference FA115 and RB 1007, respectively.

2.2 Progeny

2.2.1 Animals and local

The lambs remained with their dams for 70 days. Subsequently, only the males were sent to the feedlot for performance evaluation and slaughter after finished. A total of 23 lambs were used, being seven for CTL, nine for CR, and seven for PF. The males were not castrated and stayed in individual stalls (1.70 x 2.30 cm), with slatted flooring, access to water ad libitum, and shade for 56 days. Diet and management were the same for all lambs (Table 2), so only the dam’s diet was assessed on animal performance. They were offered twice a day, at 800 h and 1500 h, and feed consumption was controlled daily. The lambs were weighed every 14 days and the average daily weight gain was calculated. Feed efficiency was calculated by the ratio between weight gain and dry matter intake.

Table 2

Weight and body condition score (BCS), insulin, glucose, NEFA, and BHB of ewes fed different energy diets at the end of pregnancy and lactation.

135 days of gestation	Treatment					
	CTL	Cr	PF	Mean	RMSE	*P-value*
Body weight, kg	77.50	77.09	74.10	76.50	1.35	0.5033
BCS, 1–5 scale	3.82	3.91	3.75	3.83	0.55	0.7383
Insulin, µUI/mL	0.97^{b}	1.66^{ab}	2.07^{b}	1.56	0.16	0.0272
Glucose, mg/dL	70.35	66.61	63.96	66.65	10.56	0.1015
NEFA, mmol/L	0.127^{ab}	0.219^{a}	0.068^{b}	0.138	0.04	0.0446
BHB, mmol/L	1.80^{b}	3.23^{a}	3.54^{a}	1.85	0.35	0.0101

Abbreviations: CTL = with starch from corn; Cr = diet CTL plus chromium propionate; PF = diet CTL plus calcium salts of palm oil; BCS = body condition score.

$^{a-b}$ Values within a row with different superscripts differ significantly at $P < 0.05$.

2.2.2. Carcass parameters

Before slaughter, the lambs were fasted from solids for 16 hours and weighed. They were stunned with a pressure gun and bled by cutting the jugular veins and arteries. After animals were deemed dead, we removed the paws, head, leather, viscera, washed the carcass, toilet, weighed the hot carcass, and sent them to the cooling chamber, where they remained for 24 h at 2°C. After this period, the carcass was weighed again. Different carcass yields and chilling losses were calculated.

At the time of slaughter, the non-carcass components were weighed with an analytical and counterweight: feet, head, leather, lung, heart, respiratory tract, liver, intestines, and rumen. The proportion of these components about the live weight at the slaughter of the animal was made.

The chilled carcass was sectioned in half, with the left side cut between the 12th and 13th ribs, exposed to the *Longissimus dorsi* muscle, and the fat thickness was measured with the aid of a digital caliper (Digital Caliper, Western®). In this same region of the muscle, the shape of the muscle was demarcated, and measurements of A (medial-lateral direction) and B (dorsoventral direction) were used to compute the loin eye area using Eq. 1.

Loin eye area = $\left(\frac{A}{2}x\frac{b}{2}\right)x3.1416$ Eq. 1

The loin, tenderloin, and legs were removed from the half carcass. The loin corresponded to the last eight thoracic vertebrae. The tenderloin is the cut made up of the muscle masses attached to the ventral surface of the last three thoracics, six lumbar, iliac, and femur (third trochanter) vertebrae. The leg was sectioned between the femoral head and the acetabulum. All parts were weighed with a semi-analytical scale, accurate to 10 g. Subsequently, the loin and leg cuts were dissected into muscle, bone, and fat. The weight of each cut was calculated about the carcass. The muscle, bone, and fat proportions were taken from each cut.

2.2.3. Bone density

After boning the leg, the femur was weighed with a precision scale, with a capacity of 20 g to 3 kg and precision of 0.0001 g (BL3200H, Shimadzu), and its length was evaluated with a millimeter tape measure. After obtaining the weight and length of the femur, the Seedor index was calculated, which serves as an indication of bone density and corresponds to the weight of the bone (mg), divided by its length (mm) [20].

Subsequently, the femur was frozen in a freezer at -20ºC for further analysis. The femur of the left leg was radiographed, and the dorsopalmar and lateromedial measurements were recorded. For both projections, the bone was positioned on top of the digital cassette, the X-ray machine was at a distance of 100 centimeters and the beam of rays was positioned on the femur, thus emitting the X-ray waves. For the calibration of the shade system and the determination of bone density, and aluminum scale was positioned in the projection. The scale used has 25 steps, with a difference of 1 mm in height between them, with the 1st step being 1mm high and the 25th being 25mm. The standardized technique was 72 kV voltage and 3.2 mAs exposure time. A digital X-ray machine from Sound Eklin®, Carlsbad, USA, was used, and the emitter was the JOB X - Ray®, 380 HF, Job do Brazil® Sao Paulo, BR. Then, the images were analyzed using the Image J® program, and bone density was determined on an aluminum scale (mmAl) individually in the epiphysis region.

2.3. Statistical analysis

The statistical design was in randomized complete block design and treatments were considered fixed effects, according to the model:

$$Yijk = \mu + Ti + Bk + eij$$

Where: Yij is the dependent variable, (i = treatment and j = repetition), μ is the overall mean, Ti is the fixed effect of the treatment (i = 1–3), B is the block (age of the sheep) and eijk is the residual error.

Data were checked for normality (Shapiro-wilk and Levene's tests) and then analyzed using PROC GLM and the Tukey test at 5% significance [21].

3. Results

3.1. Ewes

The CTL, CR, and PF treatments did not differ ($P > 0.05$, Table 2) for BW with a mean value of 76.50 kg, BCS (mean 3.83), glucose (mean 66.65 mg/DL) at 135 days of gestation. However, the insulin concentration ($P = 0.0272$) was higher for PF than CTL. The concentration of NEFA ($P = 0.0446$) was highest in CR and lowest in PF. BHB ($P = 0.0101$) was higher in CR and PF.

3.2. Progeny

3.2.1. Performance

The birth weight (1 day postpartum, $P = 0.6864$), dry matter intake (DMI, $P = 0.4405$), average daily weight gain in confinement (ADG, $P = 0.4101$), and feed efficiency (FE, $P = 0.6861$) had no statistical difference between treatments (Table 3), and had means of 4.35 kg, 1.50 kg/d, 0.339 kg/d and 0.22 kg/kg. Lambs from ewes fed with Cr propionate or Ca salts of palm oil had higher body weight at the beginning of the feedlot ($P = 0.0150$) and slaughter ($P = 0.0040$) compared to lambs from the CTL treatment. The values for BW at the original feedlot for Cr were 26.92 kg and for PF 28.20 kg, and slaughter 46.81 kg and 47.62 kg, respectively.

Table 3
Performance of lambs from ewes fed different energy sources in late gestation and lactation.

	Treatment					
Performance	CTL	Cr	PF	Mean	RMSE	*P-value*
Body Weight, kg						
1 day postpartum	4.32	4.20	4.54	4.35	0.27	0.6864
Inicial feedlot – 70 days	22.60^{b}	26.92^{a}	28.20^{a}	25.91	0.43	0.0150
Slaughter – 126 days	42.90^{b}	46.81^{a}	47.62^{a}	45.78	0.37	0.0040
In feedlot						
Dry matter intake, kg/day	1.43	1.53	1.56	1.50	0.24	0.4405
Average daily weight gain, kg/d	0.329	0.353	0.333	0.339	0.04	0.4101
Feed efficiency, kg ADG/kg DMI	0.23	0.23	0.21	0.22	0.03	0.6861

Abbreviations: CTL = with starch from corn; Cr = diet CTL plus chromium propionate; PF = diet CTL plus calcium salts of palm oil.

$^{a-b}$ Values within a row with different superscripts differ significantly at $P < 0.05$.

3.2.2. Carcass

There was a difference in hot and cold carcass yield between treatments ($P < 0.05$, Table 4), with a higher value for lambs from CR treatment (54.58% and 52.41%, respectively), followed by CTL (52.13% and

50.13%, respectively) and lower for PF (50.74% and 48.39%, respectively). The parameter refrigerated losses (3.13% mean), loin eye area (19.59 cm^2 mean), and fat thickness (1.92 mm mean) were similar among lambs regardless of maternal diet ($P > 0.05$).

Table 4
Carcass parameters of lambs from ewes fed different energy sources in late gestation and lactation

	Treatment					
	CTL	Cr	PF	Mean	RMSE	*P-value*
Hot carcass yield, %	52.33^{ab}	54.58^{a}	50.74^{b}	52.73	3.26	0.0432
Cold carcass yield, %	50.13^{ab}	52.41^{a}	48.39^{b}	46.69	2.00	0.0441
Refrigerated losses, %	3.10	3.07	3.26	3.13	0.07	0.7079
Loin eye area, cm^2	18.67	20.88	20.22	19.59	0.839	0.7015
Fat thickness, mm	1.92	1.81	2.06	1.92	0.44	0.6025

Abbreviations: CTL = with starch from corn; Cr = diet CTL plus chromium propionate; PF = diet CTL plus calcium salts of palm oil.

$^{a-b}$ Values within a row with different superscripts differ significantly at $P < 0.05$.

Maternal nutrition did not alter the progeny carcass ($P > 0.05$, Table 5), the weight of the half carcass (11.26 kg mean), and the proportions of loin (8.98%) and tenderloin (1.3%). However, diet treatments changed progeny's percentage of the leg ($P = 0.0255$), with a value of 34% for Cr, 32% for CTL, and 28% for PF. The percentage of leg fat ($P = 0.0359$, Table 4) was lower for Cr (19.13%) and similar between CTL (21.29%) and PF (20.22%).

Table 5
Percentage of cuts about lamb carcasses from sows fed with different energy sources in late gestation and lactation

	Treatment					
	CTL	Cr	PF	Mean	RMSE	*P- value*
Weight of half carcass, kg	10.71	11.37	11.63	11.26	1.57	0.5529
Percentage of cuts, %						
Loin	8.69	9.40	8.86	8.98	1.13	0.6822
Muscle	47.01	50.71	49.87	49.20	4.53	0.7598
Bone	37.02	32.78	33.45	34.42	7.32	0.4334
Fat	16.00	16.51	16.68	16.40	8.16	0.7291
Tenderloin	1.25	1.36	1.28	1.3	0.27	0.7333
Leg	32.12^{ab}	34.04^{a}	28.35^{b}	31.50	3.61	0.0255
Muscle	61.76	63.40	63.70	53.95	3.36	0.2584
Bone	16.95	17.47	16.08	16.83	3.69	0.6867
Fat	21.29^{ab}	19.13^{b}	20.22^{a}	20.21	2.18	0.0359

Abbreviations: CTL = with starch from corn; Cr = diet CTL plus chromium propionate; PF = diet CTL plus calcium salts of palm oil.

$^{a–b}$ Values within a row with different superscripts differ significantly at $P < 0.05$.

Maternal diet altered the proportion of the spleen ($P = 0.0216$) and the respiratory tract (lung + trachea + esophagus, $P = 0.0462$). The spleen was larger for the CTL (0.22%), intermediate for Cr (0.19%), and smaller for PF (0.16%). The respiratory tract was higher for CTL (2.04%), intermediate for PF (1.86%), and lower for Cr (1.71%). There was no difference ($P > 0.05$, Table 6) between the maternal diets for the other non-carcass components of lambs, averaging 1.70% for testicles, 0.48% for heart, 2.18% for the liver, 1.72% for kidneys, 6.08% for the head, 4.31% for feet, 11.38% for the hide, 4.29% for intestines, and 2.62% for the reticulorumen.

Table 6
The proportion of non-carcass components of lambs from ewes fed with different levels and sources of energy in late gestation and lactation

	Treatment					
Proportion (%)	CTL	Cr	PF	Mean	RMSE	*P- value*
Spleen	0.22[a]	0.19[ab]	0.16[b]	0.199	0.044	0.0216
Respiratory tract	2.04[a]	1.71[b]	1.86[ab]	1.85	0.312	0.0462
Testicles	1.66	1.34	2.41	1.70	1.52	0.4669
Heart	0.52	0.45	0.49	0.48	0.098	0.4025
Liver	2.36	2.12	2.03	2.18	0.35	0.2616
Kidneys	1.80	1.69	1.66	1.72	0.45	0.8319
Head	6.58	5.50	6.42	6.08	1.70	0.4136
Feet	6.96	2.64	3.64	4.31	5.07	0.2521
Leather	12.16	11.73	9.44	11.38	3.58	0.5646
Intestines	4.65	3.92	4.45	4.29	1.20	0.4738
Rumen	2.54	2.61	2.73	2.62	0.4581	0.7859
Abbreviations: CTL = with starch from corn; Cr = diet CTL plus chromium propionate; PF = diet CTL plus calcium salts of palm oil.						
[a–b] Values within a row with different superscripts differ significantly at $P < 0.05$.						

There was a tendency ($P = 0.0704$, Table 7) for lambs from the CR and PF treatments to have better muscle conformation (average of 3) compared to the CTL (2.8). The maternal diet did not change the fat conformation, the internal and external length of the carcass, the measurements of the thorax, and the length of the leg ($P > 0.05$). However, the diet altered the leg dimensions: perimeter was 22 cm for CR and PF groups and 20 for CTL, and the width was approximately 70 cm for Cr and PF and 65 cm for CTL ($P = 0.0379$).

Table 7
Lamb carcass grade and measurements from ewes fed with different levels and sources of energy in late gestation and lactation

	Treatment					
	CTL	Cr	PF	Mean	RMSE	*P-value*
Muscle conformation, 1–5	2.83	3.66	3.66	3.42	0.82	0.0704
Fat conformation, 1–5	2.66	3.00	3.35	3.00	0.9	0.457
Internal carcass length, cm	67.5	66.22	70.00	67.60	3.48	0.149
External carcass length, cm	65.66	65.33	67.66	66.09	2.87	0.3022
Thoracic perimeter, cm	23.28	24.95	24.6	24.37	2.03	0.3047
Thoracic width, cm	75	76.11	75.66	75.66	3.59	0.8431
Hind perimeter, cm	20.7^{a}	22.71^{b}	22.45^{b}	22.06	1.04	0.005
Hind width, cm	65.66^{a}	70.11^{b}	69.66^{b}	67.71	3.15	0.0379
Leg length, cm	47.66	44.66	48.16	46.52	3.66	0.1582

Abbreviations: CTL = with starch from corn; Cr = diet CTL plus chromium propionate; PF = diet CTL plus calcium salts of palm oil.

$^{a-b}$ Values within a row with different superscripts differ significantly at $P < 0.05$.

3.2.3. Bone density

The thickness of the femoral epiphysis was altered by the diet ($P = 0.0307$, Table 8), with a value of 5 for CTL, intermediate for PF with 4.6, and lower for Cr with 4.5. Bone length ($P = 0.0343$) was similar to the epiphysis, that is, longer for CTL with 186.85 mm, intermediate for PF 182.83 mm, and shorter for CR 175.44 mm. Weight measurements and Seedor index were not influenced by diet ($P > 0.05$), with 1.92 kg and 106 mg/mm, respectively.

Table 8
Bone parameters of lambs from ewes fed with different levels and sources of energy in late gestation and lactation.

	Treatment					
	CTL	Cr	PF	Mean	RMSE	*P- value*
Proximal epiphysis, mmAl	5.28a	4.53b	4.66ab	4.84	0.84	0.0307
Length, mm	186.85[a]	175.44[b]	182.83[ab]	181.00	8.14	0.0343
Weight, kg	1.88	1.92	1.96	1.92	0.23	0.8399
IS, mg/mm	100.57	109.65	106.86	106.00	10.67	0.2593
Abbreviations: CTL = with starch from corn; Cr = diet CTL plus chromium propionate; PF = diet CTL plus calcium salts of palm oil.						
[a–b] Values within a row with different superscripts differ significantly at $P < 0.05$.						

4. Discussion And Conclusion

4.1. Ewes

At the late gestation and early lactation, the nutritional requirement of the ewe increases [22]. Therefore, nutritional strategies are used in order to meet these demands and decrease negative energy balance. The body condition score (BCS) is an easy and accurate method to estimate the nutritional condition of sheep. The BCS suitable for late pregnancy is between 3.5 to 4 and at the end of lactation between 2.5 to 3 [19]. The results obtained for BCS and BW in CTL, CR, and PF diets were the same and are within the recommended range.

The use of grain in the diet of ruminants at the late of gestation and lactation aims to provide ingredients with a higher concentration of non-structural carbohydrates and thus meet the nutritional requirements. A commonly used energy ingredient in ruminant production is corn due to the high amount of starch in its composition. The digestive processes of starch by the rumen microbiota result in an increase in propionate production, which has an important role in maintaining plasma glucose levels. Shortly after eating, serum glucose levels rise, and the pancreatic beta cells react by releasing insulin into the circulation. By binding to insulin receptors on the cell wall, the hormone activates the glucose transport pathways, enabling its circulation inside the cells.

In sheep, the placenta-fetal unit uses 30 to 40% of the maternal glucose [23]. During periods of inadequate dietary energy consumption, the concentration of hormones that stimulate lipolysis, such as glucagon, beta-adrenergic catecholamines, and cortisol, increases while the concentration of insulin decreases. The high mobilization of triglycerides substantially increases the amount of free fatty acids (NEFA) [24]. Typically, a small amount of acetyl-CoA is transformed into acetone, acetoacetate, and beta-hydroxybutyrate in the liver. The production of ketone bodies is abnormally high when the degradation of carbohydrates does not accompany the degradation of triglycerides.

Calcium salts of palm oil increased the concentration of insulin and BHB in the blood, indicating energy generation by lipid metabolism. Supplementation with chromium propionate increased the concentration of NEFA and BHB, which are common findings in a high metabolizable energy diet [25]. *The use of chromium propionate days before parturition increases lipolysis* [26].

4.2. Progeny

4.2.1. Performance

The sheep's diet had the same concentration of metabolizable energy but with different feeds, such as chromium propionate and calcium salts of palm oil. During pregnancy and lactation, ewes fed these two foods produced heavier lambs at the beginning and the end of the feedlot, with approximately a difference of 4 kg of body weight more than the lamb in the CTL treatment. Therefore, there was a beneficial effect of fetal programming for weight in the progeny growth phase.

Daily weight gain and feed efficiency in the confinement of lambs were similar between diets ($P > 0.05$). However, the lambs entered the feedlot with different body weights, and there was no compensatory gain, so the difference in body weight remained until slaughter.

CR and PF increased the energy availability of ewes during the gestation phase, which promotes greater fetal growth at the end of pregnancy. According to Robinson et al. (2013) the nutrition of ewes during pregnancy has an influence of 20% on the birth weight of the progeny, is also influenced by breed (8.1%), age of the mother (5.5%), time of delivery (4.7%) and sex (4.2%). The better availability of feed energy for the ewe during pregnancy can affect milk production and consequently the weaning weight of the lamb. The effect of fetal programming can extend to the lactation period. Therefore, the best energy use of the diet by the ewe fed either with CR or with PF may have increased the milk production of the ewes, which allowed better nutrition of the lambs and thus influenced the weight at weaning and slaughter. The beneficial effect of chromium propionate or calcium salts of palm oil has already been proven with dairy cows [25].

4.2.2. Carcass parameters

The carcass yield (hot and cold) of lambs whose mothers were fed with chromium propionate were higher than those treated with calcium salts of palm oil and similar to CTL. The Cr ingested by the ewe may have enabled better energy partition between the ewe and the fetus, with greater energy support for fetal. In the adult phase, this greater muscular development results in lambs with higher carcass yield.

Fat thickness and loin eye area were not influenced by maternal diet. Animals fed directly with Cr may have a reduction in the amount of fat in the carcass, despite this being controversial information. Moreno-Camarena et al. (2015) observed that lambs fed different doses of Cr did not have a different fat thickness but had a change in mesenteric fat. Castro et al. (2005) studied the increasing supply of rumen-protected fat in the diet of lambs and did not observe any difference in the measure of fat thickness.

Maternal diets did not change the proportions of loin and tenderloin cuts about half carcass weight ($P >$ 0.05). But they changed the leg proportion, being higher for CR, intermediate for CTL, and lower for PF (P = 0.0255). Within the leg cut, there was a difference in the proportion of fat, with a higher value for PF, intermediate for CTL, and lower for CR ($P = 0.0359$). Mostafa-Tehrani et al. (2006) also observed a higher proportion of the hind limb in the carcass of lambs fed with Cr, with the explanation of greater insulin activity and glucose utilization for muscle development.

Regarding the non-carcass components, the maternal diet influenced the spleen and the respiratory tract ($P < 0.05$). The animals in the control group had a larger spleen, intermediate for CR and smaller for PF. Among the main functions of the spleen is the important formation of cells of the immune system and filter the body's red cells. Dietary Cr supplementation can stimulate genes present in the spleen that stimulate the production of defense cells in animals under some stress conditions. The chromium requirement for sheep is not yet established [22], which demonstrates the need for further studies on the subject. Badee and Hidaka (2014) studied different sources and concentrations of rumen-protect fat in the diet of lambs and observed changes in spleen weight as in this experiment.

Dallago et al. (2016) studied Cr supplementation and its deposit in different tissues of the body of lambs and concluded that Cr accumulated in greater amounts in the heart, testes, and lungs. In our study, ewes fed with Cr had lamb with a smaller respiratory tract than the others, and there may be an action from the mother to the fetus and the fetus to adulthood.

The hind perimeter and hind width were greater for the CR and PF than for the CTL ($P > 0.05$, Table 7). The largest leg influenced the evaluation of the carcass, there was a tendency for these carcasses to be better evaluated in terms of their conformation ($P = 0.0704$). The highest values of hind width and perimeter agree with the greater weight of the animals at slaughter in the CR and PF groups (Table 3) and the greater leg in the CR (Table 5). Maternal nutrition in early pregnancy affects the number of muscle fibers, at the end of pregnancy, it interferes with muscle hypertrophy, that is, fiber size. The diets that allowed the best energy use for the ewe were CR and PF, which resulted in heavier lambs at weaning, and this advantage extended until slaughter, hence the greater measures of the shank. The allometric growth of the leg can be affected by genetics, but in this study, the racial pattern was similar; therefore, the positive effect of the larger size of this cut is related to maternal nutrition.

4.2.3.Density bone

Bone tissue presents an intense vascularization that allows the exchange of nutrients and minerals, therefore, helping to regulate mainly the amount of calcium and plasma phosphorus by the action of hormones, such as parathyroid hormone (PTH), calcitonin, and vitamin D. Bone cells, osteoclast, act on bone resorption, and present a large number of mitochondria, supporting the premise that energy utilization is an essential element in bone remodeling [33], reaffirming the hypothesis that diets with more energy are capable of produce the most offspring and bone heavy. The smaller density bone in the epiphysis region found in treatments with CR and PF may be related to increased production or activation of insulin and better energy use of the sheep with these diets benefiting the progeny.

The primary event leading to insulin secretion is increased ionized calcium in the beta-cell cytosol. At the beginning of pregnancy, the treatment with PF contained a higher concentration of Ca since the protection of fatty acids was through adding calcium salts. This may have caused this group to release more insulin.

In theory, Cr supplementation had better glucose activation. Insulin is released rapidly into the bloodstream when blood sugar levels rise and bind to an outer α subunit of the transmembrane protein insulin receptor, causing a change in receptor conformation. The receptor autophosphorylates tyrosine residues in the inner portion of its β subunit, transforming the receptor into an active kinase. Chromodulin is stored in its apo form in the cytosol and the nucleus of insulin-sensitive cells. Chromium enhances insulin receptor activation so that more glucose enters the cell. A maximum of four Cr molecules can bind to an insulin receptor, leading to an eight-fold difference in insulin receptor activation [34].

Insulin acts on bone cells by inhibiting the expression of Opg (osteoprotegerin). This, in turn, acts by inhibiting the activation and maturation of osteoclasts. Therefore, insulin, by inhibiting Opg, stimulates the action of osteoclasts and, consequently, bone resorption. Diets with Cr and rumen-protected fat may have had an imbalance in bone remodeling due to greater insulin activation and, therefore, greater bone resorption, resulting in lower bone density by X-ray. McCarty (1995) concludes that bone resorption is not greater than bone formation, thus causing osteoporosis, it is necessary to assess the amount of Cr better to be ingested together with calcium and vitamin D. This is important information in this work and should be further studied.

However, bone formation is regulated in the fetal phase, and maternal undernutrition during pregnancy can result in a lighter, smaller femur with less bone density [35]. Even with restriction in pregnancy, the lamb's femur can present compensatory growth, increase in weight, and become equal to lambs without fetal restriction, especially after weaning [6]. In this study, however, the sheep had no difference in the level of nutrition, only the source of energy used was different. This is likely the reason there was no effect on the weight and length of the femur.

We concluded that despite maternal diets having the same amount of metabolizable energy, supplementation with chromium propionate or calcium salts of palm oil altered the progeny, producing heavier lambs at slaughter, with better carcass yield a better proportion of prime cut. It is important to better understand the effect of these feeds on the spleen and their effects on the immune system and the density and length of the femur.

Abbreviations

CTL
with starch from corn

Cr
diet CTL plus chromium propionate
PF
diet CTL plus calcium salts of palm oil
EE
ether extract
ME
metabolizable energy
ADF
acid detergent fiber
NDF
neutral detergent fiber.

Declarations

Ethics approval

Animal investigations were carried out by the Institutional Committee on the Use of Animals (Protocol No CEUA 2700201218).

Author ORCIDs

L. Brochine https://orcid.org/0000-0001-6069-7223

F. F. dos Santos https://orcid.org/0000-0002-7086-1311

F. M. Moreira https://orcid.org/0000-0002-5046-1871

P. R. Leme https://orcid.org/0000-0002-5210-275X

L. O. Tedeschi https://orcid.org/0000-0003-1883-4911

S. B. Gallo https://orcid.org/0000-0001-7584-4548

Author contributions

L. Brohcine investigation, data curation, writing, and resources.

F. F. Santos investigation, data curation formal analysis, writing, and resources.

F. M. Moreira investigation and resources.

P. R. Leme conceptualization, methodology, data curation.

L. O. Tedeschi conceptualization, methodology, data curation, writing, revising.

S. B. Gallo conceptualization, methodology, formal analysis, investigation, resources data curation, writing, revising, supervision, project administration, funding acquisition.

Declaration of interest

None.

Financial support statement

This work was supported by FAPESP - São Paulo Research Foundation, process number 2017/20555-8, and CAPES for the scholarship.

References

1. NRC - National Research Council, Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids, National Academies Press, Washington, D.C., 2007. https://doi.org/10.17226/11654.
2. C. Brozos, V.S. Mavrogianni, G.C. Fthenakis, Treatment and Control of Peri-Parturient Metabolic Diseases: Pregnancy Toxemia, Hypocalcemia, Hypomagnesemia, Vet. Clin. North Am. - Food Anim. Pract. 27 (2011) 105–113. https://doi.org/10.1016/j.cvfa.2010.10.004.
3. E.D. Sartori, A.G. Sessim, D.D. Brutti, J.F. Lopes, C.M. McManus, J.O.J. Barcellos, Fetal programming in sheep: effects on pre- and postnatal development in lambs, J. Anim. Sci. 98 (2020). https://doi.org/10.1093/jas/skaa294.
4. F. Gao, Y. Liu, L. Li, M. Li, C. Zhang, C. Ao, X. Hou, Effects of maternal undernutrition during late pregnancy on the development and function of ovine fetal liver, Anim. Reprod. Sci. 147 (2014) 99–105. https://doi.org/10.1016/j.anireprosci.2014.04.012.
5. S. Luzardo, G. de Souza, G. Quintans, G. Banchero, Refeeding ewe's ad libitum after energy restriction during mid-pregnancy does not affect lamb feed conversion ratio, animal performance and meat quality, Small Rumin. Res. 180 (2019) 57–62. https://doi.org/10.1016/j.smallrumres.2019.09.020.
6. M.P. Tygesen, A.P. Harrison, M. Therkildsen, The effect of maternal nutrient restriction during late gestation on muscle, bone and meat parameters in five month old lambs, Livest. Sci. 110 (2007) 230–241. https://doi.org/10.1016/j.livsci.2006.11.003.
7. M. Du, B. Wang, X. Fu, Q. Yang, M.J. Zhu, Fetal programming in meat production, Meat Sci. 109 (2015) 40–47. https://doi.org/10.1016/j.meatsci.2015.04.010.
8. S.B. Gallo, F. de Almeida Merlin, C.M. de Macedo, R.D. de Oliveira Silveira, Whole grain diet for Feedlot Lambs, Small Rumin. Res. 120 (2014) 185–188. https://doi.org/10.1016/j.smallrumres.2014.05.014.
9. J.M. Santos Neto, J. de Souza, A.L. Lock, Effects of calcium salts of palm fatty acids on nutrient digestibility and production responses of lactating dairy cows: A meta-analysis and meta-regression,

J. Dairy Sci. 104 (2021) 9752–9768. https://doi.org/10.3168/JDS.2020-19936.

10. S. Sgorlon, G. Stradaioli, G. Gabai, B. Stefanon, Variation of starch and fat in the diet affects metabolic status and oxidative stress in ewes, Small Rumin. Res. 74 (2008) 123–129. https://doi.org/10.1016/J.SMALLRUMRES.2007.04.004.
11. J. Simões, J.A. Abecia, A. Cannas, J.A. Delgadillo, D. Lacasta, K. Voigt, P. Chemineau, Review: Managing sheep and goats for sustainable high yield production, Animal. 15 (2021) 100293. https://doi.org/10.1016/J.ANIMAL.2021.100293.
12. K. Ognik, W. Dworzański, I. Sembratowicz, B. Fotschki, E. Cholewińska, P. Listos, J. Juśkiewicz, The effect of the high-fat diet supplemented with various forms of chromium on rats body composition, liver metabolism and organ histology Cr in liver metabolism and histology of selected organs, J. Trace Elem. Med. Biol. 64 (2021) 126705. https://doi.org/10.1016/J.JTEMB.2020.126705.
13. T. Leiva, R.F. Cooke, A.P. Brandão, U. Pardelli, R.O. Rodrigues, F.N. Corrá, J.L.M. Vasconcelos, Effects of concentrate type and chromium propionate on insulin sensitivity, productive and reproductive parameters of lactating dairy cows consuming excessive energy, Animal. 11 (2017) 436–444. https://doi.org/10.1017/S1751731116001713.
14. S. Lashkari, M. Habibian, S.K. Jensen, A Review on the Role of Chromium Supplementation in Ruminant Nutrition—Effects on Productive Performance, Blood Metabolites, Antioxidant Status, and Immunocompetence, Biol. Trace Elem. Res. 186 (2018) 305–321. https://doi.org/10.1007/s12011-018-1310-5.
15. L.O. Tedeschi, D.G. Fox, The Ruminant Nutrition System: Volume I - An Applied Model for Predicting Nutrient Requirements and Feed Utilization in Ruminants., Third Edit, XanEdu, Acton, MA, Acton, MA, 2020.
16. AOAC, Official Methods of Analysis of AOAC International, Assoc. Off. Anal. Chem. Int. (2000). https://doi.org/10.3109/15563657608988149.
17. D.R. Mertens, M. Allen, J. Carmany, J. Clegg, A. Davidowicz, M. Drouches, K. Frank, D. Gambin, M. Garkie, B. Gildemeister, D. Jeffress, C.S. Jeon, D. Jones, D. Kaplan, G.N. Kim, S. Kobata, D. Main, X. Moua, B. Paul, J. Robertson, D. Taysom, N. Thiex, J. Williams, M. Wolf, Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study, J. AOAC Int. 85 (2002) 1217–1240.
18. L.O. Tedeschi, A. Cannas, D.G. Fox, A nutrition mathematical model to account for dietary supply and requirements of energy and other nutrients for domesticated small ruminants: The development and evaluation of the Small Ruminant Nutrition System, Small Rumin. Res. 89 (2010) 174–184. https://doi.org/10.1016/J.SMALLRUMRES.2009.12.041.
19. P.R. Kenyon, S.K. Maloney, D. Blache, Review of sheep body condition score in relation to production characteristics, New Zeal. J. Agric. Res. (2014). https://doi.org/10.1080/00288233.2013.857698.
20. J.G. Seedor, H.A. Quartuccio, D.D. Thompson, The bisphosphonate alendronate (MK-217) inhibits bone loss due to ovariectomy in rats, J. Bone Miner. Res. 6 (1991) 339–346. https://doi.org/10.1002/jbmr.5650060405.

21. SAS Institute Inc., Base SAS® 9.4 Procedures Guide, 2013. https://doi.org/10.1016/B978-0-444-59425-9.00014-7.
22. NRC, Nutrient Requirements of Small Ruminants, National Academies Press, Washington, D.C., 2007. https://doi.org/10.17226/11654.
23. R.L. Prior, R.K. Christenson, Insulin and Glucose Effects on Glucose Metabolism in Pregnant and Nonpregnant Ewes, J. Anim. Sci. 46 (1978) 201–210. https://doi.org/10.2527/jas1978.461201x.
24. U. Moallem, A. Rozov, E. Gootwine, H. Honig, Plasma concentrations of key metabolites and insulin in late-pregnant ewes carrying 1 to 5 fetuses, J. Anim. Sci. 90 (2012) 318–324. https://doi.org/10.2527/jas.2011-3905.
25. T. Leiva, R.F. Cooke, A.P. Brandão, R.D. Bertin, E.A. Colombo, V.F.B. Miranda, L.A.C. Lourenço, S.M.B. Rodrigues, J.L.M. Vasconcelos, Effects of supplemental calcium salts of palm oil and chromium-propionate on insulin sensitivity and productive and reproductive traits of mid- to late-lactating Holstein × Gir dairy cows consuming excessive energy, J. Dairy Sci. 101 (2018) 491–504. https://doi.org/10.3168/jds.2017-13081.
26. J.P. McNamara, F. Valdez, Adipose Tissue Metabolism and Production Responses to Calcium Propionate and Chromium Propionate, J. Dairy Sci. 88 (2005) 2498–2507. https://doi.org/10.3168/JDS.S0022-0302(05)72927-1.
27. D.L. Robinson, L.M. Café, P.L. Greenwood, Meat Science and muscle Biology Symposium: Developmental programming in cattle: Consequences for growth, efficiency, carcass, muscle, and beef quality characteristics, J. Anim. Sci. (2013). https://doi.org/10.2527/jas.2012-5799.
28. L. Moreno-Camarena, I. Domínguez-Vara, J. Bórquez-Gastelum, J. Sánchez-Torres, J. Pinos-Rodríguez, A. Mariezcurrena-Berasain, E. Morales-Almaráz, A.Z.M. Salem, Effects of organic chromium supplementation to finishing lambs diet on growth performance, carcass characteristics and meat quality, J. Integr. Agric. 14 (2015) 567–574. https://doi.org/10.1016/S2095-3119(14)60835-2.
29. T. Castro, T. Manso, A.R. Mantecón, J. Guirao, V. Jimeno, Fatty acid composition and carcass characteristics of growing lambs fed diets containing palm oil supplements, Meat Sci. 69 (2005) 757–764. https://doi.org/10.1016/j.meatsci.2004.11.008.
30. A. Mostafa-Tehrani, G. Ghorbani, A. Zare-Shahneh, S.A. Mirhadi, Non-carcass components and wholesale cuts of Iranian fat-tailed lambs fed chromium nicotinate or chromium chloride, Small Rumin. Res. 63 (2006) 12–19. https://doi.org/10.1016/j.smallrumres.2005.01.013.
31. G. Badee, S. Hidaka, Growth performance, carcass characteristics, fatty acid composition and CLA concentrations of lambs fed diets supplemented with different oil sources, Anim. Sci. J. (2014). https://doi.org/10.1111/asj.12094.
32. B.S.L. Dallago, B.A.F. Lima, S.V. Braz, V. da S. Mustafa, C. McManus, T. do P. Paim, A. Campeche, E.F. Gomes, H. Louvandini, Tissue accumulation and urinary excretion of Cr in chromium picolinate (CrPic)-supplemented lambs, J. Trace Elem. Med. Biol. 35 (2016) 30–35. https://doi.org/10.1016/J.JTEMB.2016.01.004.

33. M.F. McCarty, Anabolic effects of insulin on bone suggest a role for chromium picolinate in preservation of bone density, Med. Hypotheses. 45 (1995) 241–246. https://doi.org/10.1016/0306-9877(95)90112-4.
34. J.B. Vincent, Recent Developments in the Biochemistry of Chromium(III), Biol. Trace Elem. Res. 99 (2004) 001–016. https://doi.org/10.1385/bter:99:1-3:001.
35. X. Li, H. Li, Z. He, Z. Tan, Q. Yan, Effects of maternal intake restriction during early pregnancy on fetal growth and bone metabolism in goats, Small Rumin. Res. 36 (2019) 57–65. https://doi.org/10.1016/j.cois.2019.08.004.