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Effect of Calcium Propionate and Chromium-Methionine Supplementation: Growth Performance, Body Fat Reserves, and Blood Parameters of High-Risk Beef Calves

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Simple Summary: Much of the cattle received in feedlots are considered "high-risk", and in the first weeks of their arrival they have low feed intake, presenting a negative energy balance, reducing body fat reserves, and thus negatively influencing their immune system. For that reason, it is necessary to (1) increase the energy density of the diet with the supplementation of calcium propionate (CaPr) or (2) improve the insulin response with the inclusion of chromium (Cr). Therefore, the effect of CaPr and Cr, as well as the possible synergy, were evaluated in this study. The results indicate that supplementation with CaPr or Cr improves growth performance, dietary energetics, and body fat reserves, but CaPr + Cr did not surpass these results.

Abstract: Energy availability is a critical point in newly received calves. This study was conducted to examine the effect of daily calcium propionate (CaPr), chromium-methionine (Cr-Met), or CaPr plus Cr-Met (CaPr + Cr-Met) supplementation on growth performance, dietary energetics, body fat reserves, serum metabolites, and hematological responses in high-risk beef calves. Forty-eight crossbred bull calves (148.7 ± 2.05 kg body weight) were involved in a fully randomized experimental design. Calves which were individually pen allocated (12 repetitions/treatment) were subjected to one of the following treatments daily over 56 d: (1) Control, no additives; (2) CaPr, 19 g CaPr; (3) Cr-Met, 4 g Cr-Met; and (4) CaPr + Cr-Met, 19 g CaPr plus 4 g Cr-Met. Compared to controls, feed additive supplementation alone or in combination did not modify dry matter intake (DMI), but increased average daily gain (ADG), improving the ADG/DMI ratio. However, no synergistic effect on dietary energy utilization efficiency was observed with the combination of CaPr and Cr-Met; individual supplementation proved more effective. Because of the magnitude of the effects of Cr-Met on the efficiency of dietary energy utilization, this resulted in an increase ($p < 0.05$) in rump fat thickness (RFT). The supplementation of CaPr + Cr-Met decreased ALB/GLO ratio, MPV, and RBC, but increased TCHO, GLU, and MCH ($p > 0.05$). It is concluded that supplementation with CaPr or Cr-Met independently is an effective strategy to improve growth performance, energy utilization and retention, and body fat reserves, without adverse effects on health among high-risk beef calves.

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1. Introduction

Low dry matter intake (DMI) at the calf reception stage is the main challenge in the feedlot during the first weeks of arrival. Low feed intakes promote a negative energy balance, making it difficult to recover the original weight and reducing body fat reserves [1]. Energy is a key nutritional element for the immune system [2]. Therefore, in low-intake calves, the immune system is weakened, increasing the risk of high morbidity rate presentation [3]. In this regard, in calves with such a history, Snowder et al. [4] reported that up to 60% of newly arrived cattle presented clinical symptoms of complex respiratory bovine. Increasing the dietary energy density or improving the efficiency of dietary energy utilization are strategies that facilitate a positive energy balance during periods of low-feed intake, thus improving growth performance and health [5–9].

In regard to strategies to improve dietary energy utilization, supplementary chromium (Cr) increases insulin signaling, altering glucose and protein metabolism and affecting the protein: fat ratio in tissue composition, thus boosting feed efficiency [10–12]. In addition, supplementing the diet with Cr has been reported to alleviate stress in newly received cattle, primarily by enhancing antioxidant properties, improving insulin function, and optimizing energy metabolism. This results in increased energy reserves that help animals cope with adverse conditions [13,14]. In cattle fattening under favorable ambient conditions, supplemental organic Cr (as Cr enriched live yeast) at a dose of 1 to 1.15 mg/kg of diet improved the dietary net energy from 8 to 14% in feedlot cattle [15]. It is well known that organic forms of Cr (such as Cr-Met) are more available than inorganic forms [16,17]. The most extensively studied organic forms have been Cr yeast and Cr propionate, which have shown positive effects on growth and health [18–21]. However, separating the effects of the live yeast or propionate from the effects of Cr in those experiments is difficult. Surprisingly, research on the efficacy of Cr-Met supplementation in high-risk beef calves is quite limited.

On the other hand, enhancing the energy density of diets by supplementing the gluconeogenic precursor calcium propionate (CaPr) is currently an option. Increases in average daily gain (ADG, 13.3%), ADG/DMI ratio (16.7%), and body fat reserves (21%) have been observed in new-arrival calves supplemented daily with 20 g CaPr over the course of 56 d [8,9].

Increasing energy availability from diet via CaPr supplementation and facilitating improvement in metabolizable energy and N retention via Cr-Met supplementation could have a synergistic effect in newly arrived calves. Consequently, it is relevant to determine if the combination of CaPr and Cr-Met could lead to higher energy utilization and health status in calves under new arrival conditions. Hence, the objective of this study was to examine the impact of supplementing CaPr and Cr-Met separately, as well as their combination on growth performance, dietary energy, body fat reserves, serum metabolites, and hematological responses in high-risk beef calves supplemented over the course of 56 d following their arrival.

2. Materials and Methods

The experiment was carried out in the Torunos Livestock Preconditioning Center, the Grupo Exportador Pa Lante S.P.R. de R.L., in Fresnillo, Zacatecas, Mexico (23°08′56.22″ N and 102°43′48.57″ W), 2081 m above sea level with a semi-dry temperate climate. During the course of the experiment, the temperature averaged 24.3 °C. All animal care and handling techniques followed protocols approved by the Institutional Bioethics and Animal Welfare Committee (Protocol # 14 May 2024) of the Unidad Académica de Medicina Veterinaria y Zootecnia at the Universidad Autónoma de Zacatecas (UAMVZ-UAZ); furthermore, they were conducted in accordance with the Official Mexican norms for animal care [22,23]. The blood samples to determine hemogram and serum metabolites were processed at the Laboratorio de Análisis Clínicos Veterinarios of the UAZ-UAMVZ.

2.1. Animal Processing, Housing, and Feeding

The bull calves involved in this study met the high-risk criteria outlined by Carrillo-Muro et al. [24,25]: (1) unknown health and management background; (2) weight at arrival between 150 and 200 kg; (3) age between 5 and 7 months; (4) weaned for a maximum of 14 d; (5) exposed to handling and transportation; (6) co-mingled with calves from different sources; and (7) unvaccinated upon arrival to feedlot. Ninety-seven recently weaned crossbred bull calves were acquired from various sources and transported roughly 120 km (a four-hour truck journey) to the Torunos Livestock Preconditioning Center on 6 June 2024 for inclusion in this research. Upon arrival, calves (approximately 6 mo of age) were vaccinated for clostridials and *Mannheimia haemolytica* (Biovac 11 Vías®, Biozoo, Jalisco, Mexico), treated against parasites (% ivermectin, Master LP® injectable, Ourofino Salud Animal, São Paulo, Brazil), administered metaphylactic treatment with oxytetracycline (5 mg/kg BW; Emicina® líquida, Zoetis, Ciudad de Mexico, Mexico), and were individually weighed (initial body weight, IBW; electronic scale; Metrology® PBG-3000, cap. 2000 kg, Básculas y Accesorios de Peso SA de CV, Nuevo León, México). Following the evaluation and health management procedures, 49 calves were removed from the experiment because of their low initial body weight (IBW) or behavioral problems, leading to a final cohort of 48 calves for the study (n = 48, 12 calves per treatment), which had an average IBW of 148.7 $\pm$ 2.05 kg. Utilizing the processed IBW, calves were randomly assigned to 48 pens (12 pens/treatment; 1 calf/pen). Pens were 3.14 m $\times$ 5.25 m, unshaded, and equipped with waterers and line feed bunks. All calves were observed twice daily for signs of lameness or morbidity, including depression, nasal or ocular discharge, and anorexia. Throughout the study, there were no instances of morbidity or mortality.

2.2. Treatments and Diets

Dietary treatments were evaluated for 56 d (calf/d): (1) Control, no additives; (2) CaPr, 19 g CaPr; (3) Cr-Met, 4 g Cr-Met; and (4) CaPr + Cr-Met, 19 g CaPr + 4 g Cr-Met. The dose of CaPr was calculated based on the proportion of propionic acid of the product, taken as a reference from Rodríguez Cordero et al. [8] and Rivera-Villegas et al. [9], who used a dose of 20 g/calf/d of a product containing 69% propionic acid (69% $\times$ 20 g = 13.80 g propionic acid/calf/d). The product used in the present study (Propical® Dresen Química, SAPI de CV., Mexico City, Mexico) contained 73% propionic acid (73% $\times$ 19 g = 13.87 g propionic acid/calf/d); therefore, the dose was set at 19 g/calf/d. The supplemental Cr-Met source used was MiCroPlex® 1000 (Zinpro Corporation, Eden Prairie, MN, USA), containing Cr-Met 1000 ppm (Figure 1).

The diet was formulated to contain 145.1 g kg^{-1} DM of crude protein and provide 0.98 Mcal/net energy for gain (NE_g); the diet included 500 g kg^{-1} DM roughage, which included mature alfalfa hay and oats hay, along with cracked corn grain and soybean meal (Table 1). The dietary formulation was enhanced with microminerals and vitamins to fulfil NASEM [7] requirements for receiving bull calves.

To guarantee that the treated group received the complete dosage, the doses were combined with 100 g of the basal diet and offered at 800 and 1600 h. If there was any leftover portion after the calves consumed their initial servings, it was then given to them.

Fresh feed was supplied three times a day at 800, 1200, and 1600 h. Each morning, about 30 min before the morning feeding, the bunk feeders were examined to evaluate the intake from the previous day. Any leftover feed was taken out, weighed, and documented. These data were subsequently utilized to adjust the 1600 h feed to ensure that the refusal rate was below approximately 100 g per calf, while the amounts offered at 800 and 1200 h remained unchanged.

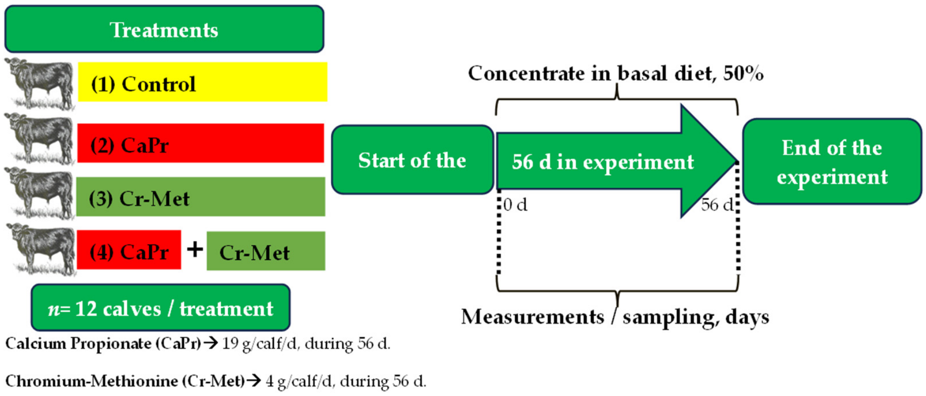

Figure 1. A completely randomized design experiment using 48 calves individually housed (12 calves/treatment). Treatments consisted of daily calcium propionate (CaPr), chromium-methionine (Cr-Met), and calcium propionate plus chromium-methionine (CaPr + Cr-Met) supplementation over a feeding period of 56 d. The prices (USD/g of product) of CaPr and Cr-Met were USD 0.0021 and USD 0.0047, respectively.

Table 1. Composition of experimental diet (dry matter basis).

Ingredients	g kg^{-1} DM
Alfalfa hay mature	250
Oats hay	250
Corn grain cracked	280
Soybean meal-44	105
Molasses cane	50
Vegetable fat	21.5
Sodium bentonite	7.5
Sodium Sesquicarbonate	15
Calcium carbonate	8
Monocalcium phosphate	2
Urea	5
Salt	5
Premix [a]	1
Chemical composition, g kg^{-1} DM	
Dry matter	843.0
Crude protein	145.1
Ether extract	42.9
Neutral detergent fiber	343.7
Calcium [b]	9.5
Phosphorus [b]	2.9
Ca/P ratio	3.3
Calculated net energy, Mcal/kg	
Maintenance	1.57
Gain	0.98

[a] Contained per kilogram of premix: 0.5 g of Co, 50 g of Fe, 2.5 g of I, 50 g of Mn, 50 g of Zn, 0.2 g of Se, and 15 g of Cu; 5,000,000 IU of vitamin A, 2,000,000 IU of D, and 10,000 IU of E. [b] Calculated based on the tabular values for individual feed ingredients (Ca, P, NE_m, and NE_g) NASEM [7], with the exception of DM, CP, NDF, and EE (Ankom procedures), which were determined in our laboratory.

2.3. Calculations

Samples of feed were collected every day for dry matter (DM) analysis AOAC [26]. To assess the effects of treatments on growth performance, calves were weighed at the beginning (0 d) and conclusion of the experiment (56 d). The following overall measurements were considered: (1) Average daily gain (ADG) = [(Final weight − Initial weight)/56 d], expressed in kg per day; (2) Dry matter intake (DMI) = (Feed given − Feed not consumed), which was weighed and logged daily, expressed in kg per day; and (3) ADG/DMI ratio = (ADG/DMI).

Net energy (NE) needed for maintenance (NE_m) was determined using the quadratic formula outlined by Zinn et al. [27], where $NE_m = -b \pm \sqrt{(b^2 - 4ac)}/2c$. In this formula, $a = -0.41 \times EM$; $b = (0.877 \times EM) + (0.41 \times DMI) + EG$; and $c = -0.877 \times EM$, with EM representing the energy requirement for maintenance and EG indicating the energy requirement for gain. The energy requirement for maintenance (EM, Mcal/d) was calculated using $EM = 0.077 \times SBW^{0.75}$, where SBW (initial shrunk body weight) equaled average BW × 0.96 [28], while the energy requirement for gain (EG, Mcal/d) was estimated using the equation $0.0557 \times SBW^{0.75} \times ADG^{1.097}$ [7,28]. The performance-calculated NE_g was then derived from NE_m ($NE_g = 0.877 \times NE_m - 0.41$), as previously noted by Zinn et al. [27]. The anticipated DMI was calculated based on the observed ADG, average SBW, and the estimated NE values of the diet (Table 1) using the following formula: expected DMI, kg/d = (EM/NE_m) + (EG/NE_g), where NE_m and NE_g are the tabulated NE values of the diet based on its formulation [7]. The efficiency of dietary energy use during growth performance trials was assessed through the ratio of observed to expected dietary NE.

2.4. Body Fat Reserves and Longissimus Muscle Area

The following measurements were made: (1) longissimus muscle area (LMA), measured in cm^2; (2) 12th rib fat thickness (FAT), which represented subcutaneous fat over the longissimus dorsi muscle, measured in mm, wherein both measurements were taken between the 12th and 13th ribs; and (3) rump fat thickness (RFT) at the p8 site, measured in mm. These measurements were obtained through ultrasonography on days 0 and 56. The measurements were consistently performed by the same operator using a real-time scanner equipped with a linear array transducer of 3.5 MHz (Aloka Prosound 2 instrument).

2.5. Assessment of Enzymic Activity, Metabolites, and Hematological Responses

Serum metabolite and hematological response samples were processed at the Laboratorio de Análisis Clínicos Veterinarios of the UAZ-UAMVZ. Blood samples were collected from all calves on days 0 and 56. Coinciding with individual weighing, at 700 h, and before the first feeding of the day, blood was drawn from the jugular vein using vacutainer tubes. The blood serum was collected by centrifugation (2500× *g* at 4 °C for 15 min), and enzymatic activity and metabolites were quantified using an automated analyzer (FUJI DRI-CHEM NX500®; Fujifilm, Tokyo, Japan). The following parameters were determined: (1) enzymatic activity: alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), aspartate aminotransferase (AST), and alanine aminotransferase (ALT); (2) levels of total protein (TP), albumin (ALB), globulin (TP-ALB), ALB/GLO ratio, blood urea nitrogen (BUN), and creatinine (CRE); (3) total bilirubin (TBIL), total cholesterol (TCHO), and triglycerides (TG); (4) calcium (Ca) and glucose (GLU); and (5) electrolytes: sodium (Na^+), potassium (K^+), and chlorine (Cl^-). In addition, whole blood samples were analyzed for hematological responses using an automatic cell counting machine (Exigo Veterinary Haematology Analyzer®, Boule Medical AB, Spånga, 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%; represents the coefficient of variation of the RBCs volume distribution), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV; provides information about the volume or size of individual RBCs), mean corpuscular hemoglobin (MCH; quantifies the amount of HGB per RBCs), and mean corpuscular hemoglobin concentration (MCHC; correlates the HGB content with the volume of the cell).

2.6. Statistical Analyses

All data were examined as a completely randomized design experiment utilizing SAS® software version 9.3 [29]. The individual calves served as the experimental units. Normality of the data was assessed using the Shapiro–Wilk test. The baseline data for IBW, body fat reserves, LMA, enzymatic activity, metabolites, and hematological responses, collected on day 0, were used as covariates for the measurements taken on day 56. Tukey's multiple comparison tests were applied, standard errors were reported, and treatment effects were deemed significant if the p-value was ≤ 0.05.

3. Results

3.1. Growth Performance, Dietary Energetics, Body Fat Reserves and Longissimus Muscle Area

There were no effects ($p > 0.05$) of CaPr, Cr-Met, and CaPr + Cr-Met supplementation on DMI, FAT, and LMA. The responses of calves supplemented with CaPr, Cr-Met, and CaPr + Cr-Met were similar ($p > 0.05$) in ADG but higher ($p < 0.05$) compared to the control, which promoted an increase in ADG/DMI ratio ($p < 0.05$): 22% CaPr, 24.1% Cr-Met, and 19.9% CaPr + Cr-Met (Table 2). Likewise, the effects of CaPr and Cr-Met supplementation were similar ($p > 0.05$) in regards to dietary energy and the efficiency of dietary NE utilization and retention, but higher than the control treatment ($p < 0.05$): 10.4, 10.2, and 12.5% for CaPr, and 11.7, 11.4, and 13.8% for Cr-Met, respectively (Table 3). The positive effect on ADG/DMI ratio, efficiency of dietary NE utilization, and retention promoted a 25.0% increase in RFT with Cr-Met supplementation ($p < 0.05$).

Table 2. Growth performance of high-risk beef calves fed with supplemental calcium propionate (CaPr) and chromium-methionine (Cr-Met) for 56 d (n = 12 calves/treatment).

Item [a]	Treatments [a]				SEM [b]
	Control	CaPr	Cr-Met	CaPr + Cr-Met	
Initial body weight, kg	154.00	147.56	148.02	143.33	4.38
Final body weight, kg	205.67 [b]	210.11 [a]	212.44 [a]	206.17 [ab]	1.64
Average daily gain, kg/d	0.922 [b]	1.117 [a]	1.151 [a]	1.122 [a]	0.0288
Dry matter intake, kg/d	4.84	4.79	4.85	4.89	0.0394
Average daily gain/Dry matter intake ratio	0.191 [b]	0.233 [a]	0.237 [a]	0.229 [a]	0.0061

[a] Treatments consisted of oral administration of calcium propionate (CaPr, Propical®, Dresen Química, SAPI de CV., Ciudad de México, México) or chromium-methionine (Cr-Met, MiCroPlex® 1000, Zinpro Corporation, Eden Prairie, MN, USA): (1) No additives (Control), (2) CaPr, (3) Cr-Met, and (4) CaPr + Cr-Met. CaPr was administered at a dose of 19 g/calf/d and Cr-Met at a dose of 4 g/calf/d. [b] SEM = standard error of the mean. [a,b] Means a row with different superscripts differ ($p < 0.05$) according to Tukey's test.

Table 3. Dietary energetics and ultrasound measurement of high-risk beef calves fed supplemental calcium propionate (CaPr) and chromium-methionine (Cr-Met) for 56 d (n = 12 calves/treatment).

Item	Treatments [a]				SEM [b]
	Control	CaPr	Cr-Met	CaPr + Cr-Met	
Dietary net energy (Mcal/kg)					
Maintenance	1.612 [b]	1.780 [a]	1.796 [a]	1.725 [ab]	0.037
Gain	1.004 [b]	1.150 [a]	1.165 [a]	1.103 [ab]	0.033

Table 3. *Cont.*

Item	Treatments [a]				SEM [b]
	Control	CaPr	Cr-Met	CaPr + Cr-Met	
Observed: expected dietary net energy ratio					
Maintenance	1.027 [b]	1.132 [a]	1.144 [a]	1.099 [ab]	0.024
Gain	1.024 [b]	1.173 [a]	1.189 [a]	1.125 [ab]	0.033
Ultrasound measurements					
12th rib fat thickness, mm	2.50	2.69	2.55	2.30	0.148
Rump fat thickness, mm	3.20 [b]	3.51 [ab]	4.00 [a]	3.68 [ab]	0.208
Longissimus muscle area, cm^2	32.73	32.71	33.34	31.19	0.819

[a] Treatments consisted of oral administration of calcium propionate (CaPr, Propical®, Dresen Química, SAPI de CV., Ciudad de México, México) or chromium-methionine (Cr-Met, MiCroPlex® 1000, Zinpro Corporation, Eden Prairie, MN, USA): (1) No additives (Control), (2) CaPr, (3) Cr-Met, and (4) CaPr + Cr-Met. CaPr was administered at a dose of 19 g/calf/d and Cr-Met at a dose of 4 g/calf/d. [b] SEM = standard error of the mean. [a,b] Means a row with different superscripts differ ($p < 0.05$) according to Tukey's test.

3.2. Enzymatic Activity and Serum Metabolites

There were no effects ($p > 0.05$) of CaPr, Cr-Met, and CaPr + Cr-Met supplementation on enzymic activity (ALP, GGT, AST, and ALT; Table 4), TP (ALB and GLO), BUN, CRE, TBIL, TG, Ca (Table 5), or electrolytes (Na^+, K^+, and Cl^-; Table 6). ALB/GLO ratio was reduced ($p < 0.05$) by 18.29% with CaPr + Cr-Met supplementation, with the control treatment having the highest effect. Total cholesterol (TCHO) was increased by 12.9% with Cr-Met and 11.6% with CaPr + Cr-Met ($p < 0.05$). Finally, CaPr + Cr-Met also increased GLU concentration by 23.3%; CaPr and Cr-Met slightly elevated it, but the effect of these supplements was similar to the rest of the treatments ($p < 0.05$).

Table 4. Enzymic activity of high-risk beef calves fed supplemental calcium propionate (CaPr) and chromium-methionine (Cr-Met) for 56 d (n = 12 calves/treatment).

Item	Treatments [a]				Reference Intervals [b]	SEM [c]
	Control	CaPr	Cr-Met	CaPr + Cr-Met		
Alkaline phosphatase, U/I	159.27	181.25	182.25	193.82	14.4–469.6 (209.7 ± 107)	17.34
Gamma glutamyltransferase, U/I	14.27	11.41	13.45	14.60	10.0–47.9 (17.0 ± 8.9)	1.18
Aspartate aminotransferase, U/I	81.72	70.66	73.54	77.51	38.9–124.2 (68.8 ± 21.9)	5.12
Alanine aminotransferase, U/I	34.90	29.01	30.00	31.90	16.6–44.4 (26.5 ± 7.3)	1.73

[a] Treatments consisted of oral administration of calcium propionate (CaPr, Propical®, Dresen Química, SAPI de CV., Ciudad de México, México) or chromium-methionine (Cr-Met, MiCroPlex® 1000, Zinpro Corporation, Eden Prairie, MN, USA): (1) No additives (Control), (2) CaPr, (3) Cr-Met, and (4) CaPr + Cr-Met. CaPr was administered at a dose of 19 g/calf/d and Cr-Met at a dose of 4 g/calf/d. [b] Reference intervals reported here are from the publication by Carrillo-Muro et al. [24]. [c] SEM = standard error of the mean.

Table 5. Serum metabolites of high-risk beef calves fed supplemental calcium propionate (CaPr) and chromium-methionine (Cr-Met) for 56 d (n = 12 calves/treatment).

Item	Treatments [a]				Reference Intervals [b]	SEM [c]
	Control	CaPr	Cr-Met	CaPr + Cr-Met		
Total protein, g/dL	6.41	6.45	6.58	7.12	4.4–7.71 (6.22 ± 0.83)	0.19
Albumin, g/dL	3.14	3.09	3.02	3.11	1.9–3.7 (2.97 ± 0.50)	0.14
Globulins, g/dL	3.32	3.35	3.58	3.77	2.2–4.11 (3.18 ± 0.50)	0.14
Albumin/Globulins ratio	0.97 [a]	0.94 [ab]	0.85 [ab]	0.82 [b]	0.68–1.32 (0.94 ± 0.17)	0.03
Blood urea nitrogen, mg/dL	15.79	14.17	15.75	14.68	6.91–16.1 (11.08 ± 2.31)	0.59
Creatinine, mg/dL	0.71	0.79	0.71	0.75	0.52–1.35 (0.81 ± 0.20)	0.06
Total bilirubin, mg/dL	0.54	0.39	0.34	0.39	0.20–1.30 (0.34 ± 0.29)	0.08

Table 5. *Cont.*

Item	Treatments [a]				Reference Intervals [b]	SEM [c]
	Control	CaPr	Cr-Met	CaPr + Cr-Met		
Total cholesterol, mg/dL	71.30 [b]	73.16 [b]	80.53 [a]	79.54 [a]	50.0–127.7 (78.6 ± 22.0)	4.09
Triglycerides, mg/dL	19.59	20.58	21.53	17.45	10.0–360.7 (36.5 ± 77.1)	3.01
Calcium, mg/dL	9.13	9.62	9.87	10.12	7.12–12.5 (10.28 ± 1.42)	0.25
Glucose, mg/dL	63.41 [b]	70.00 [ab]	72.58 [ab]	78.20 [a]	26.1–126.0 (89.0 ± 22.5)	3.53

[a] Treatments consisted of oral administration of calcium propionate (CaPr, Propical®, Dresen Química, SAPI de CV., Ciudad de México, México) or chromium-methionine (Cr-Met, MiCroPlex® 1000, Zinpro Corporation, Eden Prairie, MN, USA): (1) No additives (Control), (2) CaPr, (3) Cr-Met, and (4) CaPr + Cr-Met. CaPr was administered at a dose of 19 g/calf/d and Cr-Met at a dose of 4 g/calf/d. [b] Reference intervals reported here are from the publication by Carrillo-Muro et al. [24]. [c] SEM = standard error of the mean. [a,b] Means a row with different superscripts differ ($p < 0.05$) according to Tukey's test.

Table 6. Electrolytes of high-risk beef calves fed supplemental calcium propionate (CaPr) and chromium-methionine (Cr-Met) for 56 d (n = 12 calves/treatment).

Item	Treatments [a]				Reference Intervals [b]	SEM [c]
	Control	CaPr	Cr-Met	CaPr + Cr-Met		
Sodium, mEq/L	123.08	126.27	126.67	128.92	98.2–143.0 (126.3 ± 12.1)	2.51
Potassium, mEq/L	6.83	6.61	6.65	6.79	3.11–8.59 (4.93 ± 1.21)	0.37
Chlorine, mEq/L	88.53	91.17	91.84	95.72	71.1–109.0 (90.8 ± 9.8)	1.93

[a] Treatments consisted of oral administration of calcium propionate (CaPr, Propical®, Dresen Química, SAPI de CV., Ciudad de México, México) or chromium-methionine (Cr-Met, MiCroPlex® 1000, Zinpro Corporation, Eden Prairie, MN, USA): (1) No additives (Control), (2) CaPr, (3) Cr-Met, and (4) CaPr + Cr-Met. CaPr was administered at a dose of 19 g/calf/d and Cr-Met at a dose of 4 g/calf/d. [b] Reference intervals reported here are from the publication Carrillo-Muro et al. [24]. [c] SEM = standard error of the mean.

3.3. Hematological Responses

The supplementation of CaPr, Cr-Met, and CaPr + Cr-Met did not cause significant effects ($p > 0.05$) in WBC, LYM, LYM%, MON, MON%, GRA, GRA% (Table 7), PLT, RDW%, HGB, HCT%, and MCHC (Table 8). However, the supplementation resulted in a significant increase ($p < 0.05$) in MPV, with CaPr showing a 10.3% increase, Cr-Met showing a 3.9% increase, and CaPr + Cr-Met showing a 1.9% increase. Additionally, the inclusion of CaPr led to a 6.2% rise in RBC, while the combination of CaPr + Cr-Met caused a 7.7% decrease in RBC ($p < 0.05$). Finally, CaPr + Cr-Met resulted in a 2.9% increase in MCH ($p < 0.05$).

Table 7. White blood cells of high-risk beef calves fed supplemental calcium propionate (CaPr) and chromium-methionine (Cr-Met) for 56 d (n = 12 calves/treatment).

Item	Treatments [a]				Reference Intervals [b]	SEM [c]
	Control	CaPr	Cr-Met	CaPr + Cr-Met		
Total white blood cells, $\times 10^3$ cells/µL	8.43	8.45	8.41	7.05	4.6–15.2 (9.65 ± 2.62)	0.535
Lymphocytes, $\times 10^3$ cells/µL	5.67	5.19	4.97	5.03	2.6–9.0 (5.87 ± 1.6)	0.431
Lymphocytes, %	67.29	59.54	59.8	59.38	33.6–74.61 (59.2 ± 9.48)	2.796
Monocytes, $\times 10^3$ cells/µL	0.80	0.81	0.75	0.87	0.30–1.40 (0.83 ± 0.26)	0.071
Monocytes, %	8.71	9.41	8.16	9.65	5.54–12.0 (8.28 ± 1.5)	0.431

Table 7. *Cont.*

Item	Treatments [a]				Reference Intervals [b]	SEM [c]
	Control	CaPr	Cr-Met	CaPr + Cr-Met		
Granulocytes, ×10³ cells/µL	1.99	2.41	2.35	2.27	1.10–6.74 (3.33 ± 1.42)	0.261
Granulocytes, %	24.33	28.31	32.13	30.52	17.51–85.57 (35.3 ± 14.8)	2.764

[a] Treatments consisted of oral administration of calcium propionate (CaPr, Propical®, Dresen Química, SAPI de CV., Ciudad de México, México) or chromium-methionine (Cr-Met, MiCroPlex® 1000, Zinpro Corporation, Eden Prairie, MN, USA): (1) No additives (Control), (2) CaPr, (3) Cr-Met, and (4) CaPr + Cr-Met. CaPr was administered at a dose of 19 g/calf/d and Cr-Met at a dose of 4 g/calf/d. [b] Reference intervals reported here are from the publication by Carrillo-Muro et al. [24]. [c] SEM = standard error of the mean.

Table 8. Platelets and red blood cells of high-risk beef calves fed supplemental calcium propionate (CaPr) and chromium-methionine (Cr-Met) for 56 d (*n* = 12 calves/treatment).

Item	Treatments [a]				Reference Intervals [b]	SEM [c]
	Control	CaPr	Cr-Met	CaPr + Cr-Met		
Platelets, ×10³ cells/µL	276.81	253.74	239.00	269.52	91.2–444.9 (239.8 ± 90.7)	28.169
Mean platelet volume, fL	6.75 [d]	7.44 [a]	7.02 [b]	6.88 [c]	6.14–9.08 (7.22 ± 0.97)	0.0001
Red blood cells, ×10⁶ cells/µL	7.92 [ab]	8.41 [a]	7.86 [ab]	7.31 [b]	7.88–11.90 (9.77 ± 1.07)	0.244
Red blood cells distribution width test, %	25.83	26.23	26.11	27.18	19.11–30.13 (26.4 ± 2.54)	0.436
Hemoglobin, g/100 mL	11.01	11.24	11.15	10.62	9.40–14.4 (12.05 ± 1.31)	0.258
Hematocrit, %	29.87	30.21	30.04	28.19	26.65–41.93 (34.27 ± 3.92)	0.881
Mean corpuscular volume, fL	37.27	36.33	36.18	37.53	29.1–41.8 (35.12 ± 3.1)	0.474
Mean corpuscular hemoglobin, pg	13.86 [b]	13.50 [b]	13.81 [b]	14.26 [a]	10.6–14.4 (12.36 ± 1.03)	0.178
Mean corpuscular hemoglobin concentration, g/dL	37.17	37.36	37.37	37.65	30.58–38.93 (35.35 ± 1.98)	0.295

[a] Treatments consisted of oral administration of calcium propionate (CaPr, Propical®, Dresen Química, SAPI de CV., Ciudad de México, México) or chromium-methionine (Cr-Met, MiCroPlex® 1000, Zinpro Corporation, Eden Prairie, MN, USA): (1) No additives (Control), (2) CaPr, (3) Cr-Met, and (4) CaPr + Cr-Met. CaPr was administered at a dose of 19 g/calf/d and Cr-Met at a dose of 4 g/calf/d. [b] Reference intervals reported here are from the publication by Carrillo-Muro et al. [24]. [c] SEM = standard error of the mean. [a–d] Means a row with different superscripts differ ($p < 0.05$) according to Tukey's test.

4. Discussion

The hypothesis was that supplementing with 19 g of CaPr or 4 g of Cr-Met would have similar effects in high-risk beef calves, and that combining CaPr and Cr-Met could enhance growth performance, dietary energy, and body fat reserves. However, no synergistic effects were detected; instead, similar results were observed for all variables with individual supplementation.

Although their modes of action act at different levels, both CaPr and Cr have important effects on the physiological processes and pathways related to obtaining energy for maintenance, productivity, and health. CaPr has an effect at the ruminal level, promoting greater DM digestibility and N retention and favoring more efficient ruminal fermentation processes (by increasing propionate production and reducing methane production) [5,30–32]. At the systemic level, CaPr increases the synthesis of GLU via gluconeogenesis in the liver by increasing the energy status [33], whereas the effect of Cr is predominantly systemic, since it improves the response to insulin, due to its potential to assist in the cellular absorption of GLU and amino acids in tissues sensitive to insulin. In such a way, it can be stated that CaPr improves the energy status, and Cr improves the use of that energy for cell maintenance and growth [10,34]. In addition, Cr improves immune function, reduces serum cortisol concentration, and modulates the inflammatory response in stressed animals [35]. Therefore, the hypothesis arises that the combination of CaPr with Cr could have a synergistic effect on optimizing the energy from food to enhance productivity and health in newly received high-risk beef calves.

4.1. Growth Performance, Dietary Energetics, Body Fat Reserves and Longissimus Muscle Area

Calves that received the combination of CaPr + Cr-Met exhibited an improved ADG and ADG/DMI ratio, similar to the individual effects of CaPr and Cr-Met supplemented separately. There are no previous reports on high-risk beef calves supplemented with CaPr + Cr-Met, and those surrounding Cr-Met are also very limited, since it is used more in the finishing stage. Previous reports regarding supplementation with 20 g of CaPr [8,9] have indicated improvements in ADG and ADG/DMI ratio, with no negative effects on DMI. On the other hand, supplementation for 28 d of CrPr or Cr-Yeast has been shown to significantly improve DMI, ADG, and ADG/DMI ratio [19,36,37]; however, other authors did not observe effects with the inclusion of Cr-Met [38]. The efficiency of dietary NE utilization improved with CaPr or Cr-Met supplementation by 10.2 and 11.4%, respectively, but the combination (CaPr + Cr-Met) produced very similar results, and this coincided with the observed expected dietary NE ratio; likewise, energy retention had the same behavior. Expected DMI increased by 12.5% for CaPr, and 13.8% for Cr-Met.

The presence of body fat reserves in calves upon arrival is an important indicator of their nutritional status. Therefore, it is crucial to encourage the accumulation of these reserves [39]. In our study, the inclusion of CaPr or CaPr + Cr-Met increased RFT, but the increase was greater with Cr-Met. Previous observations in calves have shown that with CaPr, there were maximum increases of 24.9% of FAT at 42 d and 21% of RFT at 56 d [8]. Additionally, the inclusion of CaPr, along with an increase in the concentrate level, led to a RFT increase of up to 16.6% [9]. This is due to the fact that diets with a higher energy or protein content stimulate the deposition of body fat reserves [40,41], coupled with the increase in adipogenesis with the inclusion of CaPr [42].

4.2. Enzymatic Activity and Serum Metabolites

The supplementation of CaPr, Cr-Met, or CaPr + Cr-Met in high-risk beef calves has been found to have no pathological effect or improvement in hepatic and renal metabolism. This is evidenced by the maintenance of enzymatic activity within the reference intervals (ALP, GGT, AST, and ALT) [24]. Regarding ALP, it has been reported that CrPr in high-risk beef calves [21], Cr-Met in finishing cattle [43], and doses of 20 g of CaPr do not modify its activity, but when inclusion of CaPr is raised above 80 g, it causes a reduction in ALP [8,9]; these reductions in young growing calves are associated with a decrease in osteoblasts (reduced bone growth) [44]. Other authors have also pointed out that GGT activity is not modified by CaPr supplementation [8,9], but the increase is related to greater hepatic activity [45]. AST activity has also not been modified by CaPr supplementation [8,9] or with CrPr [21], CrPic, or Cr Yeast [14]; increases are considered a nonspecific indicator of tissue injury [46]. Finally, ALT activity is not modified with increasing levels of concentrate and CaPr supplementation [9] or with CrPr [21], CrPic, or Cr Yeast [14].

TP is the main solid component of serum and is made up of ALB and GLO, which are indicative of the nutritional status of cattle. In the present research, TP, ALB, and GLO were found to remain within the established RIs despite supplementation with CaPr, Cr-Met, or CaPr + Cr-Met [24]. In high-risk beef calves, Rivera-Villegas et al. [9] provided CaPr supplementation and a diet with 50% concentrate, and their results indicated that TP and ALB concentrations were reduced, although they remained within the RIs. However, following supplementation with different levels of CaPr [8] or with CrPr [21], CrPic, and

Cr Yeast [14], or Cr-Met in finishing cattle [43], no effects were observed. Supplementation with doses lower than 60 g of CaPr [8,9] or with CrPr [21] did not alter GLO, while an increase in its concentration was noted with 80 g of CaPr [8], with the final value still within the RIs; this could possibly be due to chronic inflammation of the liver [47]. Additionally, the ALB/GLO ratio decreased with the supplementation of CaPr and Cr-Met, with the lowest value observed when both products were combined (CaPr + Cr-Met). Nevertheless, these changes remained within the RIs, indicating the overall health of the cattle.

Serum BUN levels help to estimate the amount of N excreted and the efficiency with which it is utilized [48]. This is influenced by the level of crude protein intake, rumen degradability, and liver and kidney function [49]. In the current study, N utilization efficiency was not affected by the treatments. Similarly, in high-risk beef calves, studies using low doses of CaPr [8,9] or CrPr [21], CrPic and Cr Yeast [14], or Cr-Met in finishing cattle [43] did not show significant changes. However, with high doses of 80 g of CaPr, increases were observed without exceeding RIs [8,24]. Waggoner et al. [50] noted that calves with immunological problems have lower nitrogen retention due to increased muscle catabolism to obtain proteins and improve the immune response. Protein catabolism is higher (indicated by high BUN levels) at the beginning of the reception phase (first 20 d), but it decreases as the animal becomes more dependent on dietary protein rather than muscle catabolism [51].

Supplementation with CaPr, Cr-Met, or CaPr + Cr-Met did not affect the CRE concentration, which remained within the RIs [24]; these results coincide with those reported by other authors in high-risk beef calves who were administered low doses of CaPr [8,9] or with CrPr [21], as well as with Cr-Met supplementation in finishing cattle [43], indicating the maintenance of an appropriate renal glomerular filtration rate and the absence of treatment interference. However, it has been observed that escalating the inclusion of CaPr from 20 to 80 g results in elevated CRE levels [8].

TBIL is an important indicator of liver function, exhibiting an increase during severe lipidosis and a decrease in the presence of a healthy liver [52,53]. In this study, TBIL concentrations remained unaffected by the treatments, falling within the normal range (0.20–1.30; [24]), signifying the absence of adverse effects of the treatments on liver function. This aligns with the findings of other studies involving increased concentrate levels and CaPr supplementation [9] or CrPr [21].

Lipids primarily consist of TCHO and TG, reflecting the liver's energy metabolism [54]. Increases in lipid levels are associated with a rise in propionic acid production in the rumen, leading to increased TCHO production in the liver, while decreases indicate an energy deficit [55]. The supplementation of Cr-Met or CaPr + Cr-Met has been found to boost the energy of high-risk beef calves, observed with the increase in TCHO concentration, which did not exceed the RIs [24]. Similar effects were observed with 80 g CaPr supplementation [8]. However, no significant effects were noted with 20 g CaPr supplementation [8,9], CrPic and Cr Yeast [14], or Cr-Met in finishing cattle [43].

The supplementation of CaPr, Cr-Met, or CaPr + Cr-Met did not alter the Ca concentration beyond the RIs [24]. This finding is consistent with various studies on high-risk beef calves that also received CaPr supplementation [8,9,56,57] or CrPr [21], as well as Cr-Met in finishing cattle [43]. However, other authors have mentioned that as the level of CaPr supplementation increases, it leads to an increase in Ca concentration [8,41].

Blood GLU concentration is often used as a metabolic indicator of nutrient intake in beef cattle [58]. CaPr and Cr-Met supplementation increased GLU levels, with the highest levels observed with CaPr + Cr-Met, while still remaining within the RIs [24]. Ermita et al. [59] also noted increases with CaPr administration, whereas Bernhard et al. [19] observed reductions with CrPr. The increase may be linked to adequate DMI, since circulating GLU is influenced by nutrient availability and utilization [60,61]. However, different authors have pointed out that in high-risk beef calves supplemented with CaPr [8,57,62], CrPr [21], CrPic, and Cr Yeast [14], or Cr-Met in finishing cattle [43] exhibited no change in glycemia.

Supplementation with CaPr, Cr-Met, or CaPr + Cr-Met did not alter the values of electrolytes (Na^+, K^+, and Cl^-), and they remained within the established RIs [24]. This finding is consistent with other reports on the effects of CaPr supplementation [8,9] or CrPr [21] in high-risk beef calves. All of the above indicates that the basal diets adequately met the nutritional electrolyte requirements of the calves [63]. Low Na^+ and Cl^- values are often due to diarrhea [46], and K^+ deficiency is commonly associated with stressed cattle due to dehydration and loss of K^+ in the tissues [64].

4.3. Hematological Responses

Supplementation with CaPr, Cr-Met, or CaPr + Cr-Met helped maintain WBC, PLT, and RBC (hematological response) values within the established RIs [24]. This indicates that high-risk calves have healthy immunological status [65].

WBC values were not affected by CaPr, Cr-Met, or CaPr + Cr-Met supplementation, which aligns with findings from Rodríguez-Cordero et al. [8] and Rivera-Villegas et al. [9] with 20 g CaPr, and Smock et al. [21] with CrPr supplementation. However, increasing CaPr inclusion from 40 to 80 g reduced LYM%, and increased GRA and GRA% [8].

Regarding PLT, MCHC, RBC, RDW%, HGB, HCT, and MCV, no discernible effects of CaPr, Cr-Met, or CaPr + Cr-Met were noted, which coincides with what was observed by Rodríguez-Cordero et al. [8] and Rivera-Villegas et al. [9] with CaPr supplementation. Additionally, Smock et al. [21] also found no significant impact on most of these variables with CrPr supplementation, although they did observe reductions in HGB, HCT, and MCV. Only CaPr supplementation had an effect on MPV and RBC, resulting in elevated values. However, other authors have observed no effect with CaPr [8,9] or CrPr [21] supplementation. Likewise, CaPr + Cr-Met supplementation increased MCH values, which coincides with Rodríguez-Cordero et al. [8], who also observed an increase when CaPr inclusion was raised from 40 to 80 g; conversely, Smock et al. [21] reported that CrPr supplementation led to a decrease in MCH values.

5. Conclusions

Supplementation with the gluconeogenic precursor CaPr or Cr-Met improved growth performance and dietary energy utilization in newly arrived high-risk calves, with no negative impact on hematological parameters and minimal effect on serum metabolites. Thus, either CaPr or Cr-Met can be used individually to enhance the performance of high-risk calves at arrival, and their combination does not appear to be required under these experimental conditions.

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