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Article

# Effect of Dietary Calcium Propionate Inclusion Level and Duration in High-Risk, Newly Received Stocker Calves: Growth Performance, Body Fat Reserves and Health

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**Abstract:** Fifty bull calves of the Continental × British crossbred (147.0 ± 1.67 kg body weight, BW) were used in a completely randomized design to investigate the effect varying levels and duration of calcium propionate (CaPr) supplementation on the growth performance, body fat reserves, serum metabolites, and hemogram of high-risk newly received stocker calves. These calves were individually housed and fed a received-diet for 56 d. The calves received the following treatments: 1) No CaPr (CTL), 2) 20 g CaPr/calf/d, 3) 40 g CaPr/calf/d, 4) 60 g CaPr/calf/d, and 5) 80 g CaPr/calf/d, during 14, 28, 42 and 56 d the after arrival. The supplementation with 20 g CaPr from 28 to 56 d after arrival increases average daily gain (ADG) and BW (p < 0.05), and DMI is not affected (p > 0.05). This is reflected at 28 d with increases (p < 0.05) in ADG:DMI ratio and longissimus muscle area (LMA), and at 56 d in back fat thickness (BFT) y fat thickness at the rump (FTR). Also, with 20 g, blood urea nitrogen decreased (p < 0.05); and increases were observed in the activity of gamma glutamyltransferase, monocytes (quadratic trend, p < 0.07) and granulocytes% (quadratic effect, p < 0.03). However, as the level of CaPr increased during the first 14 d after arrival, daily water intake, creatinine, total cholesterol, mean corpuscular hemoglobin concentration (linear effect, p < 0.05), globulin, calcium and mean corpuscular volume (linear trend, p = 0.08) increased, while alkaline phosphatase (linear trend, p = 0.07) and lymphocytes (linear effect, p = 0.05) decreased. Finally, the different levels of CaPr supplementation did not produce any significant effects or differences (p > 0.05), in the remaining serum metabolites and hemogram (p > 0.05). Ultimately, the inclusion of 20 g CaPr/calf/d in the diet for 28 d in newly received stocker calves increases ADG, ADG:DMI ratio and LMA. If extended to 42 or 56 d, the increases in ADG persist, but there is also a rise in body fat reserves (BFT and FTR) at the expense of a reduction in ADG:DMI ratio. Furthermore, the different supplementation levels did not impact the reference range for most serum metabolites or the health of stocker calves.

Keywords: beef calf; gluconeogenic precursor's; reception diet; serum metabolites; hemogram

# 1. Introduction

Events such as recent weaning, handling, transportation, commingling with different animals and exposure to a foreign environment, combined with a lightweight condition (< 200 kg), represent a stage of acute stress. Calves in such situations are considered high-risk [1,2], often leading to water and food deprivation [3]. These stress factors have a negative impact on the energy balance [4], resulting in a reduction of body fat reserves. This, in turn, adversely affects the immune system [5], leaving the animal vulnerable to infectious agents. As a result, morbidity and mortality rates increase during the reception period (first 6 to 8 weeks) [6].

Therefore, energy is one of the most critical nutrients for the animal's immune system [6]. Storing this energy in the form of body fat can be a valuable tool to indicate its nutritional status. Energy is

stored in the body as lipids [7], which, when catabolized, are highly efficient in energy production. However, the low dry matter intake (DMI), approximately 0.9 to 1.5% of body weight (BW), during the first 14 d after arrival [8,9], complicates the correction of nutritional deficiencies. This could further compromise immune function [10] and reduce body fat reserves. Consequently, a significant portion of the consumed energy is directed towards the production of antibodies and immune system proteins. However, this also means that less energy is available for tissue deposition or average daily gain (ADG).

As a result of this altered feeding pattern, the performance of newly received stocker calves is typically optimized with more grain-concentrated diets ( $\geq$ 60% concentrate) [11]. However, this leads to a 17% increase in morbidity rate and a 24% increase in days requiring medical treatment (morbidity severity) [12]. On the other hand, increasing the proportion of forage in the diet decreases the morbidity rate by 1.3%, but it also reduces ADG by 8.3% [13]. Despite the decrease in morbidity among the calves, it fails to compensate for the economic loss associated with the reduced productivity of calves fed with high forage proportions [14].

Considering all the aforementioned factors, it is necessary to explore ingredients that can increase the dietary energy availability to enhance both growth performance and body fat reserves without compromising the health of cattle. It has been observed that gluconeogenic precursor calcium propionate (CaPr) alters energy metabolism when supplemented in ruminant diets. Specifically, it has two key effects: 1) It alters rumen fermentation through improvements on ruminal DM digestibility, increasing the proportion of ruminal propionate, and reducing methane production [15,16]; and 2) It enhances the action of insulin on glucose (GLU) metabolism [17], promoting an increase in energy status through enhanced GLU synthesis via gluconeogenesis in the liver [18]. In this context, Carrillo-Muro et al. [19,20] in lambs finished defined that 10 g of CaPr/lamb/d for 28 d increased dry matter intake (DMI) by 1 to 13%, ADG by 28%, ADG:DMI ratio by 17 to 25%, and BW by 5 to 7%. If supplementation was extended to 42 d, it resulted in a 30% increase in body fat reserves.

However, there is currently no information available regarding the effects of the levels and duration of CaPr inclusion on the growth performance, body fat reserves, serum metabolites and hemogram, of high-risk, newly received stocker calves. Based on this, we hypothesize that increasing the available energy in the diet through CaPr supplementation in high-risk, newly received stocker calves could potentially enhance growth performance, the body fat reserves and serum metabolites, without adverse effects on the hemogram. Furthermore, the extent of these effects may be related to the level and duration of CaPr supplementation. Therefore, the objective of the present study was to investigate the impact of different levels (0, 20, 40, 60 y 80 g CaPr calf/d) and durations of CaPr inclusion (0, 14, 28, 42 o 56 d) de CaPr, in high-risk, newly received beef calves on their growth performance, the body fat reserves, serum metabolites and hemogram.

# 2. Materials and Methods

The protocol was approved (protocol # 2023/05/19) by the Animal Welfare Committee at the Unidad Académica de Medicina Veterinaria y Zootecnia at the Universidad Autónoma de Zacatecas (UAMVZ-UAZ). The cattle were handled and managed in accordance with the Officials Mexicans Standards guidelines. The experiments were conducted over two consecutive months at the Torunos Livestock Preconditioning Center, in the experimental area, located in Fresnillo, Zacatecas, Mexico (north-central Mexico), within the property of Grupo Exportador Pa Lante S.P.R. de R.L. Serum metabolite and hemogram samples were processed at the Laboratorio de Análisis Clínicos Veterinarios of the UAZ-UAMVZ. Throughout the experiment (June to July 2023), ambient air temperature averaged 22.4 °C, with a minimum of 12.2 °C and a maximum of 28.3 °C.

### 2.1. Animal Housing, Basal Diet, Management, and Feed Sampling

The health and management history of the calves used in this experiment, which had a lightweight condition (< 200 kg), were unknown. Thus, calves were considered high risk [2]. Eightyseven calves were weaned and transported approximately 120 km (4 h on a truck) from an order buyer facility in Milpillas de la Sierra, Valparaiso, Zacatecas, to the Torunos Livestock

Preconditioning Center. The cattle arrived at the preconditioning center on June 1, 2023, and experienced a 5% shrink during transit. Upon arrival, the calves were placed into pens with two calves per pen and provided access to water and long-stem alfalfa hay overnight. The next morning (0600), the calves underwent the following procedures: 1) metaphylatic antimicrobial treatment (Emicina® líquida, Zoetis, Ciudad de Mexico, Mexico); 2) eleven-way clostridial, Mannheimia haemolytica and Pasteurella multocida type A and D vaccination (Biovac 11 Vías ®, Biozoo, México); 3) deworming with 4% ivermectin (Master LP ®, Ourofino Salud Animal, Brasil) and pour on cypermethrin (Cypermil Pour On ®, Ourofino Salud Animal, Brasil); 4) each calf was assigned an individual ear tag with a unique number; and 5) individual initial body weight (IBW) was recorded. Thirty-seven calves were not used in the experiment due to low IBW or temperament issues, leaving 50 bulls for use in the experiment (n = 50). These fifty bull calves were primarily Continental × British crossbred with an average IBW of 147.0 ± 1.67 kg and 5 months of age. The IBWs of the calves were recorded, and they were accommodated in 50 soil-surfaced pens (3.14 × 5.25 m). The cattle were fed a 50% concentrate diet from d 0 to 14. Concentrate proportion was increased at d 15 and d 28 (to 60 and 70% concentrate diets, respectively). The 70% concentrate diet was fed for the remainder of the trial (d 29 to 56). Feed was offered at 90% of the amount delivered the previous day on each transition day. The diets were formulated to meet or exceed NRC [21] recommendations for nutrients (Table 1). Throughout the study, the calves had free access to the basal diets and fresh water. Fresh feed was provided three daily at 0800, 1200 and 1800 h in a 20:20:60 proportion, respectively. Feed bunks were evaluated at 0730, 1130, and 1730 daily to determine the proper quantity of feed to deliver, residual feed was collected and weighed to determine dry matter intake (DMI). Adjustments in daily feed delivery were made at the afternoon feeding. Prior to the morning feeding, calves were individually weighed at start the experiment (IBW), at intermediate points (14, 28, 42 d) and at the end of the experiment (56 d). The calves were monitored daily for signs of bovine respiratory disease, including labored breathing, nasal or ocular discharge, depression, anorexia, and lethargy. Animals expressing symptoms were removed from experiment. Daily samples of the basal diet were collected and analyzed in triplicate for the following: 1) DM%, dried for 24 h at 100 °C in a forced air-drying oven, crude protein (CP) (FP-528 LECO nitrogen analyzer) [22]; 3) neutral detergent fiber (NDF) (fiber Ankom analyzer) and 4) Ether extract (EE) (extractor of Ankom<sup>xt15</sup>).

**Table 1.** Composition and nutritional profile (DM basis) of diets offered to bull calves during the experiment (g kg<sup>-1</sup> DM).

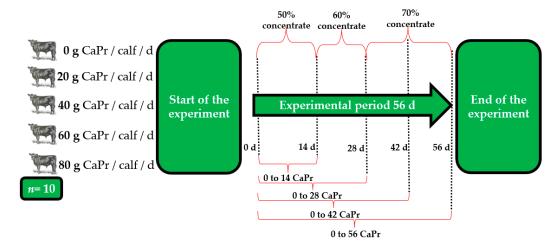
In one di onto	Cor	Concentrate in diet, % <sup>a</sup>					
Ingredients	50.0%	60.0%	70.0%				
Alfalfa hay mature	250.0	200.0	150.0				
Oats hay	250.0	200.0	150.0				
Cracked corn	280.0	380.0	480.0				
Soybean meal (44% CP)	105.0	105.0	105.0				
Liquid molasses cane	50.0	50.0	50.0				
Vegetable fat	21.5	21.5	21.5				
Sodium bentonite	10.0	10.0	10.0				
Sodium Sesquicarbonate	15.0	15.0	15.0				
Calcium carbonate	8.0	8.0	8.0				
Monocalcium phosphate	2.0	2.0	2.0				
Urea	5.0	5.0	5.0				
Salt	2.5	2.5	2.5				
Microminerals: Co, Fe, I, Mn, Zn, Se and Cu <sup>b</sup>	0.5	0.5	0.5				
Vitamins: A, D and E <sup>c</sup>	0.5	0.5	0.5				
Chemical composition	n, g kg-1 DM d						
Dry matter	872.4	862.4	852.4				
Crude protein	148.8	146.5	144.1				
Ether extract	43.6	45.8	48.0				

Neutral detergent fiber	348.4	298.7	249.0	
Calcium	9.3	8.9	8.5	
Phosphorus	2.9	2.8	2.8	
Ca: P ratio	3.2	3.2	3.0	
Calculated net ener	gy, Mcal/kg <sup>d</sup>			
Maintenance	1.6	1.7	1.8	
Gain	1.0	1.1	1.2	

 $<sup>^{</sup>a}$  50% concentrate = fed from d 0 to 14; 60% concentrate = fed from d 15 to 28; 70% concentrate = fed from d 29 to 56.  $^{b}$  Microminerals: Co (0.5 g), Fe (50 g), I (2.5 g), Mn (50 g), Zn (50 g), Se (0.2 g) y Cu (15 g). Excipient q.s. 1000 g.  $^{c}$  Vitamins A (5,000,000 IU), D (2,000,000 IU) y E (10,000 IU). Excipient q.s. 1000 g.  $^{d}$  Based on the tabular values for individual feed ingredients (Ca, P, net energy for maintenance and gain) [21], with the exception of DM, CP, NDF, and EE (Ankom procedures) which were determined in our laboratory.

# 2.2. Experimental Design and Treatments

A completely randomized design was employed to investigate the effects of varying levels and durations of CaPr supplementation in calves. The treatments included: 1) No CaPr (CTL), 2) 20 g CaPr/calf/d, 3) 40 g CaPr/calf/d, 4) 60 g CaPr/calf/d and 5) 80 g CaPr/calf/d. These treatments were administered over periods of 14, 28, 42, and 56 d after arrival (Figure 1). The source of CaPr used was Nuprocal® (Nutryplus, Mexico), originating from the same batch, comprising 20% calcium and 69% of propionic acid. Individual doses of CaPr were carefully weighed using a precision balance (Pioneer-PX523, Ohaus Corp., Parsippany, NJ, USA). To ensure that the treated group consumed the full dosage, the doses were mixed with 100 g of the basal diet, offered at 0800 and 1600. Any remaining portion of the diet was administered to the calves once consumed.



**Figure 1.** Completely randomized design, level (0, 20, 40, 60 or 80 g CaPr/calf/d) and duration (14, 28, 42 and 56 d) of calcium propionate (CaPr) supplementation in newly received stocker calves.

# 2.3. Growth Performance

Using the collected individual data during feeding trial, the following averages were calculated for different time periods (d 0 to 14, d 0 to 28, d 0 to 42 and d 0 to 56): 1) ADG= [(Weight out – Weight in / Days on period] expressed as kg/d; 2) DMI= (Feed offered – Feed refused), which was weighed and recorded daily, expressed as kg/d; 3) ADG:DMI ratio= (ADG / DMI); and 4) Daily water intake (DWI) = (Water offered – Water refused), which was determined and recorded daily, expressed as L/d. For this purpose, a drinking cup with a capacity of 30 L was graduated to determine intake.

# 2.4. Serum Metabolites and Hemogram

Blood samples were collected from five randomly selected calves from each treatment, on d 0, 14, 28, 42, and 56. Concurrently with individual weighing, at 0700, and before the first feeding of the

d, blood was drawn from the jugular vein. The blood samples (6.0-mL BD Vacutainer con EDTA K2-Dikysa), were later analyzed for a complete blood count (CBC) using an automatic cell counting machine (Exigo veterinary haematology analyser, Boule Medical AB, Sweden). The following parameters were determined: total white blood cells (WBC), lymphocytes (LYM), lymphocytes % (LYM%), monocytes (MON), monocytes % (MON%), granulocytes (GRA), granulocytes % (GRA%), platelets (PLT), mean platelet volume (MPV), red blood cells (RBC), red blood cells distribution width test % (RDW%), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). In addition, the blood serum was collected by centrifugation (2,500 × g for 30 min; 4 °C) and metabolites were quantified using an automated analyzer (FUJI DRI-CHEM NX500; Fujifilm, Tokyo, Japan). The following parameters were determined: activity of alkaline phosphatase (ALP), gamma glutamyltransferase (GGT) and aspartate aminotransferase (AST); levels of albumin (ALB), blood urea nitrogen (BUN), calcium (Ca), creatinine (CRE), GLU, total bilirubin (TBIL), total cholesterol (TCHO), triglycerides (TG), total protein (TP), sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>) and chlorine (Cl<sup>-</sup>). The globulin fraction (GLO) is a calculated value obtained by subtracting the ALB concentration from the TP concentration [23].

#### 2.5. Body Fat Reserves and Longissimus Muscle Area

The longissimus muscle area (LMA, measured in cm²) and back fat thickness (BFT, subcutaneous fat over the longissimus dorsi muscle, measured in mm, were determined between the 12th and 13th ribs; and fat thickness at the rump at the p8 site (FTR, subcutaneous rump fat, measured in mm), which is located over the gluteus muscle on the rump, at the intersection of a line through the pin bone parallel to the chine and perpendicular through the third sacral crest [24], were estimated by ultrasonography, every 0, 14, 28, 42, and 56 d, by the same operator using a real time, scanner with a linear array transducer of 3.5 MHz (Aloka Prosound 2 instrument).

#### 2.6. Statistical Analyses

Statistical analyses were conducted using SAS University software. A normality test was performed using the UNIVARIATE procedure. The data on growth performance were analyzed assuming a completely random design with calf as the experimental unit. The GLM procedure of SAS was used, employing a model that considered the effects of treatment (PrCa level), with IBW introduced as a covariate for the analysis of growth performance. When significant effects were detected, mean comparisons were conducted using the Tukey method with the LSMEANS instruction. Orthogonal polynomials were used to evaluate linear and quadratic responses among levels of CaPr. Significance was established when the p-value was  $\leq 0.05$ , and a trend if the p-value was  $\geq 0.05$  and  $\leq 0.10$ .

# 3. Results

# 3.1. Growth Performance

Most of the variables under study were not affected by the different levels during the first 14 d (p > 0.05; Table 2). However, regarding DWI, an effect was observed during this period, with a 4.6% increase as the inclusion level increased (linear effect, p = 0.04). For DMI, reductions were observed from d 28 to d 56 as the level of inclusion increased (linear effect, p < 0.05). The lowest consumption was recorded on d 56 with 80 g, but the rest of the levels were similar to the CTL (p < 0.05). Starting from d 28, differences were observed with 20 g, showing an 11.8% increase in ADG (quadratic trend, p = 0.07) and a 4.6% increase in BW, compared to the CTL (p < 0.05). This trend continued for d 42 and 56, with the highest increase occurring on d 42, with a 13.3% increase in ADG and a 4.9% increase in BW (p < 0.05). Nonetheless, it was also observed that as the level of inclusion increased, ADG decreased on d 42 and 56 (linear effect, p < 0.05), and the same trend was observed for BW on d 56 (linear trend, p = 0.06). This was reflected in a 16.7% increase in the ADG:DMI ratio on d 28 (p < 0.05)

with 20 g. However, on days 42 and 56, no significant differences were observed between the treatments (p > 0.05).

**Table 2.** Effect of dietary calcium propionate inclusion level and duration on the growth performance of high-risk, newly received stocker calves.

Ti		Calcium	propior	nate level	S a	CENA	Effects	(p-value)
Item	0	20	40	60	80	SEM b	Linear	Quadratic
Experiment days	56	56	56	56	56	-	-	-
No. of calves	10	10	10	10	10	-	-	-
			Body	weight,	kg			
Initial	145.3	147.8	143.4	146.4	152.3	1.67	0.93	0.92
Day 14	173.1	177.8	167.9	172.2	175.4	1.85	0.59	0.96
Day 28	192.3b	201.1a	$189.0^{b}$	190.2 <sup>b</sup>	190.6bc	1.65	0.41	0.45
Day 42	209.1b	219.3a	200.1 <sup>b</sup>	202.6b	198.9bc	2.81	0.18	0.54
Day 56	232.8b	239.1a	223.3b	220.2b	214.5bc	2.70	0.06	0.45
			Average	e daily ga	in, kg			
D 0 to 14	2.0	2.2	1.8	1.8	1.7	0.13	0.20	0.70
D 0 to 28	$1.7^{b}$	1.9a	$1.6^{b}$	$1.6^{b}$	1.4bc	0.07	0.12	0.07
D 0 to 42	1.5 <sup>b</sup>	$1.7^a$	1.3 <sup>b</sup>	1.3 <sup>b</sup>	1.1bc	0.08	0.03	0.20
D 0 to 56	1.5 <sup>b</sup>	$1.6^{a}$	$1.4^{b}$	1.3 <sup>b</sup>	1.1bc	0.07	0.01	0.25
			Dry mat	ter intak	e, kg/d			
D 0 to 14	4.0	4.0	3.9	3.9	3.9	0.07	0.18	0.98
D 0 to 28	4.9	4.8	4.7	4.4	4.3	0.16	0.04	0.54
D 0 to 42	5.1	5.1	4.9	4.7	4.6	0.17	0.05	0.57
D 0 to 56	5.5a	$5.4^{a}$	5.2ab	5.0ab	$4.6^{b}$	0.17	0.05	0.80
			Daily w	ater intak	e, L/d			
D 0 to 14	23.5	23.9	24.1	24.6	23.7	0.38	0.04	0.87
D 0 to 28	23.3	23.8	23.4	23.5	23.2	0.25	0.88	0.45
D 0 to 42	25.1	25.1	24.7	25.5	24.2	0.42	0.63	0.36
D 0 to 56	26.7	26.8	26.0	26.8	24.9	0.59	0.84	0.53
			ADO	G:DMI ra	tio			
D 0 to 14	0.48	0.53	0.44	0.46	0.42	0.035	0.34	0.74
D 0 to 28	$0.36^{b}$	$0.42^{a}$	$0.36^{b}$	$0.37^{b}$	0.32bc	0.019	0.74	0.28
D 0 to 42	0.34	0.36	0.34	0.33	0.30	0.016	0.40	0.26
D 0 to 56	0.32	0.32	0.32	0.30	0.30	0.019	0.32	0.42

<sup>&</sup>lt;sup>a</sup> Treatments consisted of oral administration of CaPr at a dose of 0, 20, 40, 60 or 80 g/calf/d at four feeding periods of 0 to 14, 0 to 28, 0 to 42 or 0 to 56 d. <sup>b</sup> SEM = standard error of the mean. <sup>a,b,c</sup> Means a row with different superscripts differ (p < 0.05) according to Tukey's test.

#### 3.2. Enzymatic Activity

The activity of ALP decreased with the increase in the level of CaPr (linear trend, p = 0.07; Table 3). It was higher in the CTL and lower with 80 g (p < 0.05). However, in the case of GGT, its activity was higher with 20 g CaPr/calf/d, showing a 37.3% increase (quadratic trend, p = 0.06) when compared to the CTL. The different levels of CaPr supplementation did not produce any effect on the activity of AST (p > 0.05).

**Table 3.** Overall effect of level calcium propionate (CaPr) inclusion on enzymes activity of high-risk, newly received stocker calves, sampled on d 0, 14, 28, 42, and 56.

Ttomb		C	aPr level	Sa		Reference	CEM	Effects	(p-value)
Item <sup>b</sup>	0	20	40	60	80	range	SEM <sup>c</sup>	Linear	Quadratic
Experiment days	56	56	56	56	56	-	-	-	-

No. of calves	5	5	5	5	5	-	-	-	-	
ALP, U/I	$379.4^{a}$	$281.1^{ab}$	$265.9^{ab}$	256.1ab	210.7b	0 - 488	37.33	0.07	0.16	
GGT, U/I	16.6	22.8	19.5	17.6	16.3	6.1 - 17.4	2.06	0.98	0.06	
AST, U/I	74.7	85.3	86.1	77.1	61.6	48 - 100	7.61	0.82	0.21	

<sup>&</sup>lt;sup>a</sup> Treatments consisted of oral administration of CaPr at a dose of 0, 20, 40, 60 or 80 g/calf/d at four feeding periods of 0 to 14, 0 to 28, 0 to 42 or 0 to 56 d. <sup>b</sup> Enzyme activities have been expressed as: ALP= alkaline phosphatase, GGT= gamma glutamyltransferase, AST= aspartate aminotransferase. <sup>c</sup> SEM = standard error of the mean. <sup>a,b</sup> Means a row with different superscripts differ (p < 0.05) according to Tukey's test.

#### 3.3. Serum Metabolites

The concentration of GLO increased with the level of CaPr (linear trend, p = 0.08; Table 4), being higher with 40 to 80 g (p < 0.05). BUN was lower with 20 g, higher with 80 g and similar with the other levels and CTL (p < 0.05). Additionally, increasing the level of CaPr increased the concentration of Ca (linear trend, p = 0.08), CRE (linear effect, p = 0.003) and TCHO (linear effect, p = 0.02) was higher with levels above 40 g (p < 0.05). The different levels of CaPr supplementation did not produce any effect or difference in ALB, GLU, TBIL, TG, TP and electrolytes (p > 0.05).

**Table 4.** Overall effect of level calcium propionate (CaPr) inclusion on serum metabolites of high-risk, newly received stocker calves, sampled on d 0, 14, 28, 42, and 56.

Thomas		Ca	Pr leve	elsa		Dafawar as war	CEMa	Effects (p-value)		
Item <sup>b</sup>	0	20	40	60	80	-Reference range	SEM	Linear	Quadratic	
Experiment days	56	56	56	56	56	-	-	-	-	
No. of calves	5	5	5	5	5	-	-	-	-	
TP, g/dl	5.9	6.6	6.0	6.1	6.5	6.74 - 7.46	0.22	0.93	0.18	
ALB, g/dl	3.9	3.0	2.9	2.8	2.6	2.8 - 3.8	0.5	0.13	0.46	
GLO, g/dl	3.1 <sup>b</sup>	$3.4^{b}$	3.7ab	3.8ab	3.9a	3.0 - 3.48	0.2	0.08	0.23	
BUN, mg/dl	11.3ab	$10.6^{b}$	10.9ab	10.9ab	12.8a	10 - 25	0.49	0.39	0.87	
CRE, mg/dl	0.75	0.79	0.80	0.83	0.87	1 – 2	0.028	0.003	0.61	
TBIL, mg/dl	0.23	0.28	0.28	0.27	0.25	0.01 - 0.5	0.018	0.15	0.21	
TCHO, mg/dl	65.5 <sup>b</sup>	65.5b	71.3ab	$74.4^{\mathrm{ab}}$	82.3a	80 - 120	4.02	0.02	0.22	
TG, mg/dl	35.1	22.9	22.8	26.9	29.6	0 - 14	5.97	0.40	0.21	
Ca, mg/dl	10.1	10.6	10.9	11.3	14.8	8.3 - 10.4	1.8	0.08	0.46	
GLU, mg/dl	106.4	104.2	100.6	91.0	90.3	45 - 75	7.31	0.36	0.43	
				Electr	olytes,	mEq/L				
Na <sup>+</sup>	121.2	124.2	122.4	124.5	123.1	132 - 152	1.90	0.35	0.80	
K <sup>+</sup>	4.4	4.6	4.3	4.4	4.2	3.9 - 5.8	0.10	0.81	0.57	
Cl-	85.7	87.6	86.5	87.1	84.7	97 – 111	1.65	0.70	0.70	

<sup>&</sup>lt;sup>a</sup>Treatments consisted of oral administration of CaPr at a dose of 0, 20, 40, 60 or 80 g/calf/d at four feeding periods of 0 to 14, 0 to 28, 0 to 42 or 0 to 56 d. <sup>b</sup> TP= total protein, ALB= albumin, GLO= globulins, BUN= blood urea nitrogen, CRE= creatinine, TBIL= total bilirubin, TCHO= total cholesterol, TG= triglycerides, Ca= calcium, GLU= glucose, Na<sup>+</sup>= sodium, K<sup>+</sup>= potassium, Cl<sup>-</sup>= chlorine. <sup>c</sup> SEM = standard error of the mean. <sup>a,b</sup> Means a row with different superscripts differ (p < 0.05) according to Tukey's test.

#### 3.4. Body Fat Reserves and Longissimus Muscle Area

BFT, FTR and LMA were not affected by the different levels during the first 14 (p > 0.05; Table 5). Regarding LMA, at 28 d its maximum value was observed, with a 23.9% increase with 20 g (quadratic effect, p = 0.05) compared to the CTL. Still, the maximum increase in BFT (p < 0.05) was observed from d 42 onwards, and for FTR, it was observed up to d 56. These increases were greater with 20 g CaPr (quadratic effect, p < 0.05), showing a 24.9% increase in BFT and a 21% increase in FTR compared to the CTL.

**Table 5.** Effect of dietary calcium propionate inclusion level and duration on the body fat reserves and longissimus muscle area of high-risk, newly received stocker calves.

Thom	C	Calcium j	propiona	te level	5 <sup>a</sup>	CEMb	Effects	s (p-value)
Item	0	20	40	60	80	SEM <sup>b</sup>	Linear	Quadratic
Experiment days	56	56	56	56	56	-	-	-
No. of calves	10	10	10	10	10	-	-	-
			Back fat	thicknes	s, mm			
Initial	2.7	2.6	2.9	2.6	2.4	0.21	0.95	0.76
Day 14	2.2	2.3	2.0	2.2	2.1	0.12	0.51	0.68
Day 28	2.5	3.0	2.7	2.8	2.6	0.15	0.43	0.27
Day 42	3.4	4.3	3.8	3.7	3.4	0.21	0.85	0.05
Day 56	3.3	4.1	3.5	3.4	3.1	0.18	0.76	0.05
		Fat	thicknes	s at the r	ump, mr	n		
Initial	2.6	2.6	2.5	2.4	2.3	0.19	0.54	0.83
Day 14	2.5	2.6	2.4	2.4	2.2	0.18	0.50	0.72
Day 28	3.0	2.9	2.7	2.9	2.9	0.2	0.66	0.71
Day 42	3.8	4.3	3.9	4.3	4.0	0.27	0.43	0.89
Day 56	3.6	4.4	3.9	3.6	3.7	0.20	0.55	0.03
		Lon	gissimu	s muscle	area, cm	$1^2$		
Initial	26.7	27.7	26.6	27.3	26.5	1.18	0.69	0.28
Day 14	33.0	33.9	33.9	31.7	34.5	1.65	0.63	0.47
Day 28	$34.7^{c}$	$43.0^{a}$	$38.0^{b}$	39.1 <sup>b</sup>	$38.2^{b}$	1.45	0.26	0.05
Day 42	36.3	41.3	37.1	40.4	35.2	1.53	0.31	0.67
Day 56	37.7	43.4	39.9	38.4	35.9	1.75	0.87	0.13

<sup>&</sup>lt;sup>a</sup> Treatments consisted of oral administration of CaPr at a dose of 0, 20, 40, 60 or 80 g/calf/d at four feeding periods of 0 to 14, 0 to 28, 0 to 42 or 0 to 56 d. <sup>b</sup> SEM = standard error of the mean. <sup>a,b,c</sup> Means a row with different superscripts differ (p < 0.05) according to Tukey's test.

#### 3.5. White Blood Cells

As the level of CaPr increased, the value of LYM decreased (linear effect, p = 0.05; Table 6). LYM% were 16.7% higher in the CTL (quadratic effect, p = 0.02). MON increased by 21.4% with 20 g (quadratic trend, p = 0.07). GRA increased by 12.9% with 20, 40 and 60 g, and GRA% increased by 19.5% with 20 g (quadratic effect, p < 0.03). The different levels of CaPr supplementation did not produce any effect or difference in WBC and MON% (p > 0.05).

**Table 6.** Overall effect of level calcium propionate (CaPr) inclusion on white blood cells of newly received stocker calves, sampled on d 0, 14, 28, 42, and 56.

Thomas b		Ca	Pr leve	ls a		Reference	CEM	Effects	(p-value)
Item <sup>b</sup>	0	20	40	60	80	range	SEM c	Linear	Quadratic
Experiment days	56	56	56	56	56	-	-	-	-
No. of calves	5	5	5	5	5	-	-	-	-
WBC, ×10 <sup>3</sup> /μL	8.9	9.3	8.9	8.3	8.2	4 - 12	0.54	0.36	0.37
LYM, ×10 <sup>3</sup> /μL	5.1	5.0	4.5	4.3	4.0	1.6 - 5.6	0.31	0.05	0.93
LYM, %	54.4	52.6	50.8	48.0	46.6	45 - 75	1.77	0.32	0.02
MON, ×10 <sup>3</sup> /μL	0.7	0.85	0.77	0.73	0.7	0 - 0.8	0.06	0.45	0.07
MON, %	7.9	8.6	8.4	8.5	8.2	2 - 7	0.34	0.33	0.43
GRA, ×10 <sup>3</sup> /μL	3.1	3.5	3.5	3.5	2.8	1.8 - 6.3	0.27	0.57	0.03
GRA, %	37.9	45.3	43.6	38.4	40.6	15 - 45	1.70	0.55	0.02

 $<sup>^{\</sup>rm a}$  Treatments consisted of oral administration of CaPr at a dose of 0, 20, 40, 60 or 80 g/calf/d at four feeding periods of 0 to 14, 0 to 28, 0 to 42 or 0 to 56 d.  $^{\rm b}$  WBC= total white blood cells, LYM= lymphocytes, LYM%= lymphocytes

%, MON= monocytes, MON%= monocytes %, GRA= granulocytes, GRA%= granulocytes %.  $^{\rm c}$  SEM = standard error of the mean.

#### 3.6. Platelets and Red Blood Cells

As the level of CaPr increased, the values of MCV (linear trend, p = 0.08) and MCH (linear effect, p = 0.02). The different levels of CaPr supplementation did not produce any effect or difference in PLT, MPV, RBC, RDW%, HGB, HCT% and MCHC (p > 0.05).

**Table 7.** Overall effect of level calcium propionate (CaPr) inclusion on platelets and red blood cells of newly received stocker calves, sampled on d 0, 14, 28, 42, and 56.

Item <sup>b</sup>		C	CaPr lev	elsª		Reference	SEM c	Effect	s (p-value)
item <sup>5</sup>	0	20	40	60	80	range	SEMI	Linear	Quadratic
PLT, ×10 <sup>3</sup> /μL	243.4	253.8	268.0	222.3	297.6	193 - 637	21.08	0.61	0.19
MPV	7.2	7.3	7.1	7.4	7.2	4.5 - 7.5	0.25	0.59	0.56
RBC, ×106/μL	10.1	9.9	9.6	9.6	9.4	5.1 - 7.6	0.25	0.11	0.71
RDW, %	26.0	25.4	25.6	25.6	26.1	16 - 20	0.48	0.67	0.52
HGB, g/100 mL	11.6	11.8	11.4	11.7	10.9	8.0 - 12.0	0.25	0.98	0.72
HCT, %	34.3	34.8	33.5	34.6	31.9	22.0 - 32.0	0.97	0.89	0.74
			I	Red bloc	d cell in	ıdex			
MCV, fL	34.1	34.1	35.3	35.7	36.3	38 - 50	0.78	0.08	0.67
MCH, pg	11.5	11.7	11.9	12.0	12.5	14 - 19	0.26	0.02	0.86
MCHC, g/Dl	34.0	33.9	34.1	34.7	34.5	38 - 43	0.64	0.43	0.61

<sup>&</sup>lt;sup>a</sup>Treatments consisted of oral administration of CaPr at a dose of 0, 20, 40, 60 or 80 g/calf/d at four feeding periods of 0 to 14, 0 to 28, 0 to 42 or 0 to 56 d. <sup>b</sup> PLT= platelets, MPV= mean platelet volume, RBC= red blood cells, RDW%= red blood cells distribution width test %, HGB= hemoglobin, HCT= hematocrit, MCV= mean corpuscular volume, MCH= mean corpuscular hemoglobin, MCHC= mean corpuscular hemoglobin concentration. <sup>c</sup> SEM = standard error of the mean.

# 4. Discussion

# 4.1. Growth Performance

The increment of energy in calf receiving diets through gluconeogenic precursors is quite limited. However, various publications concur that with receiving diets providing increasing levels of energy from concentrates, DMI, ADG and ADG:DMI ratio, significantly improve [25–27]. Moreover, different authors with various additives agree that the most significant improvements in productive behavior are observed during the first 30 d of reception [28–30]. On the other hand, when CaPr is supplemented in the diet and reaches the rumen, it undergoes hydrolysis at an acidic pH, resulting in the formation of Ca<sup>2+</sup> and propionic acid [18]. Additionally, in the rumen: 1) The pattern of volatile fatty acids is altered [16]; 2) Methane production decreases; 3) Digestibility of dry matter increases; 4) Fermentation efficiency improves [15]; 5) Insulin response capacity in GLU metabolism improves [17] and 6) Body fat reserves increase [20]. As a cumulative result of these mechanisms, there is a promotion of energy status achieved through increased GLU synthesis via gluconeogenesis [18]. This results in improvements in DMI, ADG, ADG:DMI ratio, and BW during the finishing phase [19,20].

The level of 20 g did not affect DMI at any point during the reception period. However, starting from d 28 up to d 56, there was a reduction in DMI with the increasing CaPr levels (40 to 80 g), reaching as low as 19.6% with 80 g. Similarly, when gluconeogenic precursor crude glycerin is included in the beef cattle diet at increasing proportions, DMI decreases [31–33]. Hales et al. [34] also described that DMI reduction in beef cattle, as crude glycerin concentration is increased, seems consistent throughout literature. A similar pattern has been observed with increasing levels of concentrate in the diets of receiving calves. There are maximal DMI levels with 60 to 72% concentrate and reductions with 90% concentrate during the first 28 d [11,25,35]. In agreement, but in lambs fed

finishing diets, Carrillo-Muro et al. [19] observed reductions in DMI of 14.3% with the higher levels of 30 g CaPr, whereas with 10 g, DMI increased by 1.1% during the first 28 d [20].

In this study, DWI was not affected when 20 g of CaPr was included, but it increased by 4.6% within the first 14 d as the inclusion level increased. In contrast, Carey et al. [33] added crude glycerin to the drinking water of receiving calves and did not observe any effects on DWI. However, Lofgreen et al. [25] reported that DWI was 16.6% higher at 28 d with diets containing less concentrate (20%).

Starting from day 28, there were increases of 11.8% in ADG and 4.6% in BW, with 20 g of CaPr, reaching 13.3% ADG and 4.9% BW at day 42 compared to CTL. Nevertheless, as the levels of CaPr supplementation increased, ADG decreased. In line with these findings, in lambs finished diets, Carrillo-Muro et al. [19] observed reductions of 16.0% in ADG and 2.8% in BW with the highest levels of CaPr, ranging from 20 to 30 g. Conversely, with 10 g of CaPr during the first 28 days, they observed an increase of 26.8% in ADG and 4.7% in BW [20]. Additionally, Lofgreen et al. [25], reported that ADG improved after the first 7 d of calf arrival and continued to increase until d 28, especially with higher values of ENg. Similarly, Lofgreen [27] using a diet containing 75% concentrate, detected a 6.5% increase in ADG compared to a 50% concentrate diet. In contrast, Pritchard y Méndez [35] reported increased ADG during the 28-day reception period in calves fed diets with lower energy content compared to a diet containing 60% concentrate. However, Ladeira et al. [32] who used various proportions of glycerin in bull diets, did not observe any effects on ADG and BW.

As for the ADG:DMI ratio, it improved by 16.7% during the first 28 d with 20 g of CaPr supplementation, but decreased with the CTL and the other levels. Similarly, Carrillo-Muro et al. [19] in lambs finished diets observed a 5.9% improvement with the lowest level, 10 g CaPr, and a 25% improvement during the first 28 d with 10 g [20]. In contrast, Ladeira et al. [32] used different proportions of glycerin in bulls and found that as the level increased, the ADG:DMI ratio also increased. Fluharty and Loerch [11] in receiving calves with increasing proportions of concentrate (70, 75, 80 and 85%), reported that this increase was more significant at d 14. Also, Lofgreen [27] detected a 17.5% increase in the ADG:DMI ratio with 75% concentrate compared to 50% concentrate. Contrastingly, Pritchard y Méndez [35] reported an increase in the ADG:DMI ratio during the first 28 d of the receiving period in calves fed diets with lower energy compared to a diet with 60% concentrate.

The reduced growth performance of received beef calves with higher levels of 40, 60, and 80 g CaPr can be explained by the decrease in DMI. This reduction effect on DMI is known as the hepatic oxidation theory (HOT) and was described by Allen [36] to explain the role of the ruminant liver in signaling and controlling satiety through temporal patterns of various oxidative products, including propionic acid. Signals are transmitted from the liver to the brain through afferents in the vagus nerve and are affected by hepatic oxidation and the generation of ATP [37,38]. Additionally, the elevated levels of CaPr may have increased insulin secretion, leading to a reduction in DMI. Insulin reaches the brain and binds to its specific receptors on neurons, resulting in reduced DMI [39,40].

#### 4.2. Enzymatic Activity

Blood measurements of enzyme activity including ALP, GGT, and AST were conducted to assess whether different levels of CaPr could impact liver or kidney functions or improve metabolism in these organs. It was observed that ALP activity decreased with the increase in the CaPr level, but the values remained within the established normal ranges (0 to 488 [41]). Otter [42] noted that physiologically higher ALP activity occurs in young growing cattle and is of bone origin, and increases are associated with osteoblast proliferation. Based on this, it can be inferred that received beef calves with 80 g of CaPr might reduce bone growth. As for GGT, its activity was highest with 20 g, increasing by 37.3%, slightly exceeding the reference range (6.1 to 17.4 [41]). This could be attributed to increased liver activity in these calves [43]. However, Ladeira et al. [32] used different proportions of glycerin in bulls and did not observe any effects on GGT. AST activity with different levels of CaPr supplementation did not produce any effects and remained within normal ranges (48 to 100 [44]). This aligns with Ladeira et al. [32] in bulls and de Freitas et al. [45] in ewes, who used different proportions of glycerin and also did not observe any effects on AST. In contrast, Silva et al.

[46] with crude glycerin in beef cattle, observed increments in AST with increasing inclusion. Carlson [47] states that this enzyme is a nonspecific indicator of tissue damage. Muscle injury or necrosis, especially in recumbent animals, may result in marked increases in AST activities. Finally, the enzyme activity values for ALP, GGT, and AST were below the pathological range, suggesting no liver or kidney damage or improvements in the metabolism of these organs associated with CaPr supplementation in calves reception.

#### 4.3. Serum Metabolites

The TP, which primarily includes ALB and GLO, is the main solid component of serum and serves as an indicator of an animal's nutritional status [48]. In the current study, concentrations of TP and ALB were not affected by CaPr supplementation. Nonetheless, TP was slightly below the reference range (6.74 to 7.46 [41]), while ALB values were within the normal range (2.7 to 4.2 [44]). Regarding TP, this aligns with de Freitas et al. [45] in ewes, using different proportions of crude glycerin supplementation, as they did not observe any effects on TP. Nevertheless, the concentration of GLO was slightly above the reference range (3.0 to 3.48 [41]) with the inclusion of 40 to 80 g. Hyperproteinemia results from elevated levels of ALB, GLO, or both. Dehydration is the sole cause of hyperalbuminemia; in dehydration, both ALB and GLO levels increase. However, if there is hyperproteinemia without concurrent dehydration, it is almost always the result of hyperglobulinemia. Common causes of hyperglobulinemia include chronic antigenic stimulation and liver disease. Chronic antigenic stimulation can generally be observed in various conditions such as traumatic reticuloperitonitis, liver abscesses, or chronic pneumonia [23]. Based on this, it can be assumed that with higher levels of 40 to 80 g CaPr, there may be chronic liver inflammation.

As for the BUN levels, they are typically used to estimate nitrogen excretion and utilization efficiency [49]. In ruminants, BUN concentrations are influenced by various factors, including CP intake, rumen degradability, and liver and kidney function [50]. Specifically, supplementation with 20 g reduced serum BUN levels; yet, all these values were within the normal range (10 to 25 [51]). Carrillo-Muro et al. [19] observed in lambs finished diets that with higher levels of 20 CaPr, BUN increased. Similarly, de Freitas et al. [45] in ewes and Carey et al. [33] in newly received beef calves observed increases in BUN with the highest proportions of crude glycerin. Waggoner et al. [52] pointed out that calves with immunological issues have lower N retention, probably due to increased muscle catabolism to obtain proteins and enhance the immune response. Based on the aforementioned principles, it can be inferred that supplementation with 20 g CaPr in high-risk, newly received stocker calves promotes nitrogen utilization and reduces muscle protein catabolism. Conversely, the opposite occurs with elevated levels of 40 to 80 g or 0.

Serum creatinine (CRE) concentrations were within the normal range (1 to 2 mg/dL) reported by Kaneko et al. [41]. This indicates that the renal glomerular filtration rate for CRE was adequate, without interference from CaPr. However, it was observed that CRE increased as the level of CaPr increased. In contrast to Ladeira et al. [32] with bulls and de Freitas et al. [45] in ewes, who used different levels of glycerin and did not observe effects on CRE. Otter [42] noted that CRE can be low in emaciated cattle or those with low muscle mass or elevated in heavily muscled animals. This result suggests that a higher level of CaPr (40 to 80 g) promotes more muscle deposition than fat, which aligns with the higher values of BFT and FTR observed with 20 g.

In the current study, total bilirubin (TBIL) concentrations were not affected by CaPr supplementation and remained within the normal range (0.01 to 0.5 [41]). TBIL is an important indicator of liver function, as it increases during severe lipidosis [53,54], and decreases in concentration when the liver is healthy. Therefore, based on these TBIL values, it can be concluded that different levels of CaPr do not have negative effects on liver function.

Serum lipids primarily consist of TCHO and TG. TCHO was below the range (73 to 280 [51]), but increased as the level of CaPr increased, with only the 80 g CaPr falling within the range. No treatment effects were observed on TG, but all values were above the range (0 to 14 [41]). The 80 g CaPr level increased TCHO levels, likely due to the increased production of propionic acid in the rumen, subsequently leading to increased TCHO production in the liver. The decrease in serum

TCHO levels in this study indicates an energy deficit, while increases occur in response to the ingestion of energy-rich lipid-containing foods [55]. As crude glycerin inclusion increased, the same conclusion was drawn by Silva et al. [46] in beef cattle and de Freitas et al. [45] in ewes, where they observed increases in TCHO. However, in lambs finished diets, Carrillo-Muro et al. [19] did not observe effects with levels of 10, 20, or 30 g CaPr/lamb/d on TCHO and TG; similarly, de Freitas et al. [45] did not observe effects with different proportions of crude glycerin inclusion. Ndlovu et al. [56] pointed out that TCHO concentration reflects the energy metabolism in the liver, particularly lipid export in the form of very low-density lipoproteins.

The CaPr supplemented in the present study, comprising 20% Ca and 69% propionic acid, provided additional calcium beyond the nutritional requirements of the calves, which were already met by the basal diet. Consequently, it was observed that as the levels of CaPr inclusion increased, blood calcium concentration also increased, with all levels above the reference range (8.3 to 10.4 [50]). This aligns with what Russell and Roussel [23] mentioned, stating that hypercalcemia is fairly rare in ruminants and usually occurs as a result of the administration of Ca solutions or gels.

GLU concentrations are considered metabolic indicators of nutrient intake in beef cattle [57]. No treatment effects were observed on GLU; however, all values were above the reference range (45 to 75 [41]). In line with this, in lambs fed different levels of CaPr in their diets, Carrillo-Muro et al. [19] did not observe any effects on GLU with levels of 10, 20, or 30 g CaPr. Similarly, Silva et al. [46] in beef cattle and de Freitas et al. [45] in ewes, did not observe effects on GLU with different proportions of crude glycerin inclusion. In contrast, Ladeira et al. [32] used different proportions of glycerin in bulls and observed that GLU decreased as the level of inclusion increased. These GLU concentrations within the range for all treatments are related to adequate DMI intake since circulating GLU is influenced by nutrient availability [58]. Likewise, Oosthuysen et al. [59] reported that elevated blood GLU concentrations suggest improved energy status associated with better utilization of dietary nutrients.

The different levels of CaPr supplementation did not produce any effects or differences in electrolytes (Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup>). However, the values of Na<sup>+</sup> and Cl<sup>-</sup> were below the range, 132 to 152 and 97 to 111, respectively [41]. These low values of Na<sup>+</sup> and Cl<sup>-</sup> in the calves were due to the diet not completely meeting their nutritional requirements. Radostits et al. [60] note that the most common causes of hyponatremia are the lack or inadequate level of Na<sup>+</sup> in the diet, and alterations in Cl-concentration are generally associated with proportional changes in Na<sup>+</sup> concentration, resulting from changes in relative water balance [47]. Another common reason for reduced Na<sup>+</sup> and Cl<sup>-</sup> levels in reception calves is diarrhea, which, however, did not occur in our calves [47]. Regarding K<sup>+</sup>, deficiency is commonly associated with stressed cattle due to dehydration and loss of K<sup>+</sup> from tissues [61].

#### 4.4. Body Fat Reserves and Longissimus Muscle Area

Assessing lipid reserves in received calves can provide valuable insights into their nutritional status. Energy is stored in the body in the form of lipids [7], primarily TG [62]. When catabolized, lipids are highly efficient in energy production, yielding up to 9.4 Mcal/kg, whereas carbohydrates produce 4.2 and proteins 5.6 Mcal/kg [63,64]. Therefore, energy from body fat reserves can be nearly twice as much as that derived from muscles. When energy is limited in the organism, body fat reserves are the first to be depleted through adipose tissue lipolysis, releasing TG [65]. Consequently, body fat reserves are influenced by factors such as 1) reproductive potential [66]; 2) negative energy balance [67]; 3) feeding level; and 4) nutrient composition [68].

The results regarding BFT, FTR and LMA align with expectations, as there is a specific order in tissue deposition depending on the growth curve stage of young animals. Growth initially prioritizes bone and muscle, followed by fat accumulation, with higher energy or protein content in the diet stimulating fat deposition [69,70]. Therefore, with 20 g CaPr, LMA showed a maximum increase of 23.9% up to d 28, and thereafter, from d 42 onwards, BFT increased by 24.9%, while FTR increased by 21% up to the d 56. In accordance, Carrillo-Muro et al. [20] observed in lamb finishing diets that, with the lowest level of 10 g CaPr, BFT increased by up to 30% by d 42 as the inclusion period

increased. In contrast, Ladeira et al. [32], who used different proportions of glycerin in bulls, did not observe effects on BFT. Regarding LMA, Carrillo-Muro et al. [20], Martínez-Aispuro et al. [71], Lee-Rangel et al. [34] and Mendoza-Martínez et al. [72], in lambs finishing diets did not observe effects on the 42 d; yet, Ladeira et al. [32] with different proportions of glycerin in bulls observed increases with higher levels.

#### 4.5. White Blood Cells

The values of WBC were not affected by the different levels of CaPr inclusion, and these values were within the normal WBC range (4 to 12 [74]). Lymphocyte counts (LYM) decreased with increasing CaPr levels, but these values remained within the normal range (1.5 to 5.6 [75]). Monocyte counts (MON) were slightly above the range (0 to 0.8) [42]. With these values staying within the normal range, it could suggest an overall improvement in the calves' immune status or reduced infection rates. In contrast, Silva et al. [46] and Lopez et al. [75] in beef cattle supplemented with crude glycerin, did not observe effects on any of these WBC variables. However, de Freitas et al. [45] in ewes, with different proportions of crude glycerin supplementation, observed a reduction in WBC and LYM with the highest level, and no changes in MON.

#### 4.6. Platelets and Red Blood Cells

As the CaPr level increased, the values of MCV and MCH increased as well; however, MCV was below the range (38 to 50 [76]). The different levels of CaPr supplementation did not produce any significant differences in PLT, MPV, RBC, RDW%, HGB, HCT% and MCHC. Nevertheless, RBC, RDW% and HCT remained above the range (22 to 32 [77]), while MCHC remained below ([38 to 43 [78]). Similarly, Silva et al. [46] in beef cattle and de Freitas et al. [45] in ewes did not observe effects with different proportions of crude glycerin inclusion on these variables. Yet, de Freitas et al. [45] observed an increase in MCV with the highest levels. The values of HCT% were not affected by the different CaPr inclusion doses; nonetheless, these percentages remained above the HCT% range (22% to 32%; [77]), indicating that the cattle were slightly dehydrated throughout the experiment.

## 5. Conclusions

The gluconeogenic compound CaPr can be effectively incorporated in reception diets for high-risk, newly received stocker calves. When administered at daily level of 20 g CaPr/calf for 28 d, it significantly enhances growth performance. This improvement is reflected in increased ADG, enhanced ADG:DMI ratio and LMA. However, if supplementation is extended to 42 or 56 d at this level, ADG continues to increase, and it also begins to elevate body fat reserves (BFT and FTR). This leads to a reduction in ADG:DMI ratio. Furthermore, the different levels of CaPr supplementation did not affect the reference range of most serum metabolites, and they did not have a negative effect on the health of stocker calves.

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