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Effects of Duration of Calcium Propionate Supplementation in Lambs Finished with Supplemental Zilpaterol Hydrochloride: Productive Performance, Carcass Characteristics, and Meat Quality

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Simple Summary: The finishing phase of ruminants presents lower efficiency and growth rates, due to a higher proportion of gain being fat rather than muscle. Therefore, the primary objective during finishing is to increase the muscle proportion and reduce fat content. As an alternative, the diet was supplemented with calcium propionate (CaPr) and finished with zilpaterol hydrochloride (ZH). Observations indicated that including CaPr for 28 d before slaughter improve the response to ZH supplementation on feed efficiency, growth rate, carcass weight and some whole cuts.

Abstract: Forty-five male Dorper crossbred lambs (40.17 ± 0.35 kg BW) were used in order to investigate the effects of the duration of calcium propionate (CaPr) supplementation in lambs finished with zilpaterol hydrochloride (ZH) on the productive performance, carcass characteristics, and meat quality. Lambs were individually housed and fed a finishing-diet for 42 d before being slaughtered. Lambs received the following treatments: 1) No additives (CTL), 2) ZH supplementation for 28 d (with 3 d ZH withdrawal before slaughter) at a dosage of at 7.2 mg/kg diet (ZH), for treatments 3, 4, and 5, lambs received ZH and 10g CaPr/lamb/d throughout the entire phase (42 d), or during the last 28 or 14 d before slaughter. Compared to CTL, ZH lambs exhibited a similar average daily gain (ADG) but had lower dry matter intake (DMI), leading to increased feed efficiency. Supplementing with ZH alone did not affect carcass traits, visceral mass, whole cuts, or meat quality. Lambs that received both CaPr and ZH exhibited quadratic increases ($p < 0.05$) in final BW (FBW), ADG, and dressing percentage (D%). These increases were optimal at an estimated inclusion durations of 26 d for FBW, 30 for ADG, and 39 d for D%. The ADG:DMI ratio and the longissimus muscle area (LMA) both exhibited quadratic increases ($p < 0.05$). The optimal duration of CaPr supplementation for ADG:DMI ratio was found to be 28 d, while for LMA, it was 14 d. As the period of CaPr supplementation increased, there was a linear increase ($p < 0.05$) in hot carcass weight, leg circumference, and whole cuts of breast IMPS209 and shoulder IMPS207. Cook loss percent increased quadratically ($p < 0.05$), being higher when CaPr was included for an estimated duration of 26 d. As the duration of CaPr supplementation increased, the purge loss percentage (PLR) also increased linearly ($p < 0.05$). In conclusion, including CaPr in the diet for a duration of 28 d in lambs improved the response to ZH supplementation on the productive performance, carcass weight and some whole cuts. However, it can also have a negative effect on PRL%.

Keywords: crossbred lambs; gluconeogenic precursor's; duration; β-adrenergic agonists

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1. Introduction

In recent decades, research on feedlot ruminants has focused on finding technologies that enhance feed efficiency and growth rate during finishing phase. The final phase of finishing is characterized by lower growth efficiency in cattle due to a composition of gain that is primarily fat rather than muscle [1]. Thus, one of the main objectives during finishing is to increase the proportion or weight of muscles and reduce the fat content of the carcass [2,3]. An alternative approach to achieve this goal is through the use of growth promoters, such as gluconeogenic precursors or β-adrenergic agonists (β-AA).

It has been observed that gluconeogenic precursor calcium propionate (CaPr) alters energy metabolism in two ways when supplemented in ruminant diets: firstly, by altering rumen fermentation through improvements on ruminal DM digestibility, increasing the proportion of ruminal propionate, and reducing methane production [4,5]. Another way is by improving the action of insulin on glucose metabolism [6], promoting an increase in energy status through enhanced glucose synthesis via gluconeogenesis in the liver [7]. In this context, increases in growth performance, feed efficiency and muscle growth have been reported in finishing lambs supplemented with a daily dose of 10 g of CaPr/d [8]. Carrillo-Muro et al. [9], studying finishing diets for lambs, determined that a dose of 10 g of CaPr / lamb / d, during 42 d led to the following increases: 13% dry matter intake (DMI), 28% average daily gain (ADG), 17% ADG:DMI ratio, 7% final body weight (FBW) and 4% empty body weight (EBW); in addition, cooling loss percent (CL%) was reduced by 13%, without impacting meat quality variables. Furthermore, the duration of CaPr supplementation selectively impacts the benefits on growth and carcass traits. Carrillo-Muro et al. [10] assessed varying inclusion duration of 10 g of CaPr/lamb/d, noting the following durations and their corresponding maximal increments: 15 d for DMI (1%), 25 d for FBW (5%) and ADG (27%), 28 d for ADG:DMI ratio (25%), 24 d for hot carcass weight (9%, HCW) and 20 d for dressing percentage (4%, D%). However, the extended inclusion duration (42 d) led to an increase in fat thickness (30%, FT) and a reduction in the proportion of loin (22%). Conversely, it increased the weight of leg (10%) and rack (14%). This suggests that the surplus energy provided by CaPr is stored as fat, resulting in a decrease in the proportion of certain muscle.

Zilpaterol hydrochloride (ZH) is a β-AA. When supplemented at a rate of 4 to 8 mg/kg diet during the last 20-40 d of fattening, it enhances growth performance, dietary energy utilization efficiency, and carcass traits in fattening lambs [11]. The advantages of using ZH during the finishing phase of fattening have primarily been attributed to alterations in the composition of tissue gain [2,12]. ZH activates receptors in muscle and fat, leading to augmented lipolysis, reduced lipogenesis, and increased protein accumulation, either individually or in combination [13]. However, these alterations in muscle tissue could impact the meat quality of lambs supplemented with ZH [14]. In support of this, a reduction of 8% in cook loss percentage (CKL%) has been noted in lambs supplemented with ZH [15]. Similarly, a 10% increase in Warner-Bratzler shear force has been reported in lambs that received 0.15 mg ZH/kg BW [16].

Given the mechanism of action of CaPr and ZH, their combination could be complementary. Based on this, we hypothesized that augmenting the available energy in the diet through supplemental CaPr in lambs receiving ZH during the last phase of fattening can enhance the response to ZH supplementation in terms of productive performance and carcass characteristics, while avoiding adverse effects on meat quality. Furthermore, the extent of these effects may be linked to the duration of CaPr. Therefore, the objective of the present study was to investigate impact of varying inclusion durations of CaPr (0, 14, 28, or 42 d before slaughter) at a daily dose of 10 grams in lambs finished with supplemental ZH (received ZH 28 d plus 3-d ZH withdrawal prior to slaughter) on productive performance, carcass characteristics, and meat quality.

2. Materials and Methods

The experiment was conducted at the facilities of the Small Ruminant Experimental Center the and Meat Science and Technology Laboratory, both situated within the Unidad Académica de Medicina Veterinaria y Zootecnia at the Universidad Autónoma de Zacatecas (UAMVZ-UAZ), in the state of Zacatecas, Mexico (north-central Mexico). During the experiment (April to May 2023), ambient air temperature averaged 26.2 °C, with a minimum of 9.4 °C and a maximum of 32 °C.

All experimental procedures involving the lambs were conducted in accordance with the guidelines of the approved Officials Mexicans Standards: 1) NOM-051-ZOO-1995, Humanitarian care in the mobilization of animals; 2) NOM-062-ZOO-1999, Humane care and welfare of experimental animals; 3) NOM-024-ZOO-1995, Animal health stipulations and characteristics during animal transportation; 4) NOM-033-SAG/ZOO-2014, Methods to kill domestic and wild animals; and 5) NOM-EM-015-ZOO-2002, Technical specifications for the control of the use of beta-agonists in animals. In addition, the experiment reported herein was approved of Bioethics and Animal Welfare Committee of UAMVZ-UAZ, with protocol number 2023/04.

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

Forty-five uncastrated male lambs of the Dorper crossbred, with an average initial body weight (IBW) of 40.17 ± 0.35 kg and 6 months of age, were utilized to evaluate the effects of the treatments. The lambs' IBWs were recorded, and they were accommodated in 45 individual pens (1.5 × 1.5 m). Three weeks prior to commencing the feeding trial, all lambs underwent health management, including: 1) identification with a uniquely numbered ear tag; 2) vaccination against *Clostridium* spp. and *Pasteurella* spp.; and 3) treatment for endoparasites (Closantel 5%) and ectoparasites (Doramectina 1%). Lambs were also accustomed to the facilities and the basal diet. The composition of ingredients and chemical characteristics of the basal diet are presented in Table 1. Throughout the study, lambs had free access to the basal diet and fresh water. Fresh feed was provided twice daily at 0800 and 1600 h in a 40:60 proportion, respectively. Feed offered to each lamb was adjusted to minimize refusals (~5% of the previous day's intake). Feed bunks were visually assessed between 0740 and 0750 h each morning, residual feed was collected and weighed for determination of dry matter intake (DMI). Adjustments in daily feed delivery were provided at the afternoon feeding. Prior to the morning feeding, lambs were individually weighed at start the experiment (IBW), at intermediate points (14, 28 d) and at the end of the experiment (42 d). Daily samples of the basal diet were collected and analyzed in triplicate for: 1) DM%, dried for 24 h at 100 °C in a forced air-drying oven, CP (FP-528 LECO nitrogen analyzer) [17]; 3) NDF (fiber Ankom analyzer) and 4) EE (extractor of Ankom[xt15]).

Table 1. Ingredients of the basal diet offered to lambs and nutritional composition.

Ingredients	g kg^{-1} DM
Alfalfa hay mature	100.0
Oats hay	100.0
Dry-rolled corn yellow	500.0
Dried distillers grains	130.0
Soybean meal-44	54.0
Molasses cane	80.00
Calcium carbonate	10.0
Sodium bentonite	10.0
Sodium sesquicarbonate	15.0
Microminerals: Co, Fe, I, Mn, Zn, Se and Cu[a]	0.5
Vitamins: A, D and E [b]	0.5
Chemical composition, g kg^{-1} DM[c]	
Dry matter	835.1
Crude protein	137.2
Ether extract	23.8
Neutral detergent fiber	204.0
Calcium	8.5
Phosphorus	2.4
Ca:P ratio	3.5

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Calculated net energy, Mcal/kg[d]	
Maintenance	1.9
Gain	1.3

[a] 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. [b] Vitamins A (5,000,000 IU), D (2,000,000 IU) y E (10,000 IU). Excipient q.s. 1000 g. [c] Based on the tabular values for individual feed ingredients with the exception of DM, CP, NDF [18], and EE (Ankom procedures) which were determined in our laboratory. [d] Based on the tabular energy values from NRC [18] feed composition tables.

2.2. Experimental Design and Treatments

A completely randomized design was employed to investigate the effects of varying durations of CaPr supplementation (0, 14, 28, or 42 d before slaughter) in lambs that received ZH for 28 d, followed by a 3 d withdrawal period before slaughter. The treatments included: 1) No additives (CTL), 2) ZH supplementation for 28 d (with 3 d ZH withdrawal before slaughter) at a dosage of at 7.2 mg/kg diet (ZH). In treatments 3, 4, and 5, lambs received ZH, but they also received 10g CaPr/lamb/d throughout the entire phase (42 d), or during the last 28 or 14 d before slaughter. The source of CaPr used was Nuprocal® (Nutryplus, Mexico), originating from the same batch, comprising 20% calcium and 69% of acid propionic with an estimated metabolizable energy contribution of 3.766 Mcal/kg [19]. The source of ZH was from the same batch of the patented trademark Zilmax® (MSD, Salud Animal Mexico, Estado de Mexico, Mexico). Individual dosages of CaPr (10 g/lamb/d) and ZH were hand-weighed using a precision balance (Pioneer-PX523, Ohaus Corp., Parsippany, NJ, USA). To ensure the treated group's total intake, the doses were mixed with 100 g of the basal diet, offered in the morning. Once consumed, the remaining portion of the diet was administered.

2.3. Productive Performance Calculus

Using the collected data during feeding trial, the following parameters were calculated: 1) ADG= [(FBW – IBW)/ number of d on feed]; 2) Average DMI= (Feed offered - Feed refused), which was weighed and recorded daily; and 3) ADG:DMI ratio= (ADG / DMI).

2.4. Slaughter Procedure and Visceral Organ Mass Determination

At the conclusion of the 42 d trial period, lambs underwent a fasting period (18 h) while having access to water. Pre-slaughter weights were recorded for subsequent calculations. During the slaughter process, non-carcass components, including skin, heart, lungs, liver, spleen, kidney, perirenal fat, and full and digesta-free gastrointestinal tract were removed and weighed. The EBW= (Pre-slaughter BW − Total non-carcass components weight). Visceral organ mass was expressed as g/kg of EBW.

2.5. Carcass Characteristics

The HCW was determined prior to chilling (24 h at 4°C). After the cooling process, CCW was measured, which included kidneys and internal fat. Carcass D% was calculated as = ([CCW / EBW] × 100), and the CL%= ([HCW-CCW] / HCW) × 100. After 24 h chilling period carcass dimensions were measured using a flexible tape measure: carcass length, leg length and chest circumference. Longissimus muscle area (LMA) and FT between the 12th and 13th ribs were assessed on days 0, 14, 28 and 42, employing an Aloka Prosound 2 instrument with a 3.5-MHz linear transducer.

2.6. Whole Cuts and Tissue Composition

The left sides of the carcasses were sectioned to extract the: 1) The forequarter, which was further subdivided into the neck, shoulder IMPS206, shoulder IMPS207, rack IMPS204, breast IMPS209, ribs IMPS209A; and 2) The hindquarter, comprising the loin IMPS231, leg IMPS233 and flank IMPS232, in accordance with the guidelines of the North American Meat Processors Association guidelines [20]. The weight of each cut was subsequently recorded and expressed either in g/kg of EBW or as a percentage of the CCW. The carcasses were halved, and the left side was dissected. The tissue composition of the shoulder was determined through a physical dissection process to calculate the percentage of muscle, fat, and bone.

2.7. Meat Quality

Muscle samples were obtained from the cold carcass longissimus muscle (LM, approximately 500 g) and frozen at −20 °C for subsequent meat quality analysis. Color measurements were taken from the surface of the LM exposed by the 12th/13th-rib cut, using a Minolta CR-400 spectrophotometer (Konica Minolta Sensing, Inc., Osaka, Japan). The configuration included an aperture size of 8 mm, observer 10, D65 illuminant, and a blooming time of 1.5 s. The color coordinates luminosity (Hunter L* Value), redness (Hunter a* value), and yellowness (Hunter b* value) were measured after 24 h postmortem. The pH of the right LM at the 2nd lumbar vertebra (LM) was determined using a portable digital pH meter (Hanna Instruments, Model HI–9025).

The water-holding capacity percent (WHC%) was assessed following the methodology described by Grau and Hamm, as suggested by Tsai and Ockerman [21]. In summary, 300 mg of LM were enclosed with filter papers (Whatman #1), positioned between glass plates (15 × 15 cm), and pressed under a consistent pressure of 10 kg for 20 min. LM steaks (2 cm-thick) acquired from between the 12th rib and L2 vertebrae were vacuum-sealed in plastic bags and frozen at −20 °C. Following a storage duration of 14 d, the steaks were allowed to temper for 24 h at 4 °C, then gently blotted dry, and weighed. One steak was sliced into dimensions of 15 mm × 15 mm × 30 mm, subsequently suspended at 4°C for 24 and 48 h, to calculate the percentage of purge loss (PRL%). Another steak was vacuum-sealed in a polyethylene bag and heated at 80°C until the internal temperature reached 70°C for determination of CKL%. The sealed plastic bag samples were placed individually and immersed in a water bath at 75 °C until they attained an internal temperature of 70 °C. Following the cooking process, the samples were cooled under running tap water, extracted from the packaging, gently blotted, and weighed. WHC%, PRL%, and CKL% were indicated as percentages of weight loss in comparison to the initial weight. This was calculated as [(initial weight − final weight)/initial weight] × 100.

Steaks (2.54 cm thick) were thawed for 24 h at 4°C. They were subsequently broiled on an electric grill, specifically the George Foreman Electronics (Model GR2120B), until they reached an internal temperature of 70°C [22]. The internal temperature was monitored using Kitchen thermometer (Model TP700). After the cooking process, the steaks were allowed to cool at 22°C for 4 h. In due course, six cores measuring 1.27 cm were extracted parallel to the muscle fiber using a mechanical coring device. The cores were subjected to shearing using a Texture Analyzer (G-R Manufacturing, New York, NY, USA) equipped with a Warner-Bratzler knife, and peak shear force achieved was recorded. Crosshead speed was adjusted to 200 cm min^{-1}. The measurements of shear force were then averaged and expressed in kg/cm^2.

2.8. Statistical Analyses

The statistical analysis was performed using SAS University software. The normality assumptions were validated through the UNIVARIATE procedure. The data were analyzed using a completely randomized design with the GLM procedure, except for ADG, DMI, and ADG:DMI ratio, which were subjected to analysis using the MIXED procedure for repeated measurements. For productive performance and meat characteristics, lambs and carcasses were considered as the experimental units. CCW was introduced as a covariate for the analysis of carcass characteristics. Whenever significant effects were detected, a comparison of means was carried out employing the Tukey method with the LSMEANS instruction.

Duration of CaPr supplementation were categorized into linear and quadratic orthogonal polynomials, involving four equally spaced levels, using the LSMEANS and ESTIMATE statements. In instances where quadratic polynomials exhibited significance, the quadratic equations were computed utilizing the REG procedure. Significance was established when the *p*-value was ≤ 0.05, and a trend if the *p*-value was >0.05 and ≤0.10.

3. Results

3.1. Productive Performance

In comparison to CTL, ZH lambs exhibited similar ADG but displayed a 28.5% reduction in DMI, leading to a notable 26.4% enhancement in feed efficiency. The reduction of DMI due to ZH supplementation persisted although to a lesser extent with the presence of CaPr. Among lambs that received both ZH and CaPr, a quadratic response was evident ($p = 0.0447$; Table 2) for FBW and ADG. Through regression analysis, the peak values were estimated at 26.4 d and 30.5 d, respectively. The ADG:DMI ratio displayed an increase with the inclusion of 28 d (quadratic trend, $p = 0.0963$), while no significant effects were noted for DMI ($p = 0.15$). Notably, the inclusion of CaPr for 28 d led to enhancements ($p < 0.05$) in FBW (9.5 and 8.4%%), ADG (51.4 and 42.3%) and ADG:DMI ratio (76 and 29.4%) compared to the CTL group and the ZH-only group, respectively.

Table 2. Least square means, standard error of the mean (SEM) and orthogonal polynomials of productive performance, according to the duration of calcium propionate (CaPr) supplementation in lambs finished with zilpaterol hydrochloride (ZH).

Item [b]	Control	Duration of CaPr Supplementation + ZH [a]				SEM	Effects (P-value)	
		0	14	28	42		Linear	Quadratic
IBW, kg	40.2	40.1	40.0	40.2	40.0	0.8263	0.9986	0.9628
FBW, kg	49.4[b]	49.9[b]	51.2[ab]	54.1[a]	51.5[ab]	0.8480	0.0576	0.0447
ADG, kg/d	0.220[b]	0.234[b]	0.263[ab]	0.333[a]	0.272[ab]	0.0202	0.0576	0.0447
DMI, kg/d	1.44[a]	1.12[b]	1.28[a]	1.25[ab]	1.24[b]	0.0563	0.3341	0.1498
ADG:DMI ratio	0.150[c]	0.204[b]	0.205[b]	0.264[a]	0.219[b]	0.0129	0.0705	0.0963

[a] Treatments consisted: 1) No additives (Control), 2) ZH supplementation for 28 d (0), with 3 d ZH withdrawal before slaughter, at a dosage of at 7.2 mg/kg diet (ZH), for treatments 3, 4, and 5, lambs received ZH and 10g CaPr/lamb/d throughout the entire phase (42 d), or during the last 28 or 14 d before slaughter. [b] IBW = Initial body weight, FBW = final body weight, ADG = average daily gain, DMI = dry matter intake. [a,b] Means a row with different superscripts differ ($p < 0.05$). FBW $y = -0.0052x^2 + 0.2639x + 49.545$ ($R^2 = 0.7286$), maximal value at 26.4 d of inclusion for 53.0 kg. ADG $y = -0.0001x^2 + 0.0061x + 0.2254$ ($R^2 = 0.7154$), maximal value at 30.5 d of inclusion for 0.318 kg/d.

3.2. Ultrasound Measurements, Carcass Characteristics and Shoulder Composition

ZH supplementation alone did not exert any significant impact on carcass traits, visceral mass, whole cuts, or meat quality. However, when ZH-supplemented lambs received CaPr, alterations were observed in carcass traits. Specifically, D% exhibited a quadratic response ($p = 0.05$; Table 3), with the maximum value estimated at 39.1 d of CaPr inclusion through regression analysis; Additionally, LMA increased with a 14 d inclusion period (quadratic effect, $p = 0.05$). Likewise, as the duration of CaPr inclusion extended (linear effect, $p < 0.05$) there were increments in HCW, CCW, and leg circumference. No significant effects were detected in the remaining variables ($p > 0.05$). Lambs that received both CaPr and ZH displayed an increase ($p > 0.05$) of 11.3% in LMA compared to CTL and 10.4% compared to ZH. Lambs supplemented with CaPr for a duration of 28 d exhibited higher ($p > 0.05$) HCW and CCW compared to CTL and ZH lambs ($p > 0.05$), but the differences were not statistically significant when compared to lambs receiving CaPr for 14 and 42 d. Supplementation of CaPr for any duration led to an increase ($p > 0.05$) of 9.9% in the D% relative to CTL and a 5% increase compared to ZH. No significant differences among the treatments ($p > 0.05$) were observed for the other evaluated variables.

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Table 3. Least square means, standard error of the mean (SEM) and orthogonal polynomials of ultrasound measurements and carcass characteristics, according to the duration of calcium propionate (CaPr) supplementation in lambs finished with zilpaterol hydrochloride (ZH).

Item [b]	Control	Duration of CaPr Supplementation + ZH [a]				SEM	Effects (*P*-value)	
		0	14	28	42		Linear	Quadratic
Ultrasound measurements								
Fat thickness, mm	2.9	3.1	4.0	3.4	3.8	0.32	0.9	0.2
LMA, cm^2	12.4[b]	12.5[b]	13.8[a]	12.6[ab]	12.7[ab]	0.31	0.1	0.05
Carcass characteristics								
HCW, kg	23.8[b]	24.0[b]	25.8 [ab]	26.3[a]	25.4 [ab]	0.43	0.003	0.7
CCW, kg	22.9[b]	23.1[b]	25.0[ab]	25.6[a]	24.6[ab]	0.44	0.002	0.7
Dressing percentage	53.3[b]	55.8[b]	58.0[a]	58.0 [a]	58.6[a]	0.45	0.0001	0.05
Cooling loss, %	3.2	3.5	3.2	2.4	3.1	0.46	0.2	0.5
Carcass length, cm	71.8	68.0	69.2	70.5	67.9	1.73	0.7	0.1
Leg circumference, cm	44.9	46.4	47.9	48.6	48.3	0.79	0.03	0.6
Chest circumference, cm	27.9	25.1	27.0	25.0	25.7	0.9	0.1	0.6
Shoulder composition								
Muscle, %	61.9	59.2	62.4	61.8	61.0	2.06	0.7	0.5
Fat, %	21.1	20.8	18.4	18.4	20.0	1.70	0.2	0.9
Bone, %	17.1	20.1	19.3	19.9	19.0	0.84	0.08	0.1

[a] Treatments consisted: 1) No additives (Control), 2) ZH supplementation for 28 d (0), with 3 d ZH withdrawal before slaughter, at a dosage of at 7.2 mg/kg diet (ZH), for treatments 3, 4, and 5, lambs received ZH and 10g CaPr/lamb/d throughout the entire phase (42 d), or during the last 28 or 14 d before slaughter. [b] LMA= longissimus muscle area, HCW = hot carcass weight, CCW = cold carcass weight. [a,b] Means a row with different superscripts differ ($p < 0.05$). Dressing percentage $y = -0.0032x^2 + 0.2504x + 53.205$ ($R^2 = 0.988$), maximal value at 39.1 d of inclusion for 58.1%.

3.3. Visceral Organ Mass

The supplementation of ZH alone did not have any effect on visceral mass. EBW showed a quadratic response ($p = 0.03$; Table 4), estimating the maximum value at 54.9 d for the inclusion of CaPr according to regression analysis. Overall, the inclusion of CaPr led to an average increase ($p < 0.05$) the EBW 10.7%, with respect to the CTL and 4.8% relative to ZH, without influencing visceral organ mass ($p > 0.05$).

Table 4. Least square means, standard error of the mean (SEM) and orthogonal polynomials of visceral organ mass, according to the duration of calcium propionate (CaPr) supplementation in lambs finished with zilpaterol hydrochloride (ZH).

Item [b]	Control	Duration of CaPr Supplementation + ZH [a]				SEM	Effects (*P*-value)	
		0	14	28	42		Linear	Quadratic
Empty BW, kg	53.2[b]	56.2[b]	58.0[a]	57.7[a]	58.9[a]	0.6	0.01	0.03
Skin	148.56	135.1	151.5	160.1	160.6	13.1	0.4	0.3
Limbs	24.38	26.3	28.7	26.6	28.2	0.93	0.5	0.2
Head	39.1	37.9	41.6	41.1	40.9	1.34	0.1	0.7
Heart	4.9	4.6	5.5	5.3	6.1	0.4	0.4	0.8
Lungs	23.57	27.1	25.1	26.0	23.7	1.55	0.5	0.3

Liver	21.07	20.5	22.0	22.0	21.9	1.22	0.39	0.7
Spleen	2.0	2.0	1.9	2.0	2.2	0.3	0.9	0.8
Kidney	2.7	3.0	3.3	3.1	3.5	0.3	0.1	0.3
Testicles	15.43	14.1	15.6	15.4	14.7	1.1	0.8	0.5
Visceral fat	23.1	27.0	22.9	26.0	25.7	3.87	0.8	0.9
Perirenal fat	14.63	13.3	13.7	12.4	13.7	1.38	0.4	0.9
Stomach [c]	29.93	27.3	29.7	25.3	26.1	1.82	0.2	0.6
Large intestine	10.59	11.4	12.0	9.5	10.2	1.27	0.6	0.1
Small intestine	18.32	17.0	20.5	21.6	19.8	1.63	0.1	0.4

[a] Treatments consisted: 1) No additives (Control), 2) ZH supplementation for 28 d (0), with 3 d ZH withdrawal before slaughter, at a dosage of at 7.2 mg/kg diet (ZH), for treatments 3, 4, and 5, lambs received ZH and 10g CaPr/lamb/d throughout the entire phase (42 d), or during the last 28 or 14 d before slaughter. [b] Non carcass components are expressed in g/kg of empty body weight. [c] Includes the rumen-reticulum, omasum and abomasum. [a,b] Means a row with different superscripts differ ($p < 0.05$). Empty BW $y = -0.0008x^2 + 0.0879x + 56.38$ ($R^2 = 0.8286$), maximal value at 54.9 d of inclusion for 58.8 kg.

3.4. Whole Cuts

The supplementation of ZH alone did not yield any significant effects on whole cuts. With an increase in the duration of CaPr supplementation, the values (expressed as g/kg of EBW or as percent of CCW) of breast IMPS209 (linear effect, $p < 0.05$, Table 5) and shoulder IMPS207 (linear trend, $p = 0.09$) increased. No significant effects were observed for the remaining whole cuts ($p > 0.05$). Specifically, the inclusion of CaPr (duration of 14 to 28 d) increased (p < 0.05) the rack IMPS204 19% compared to ZH and a substantial 38% increase compared to CTL.

Table 5. Least square means, standard error of the mean (SEM) and orthogonal polynomials of whole cuts, according to the duration of calcium propionate (CaPr) supplementation in lambs finished with zilpaterol hydrochloride (ZH).

Item [b]	**Control**	**Duration of CaPr Supplementation + ZH [a]**				**SEM**	**Effects (*P*-value)**	
		0	**14**	**28**	**42**		**Linear**	**Quadratic**
Whole cuts, g/kg of EBW								
Forequarter	6.19	6.09	6.42	6.71	6.06	0.16	0.1	0.3
Hindquarter	5.26	5.47	5.5	5.37	5.36	0.21	0.7	0.3
Shoulder IMPS207	2.08[b]	2.31[ab]	2.24[ab]	2.4[a]	2.2[ab]	0.06	0.09	0.6
Shoulder IMPS206	1.07	1.08	1.16	1.1	0.9	0.09	0.7	0.6
Leg IMPS233	3.04	3.15	3.17	2.99	3.14	0.10	0.8	0.1
Loin IMPS231	1.31	1.25	1.45	1.37	1.26	0.11	0.4	0.8
Rack IMPS204	0.77	0.62	0.76	0.73	0.58	0.03	0.9	0.2
Ribs IMPS209A	0.69	0.66	0.64	0.65	0.64	0.03	0.4	0.4
Flank IMPS232	0.91	1.1	0.9	0.99	0.96	0.04	0.8	0.6
Breast IMPS209	0.87[b]	0.93[b]	1.1[ab]	1.14[a]	1.04[ab]	0.03	0.004	0.4
Neck	0.70	0.47	0.62	0.69	0.68	0.11	0.8	0.1
Whole cuts, as percentage of CCW								

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Forequarter	51.0	50.1	52.8	55.3	49.9	1.82	0.1	0.3
Hindquarter	43.3	45.1	45.1	44.3	44.0	1.5	0.7	0.3
Shoulder IMPS207	8.5	9.5	9.2	9.8	9.1	0.35	0.07	0.6
Shoulder IMPS206	4.4	4.5	4.8	4.5	3.7	0.45	0.7	0.6
Leg IMPS233	12.5	13.0	13.0	12.4	12.9	0.42	0.8	0.1
Loin IMPS231	5.4	5.1	6.0	5.7	5.2	0.43	0.4	0.9
Rack IMPS204	2.18^{b}	2.53^{b}	3.12^{a}	3.01^{a}	2.39^{b}	0.21	0.9	0.1
Ribs IMPS209A	2.9	2.7	2.6	2.7	2.6	0.14	0.3	0.4
Flank IMPS232	3.7	4.4	3.6	4.1	4.0	0.25	0.7	0.6
Breast IMPS209	3.6	3.8	4.1	4.7	4.3	0.22	0.002	0.5
Neck	2.9	1.9	2.5	2.9	2.8	0.48	0.8	0.1

[a] Treatments consisted: 1) No additives (Control), 2) ZH supplementation for 28 d (0), with 3 d ZH withdrawal before slaughter, at a dosage of at 7.2 mg/kg diet (ZH), for treatments 3, 4, and 5, lambs received ZH and 10g CaPr/lamb/d throughout the entire phase (42 d), or during the last 28 or 14 d before slaughter. [b] Whole cuts are expressed in g/kg of empty body weight (EBW) and percentage of cold carcass weight (CCW). [a,b] Means a row with different superscripts differ ($p < 0.05$).

3.5. Meat Characteristics

The supplementation of ZH alone did not exert any discernible effects on meat characteristics. However, compared to CTL and ZH alone, CKL% value was higher ($p < 0.05$) in the treatments with CaPr. Within the duration period of CaPr supplementation, lambs expressed a quadratic response ($p = 0.001$; Table 6) in CKL% with the maximum value estimated at 25.8 d according to the regression analysis. The inclusion duration of CaPr showed a linear effect on PRL% values, led to rising values of PRL% at 24 h (trend, $p = 0.06$) and 48 h ($p = 0.03$). Moreover, PRL$_{48}$% was higher in lambs that received CaPr supplementation during 28 and 42 d ($p < 0.05$). No significant differences were observed between the treatments in relation to the rest of the evaluated meat variables ($p > 0.05$).

Table 6. Least square means, standard error of the mean (SEM) and orthogonal polynomials of meat characteristics and meat color, according to the duration of calcium propionate (CaPr) supplementation in lambs finished with zilpaterol hydrochloride (ZH).

Item [b]	**Control**	**Duration of CaPr Supplementation + ZH** [a]				**SEM**	**Effects (*P*-value)**	
		0	**14**	**28**	**42**		**Linear**	**Quadratic**
Meat characteristics								
pH$_{24h}$	5.26	5.4	5.66	5.7	5.58	0.19	0.08	0.7
Purge loss$_{24h}$, %	0.44	0.47	0.98	0.65	0.71	0.12	0.06	0.1
Purge loss$_{48h}$, %	0.41^{b}	1.49^{ab}	1.42^{ab}	2.14^{a}	1.94^{a}	0.37	0.03	0.6
Cook loss, %	12.4^{b}	12.2^{b}	18.2^{a}	19.1^{a}	16.7^{a}	2.17	0.1	0.001
WHC, %	11.4	13.8	14.5	14.4	14.0	2.17	0.3	0.5
WBSF, kg/cm^2	4.2	4.0	3.9	3.9	4.2	0.1	0.2	0.7
Color								
L*	44.2	43.3	47.2	44.8	42.5	2.18	0.5	0.7

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a*	16.9	16.1	13.5	16.0	16.3	1.23	0.1	0.7
b*	4.4	4.2	3.4	4.0	4.4	0.56	0.2	0.1

[a] Treatments consisted: 1) No additives (Control), 2) ZH supplementation for 28 d (0), with 3 d ZH withdrawal before slaughter, at a dosage of at 7.2 mg/kg diet (ZH), for treatments 3, 4, and 5, lambs received ZH and 10g CaPr/lamb/d throughout the entire phase (42 d), or during the last 28 or 14 d before slaughter. [b] Meat characteristics were measured on the longissimus muscle between the 12th rib and the 2nd lumbar vertebrae. WHC = water-holding capacity, WBSF = Warner-Bratzler shear force. [a,b] Means a row with different superscripts differ ($p < 0.05$). Cook loss percent $y = -0.0107x^2 + 0.5529x + 12.29$ ($R^2 = 0.9942$), maximal value at 25.8 d of inclusion for 19.4%.

4. Discussion

4.1. Productive Performance

The elevation in dietary energy stimulates a constant increase in the productive performance of finishing lambs [23–26]. When CaPr is supplemented alone, it undergoes hydrolysis within the acidic pH of the rumen, resulting in the formation of Ca^{2+} and propionic acid [7]. This process yields several effects at the rumen level: 1) alteration of the VFA pattern [5]; 2) reduction in methane production; 3) improved digestibility of the DM; and 4) enhanced fermentation efficiency [4]; and 5) improved responsiveness of insulin in glucose metabolism [6], which plays a role in both fat deposition and muscle growth. As a cumulative outcome of these mechanisms, there is a promotion of energy status achieved through the heightened synthesis of glucose via gluconeogenesis [7].

As the practice of combining feed additives during fattening is becoming more common, one of the concerns in the industry is the possible antagonism between feed additives. The findings of this study parallel those when CaPr was administered individually. In a similar vein, Carrillo-Muro et al. [10], who determined the duration of CaPr supplementation in Dorper × Katahdin lambs (IBW 39.1 kg) with diets containing 72% grains, where they observed maximum increases ($p < 0.05$) at 25 d for FBW (4.7%) and ADG (26.8%), and 28 d for ADG:DM ratio (25.8%). Interestingly, they also observed a peak in DMI (1.1%) at 15 d, with subsequent reductions in these variables observed at the 42 d mark. However, this study observed that in the presence of ZH, the peak values were apparent within the range of 25 to 30 d and declined at 42 d; nevertheless, the observed increments surpassed 8.4% for FBW, 42.3% for ADG and 29.4% for ADG:DMI ratio. Surprisingly, no discernible differences were noted in DMI ($p > 0.05$). This observation aligns with the findings of Martínez-Aispuro et al. [8], yet it contrasts with the results reported by Carrillo-Muro et al. [9] who reported an increase in DMI ($p < 0.05$). Both studies, however, corroborate that the inclusion of CaPr for 42 d in the finishing phase enhances FBW, ADG, and ADG:DMI ratio compared to not incorporating CaPr.

The utilization of β-AA enhances ADG and ADG:DMI ratio [2,27] stimulating protein synthesis in skeletal muscle and inhibiting its degradation, while in adipose tissue, they amplify lipolysis and curtail lipogenesis [28]. In our study, the improvements in ADG and ADG:DMI ratio achieved by supplementing lambs with CaPr for 28 d in the presence of ZH surpassed those observed in previous reports when ZH was administered individually [2,12,29]. The literature states that ZH supplementation in finishing lamb diets brings about ADG enhancements ranging from 20.1 % to 40.6 % alongside ADG:DM ratio increases ranging from 16.5% to 43.3%. However, our research yielded even greater gains, revealing a 9.5% upsurge in FBW, a remarkable 51.4% escalation in ADG, and an impressive 76% increase ADG:DMI ratio. Likewise, the decrease in DMI reported by other authors upon ZH supplementation (13.3 % to 16.2 %) [12,14,29,30] aligns with the present study’s findings (13.2%, $p = 0.0265$). This drop in DMI, coupled with the heightened ADG:DMI ratio likely signifies ZH’s direct influence on net protein retention, consequently impacting the growth of lean tissue [31]; ultimately leading to a more efficient use of nutrients for ADG, with more significant feed savings (13.2%) compared to CTL.

4.2. Ultrasound Measurements, Carcass Characteristics and Shoulder Composition

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Piola-Junior et al. [26] mentioned that elevating the energy level in the diet of fattening lambs has positive effects on carcass characteristics, revealing a linear connection between ME and HCW ($R^2 = 0.9$), CCW ($R^2 = 0.95$) and D% ($R^2 = 0.91$). Furthermore, Moloney [32] incorporating sodium propionate (0 or 40 g/kg DM) into the diet, pointed out that it alters the growth and composition of lamb carcasses. In addition, they observed a decrease in fat deposition, an increase in skeletal muscle growth, and a change in the acetate:propionate ratio in rumen fluid. This shift was attributed to increased protein accumulation to absorbed propionate, which is used for gluconeogenesis, thereby sparing amino acids to fuel protein synthesis.

A study conducted by Carrillo-Muro et al. [10] aimed to ascertain the duration of CaPr supplementation in lambs and found peak enhancements ($p < 0.05$) at 24 d for HCW and CCW, and 20 d for D%, yet no effects on FT and LMA ($p > 0.05$), and all these variables were reduced at 42 d. Notably, in the present study, by combining CaPr with the same duration with ZH, the optimal values are observed in the range of 14 to 28 d and reduced at 42 d. Additionally, an increase in LMA with CaPr was observed by 14 d combining ZH. This alignment corresponds with the findings of Cifuentes-López et al. [33] regarding the fact that sheep fed with CaPr for 42 d improve ($p < 0.05$) D% and LMA. Additionally, Carrillo-Muro et al. [10] reported an elevation in HCW ($p = 0.09$) and CCW ($p = 0.08$) along with a lower CL ($p < 0.05$). However, no significant effect ($p > 0.05$) on D%. In contrast, Martínez-Aispuro et al. [8] did not find any noticeable impact ($p > 0.05$) on LMA in lambs over a 42 d period. Furthermore, in lambs fed finishing diets with CaPr supplementation for 42 d Lee-Rangel et al. [34] and Mendoza-Martínez et al. [19], reported no discernible differences ($p > 0.05$) in HCW or LMA (CaPr vs. CTL).

The consistent response of increased LMA, HCW, CCW, and D% through ZH supplementation has been well-established in finishing lambs within the feedlot setting [2,11,27]. The increase in LMA is mainly due to the greater ADG [35] and the increase in shoulder muscle [Partida et al., 2015]. While the D% increase of 3.9% ($p < 0.05$) with CaPr was observed by 28 d combining ZH, compared to ZH, caused by CaPr supplementation, meant 2.2 kg more salable meat than ZH. This increase in D% coincides with several previous studies in which ZH was supplemented [3,36]. This outcome can be attributed to muscle hypertrophy caused by increased protein synthesis and reduced protein breakdown [37,38], leading to reduced fat [39], a parameter unaffected by any treatment in our study ($p > 0.05$).

4.3. Visceral Organ Mass

The results of the present study revealed maximum increases at 55 d of 10.7% of EBW compared to CTL and 4.8% with ZH ($p < 0.05$). However, Carrillo-Muro et al. [10] investigating lambs supplemented over different periods, identified peak increases ($p < 0.05$) at 28 d, followed by a decline at 42 d. This suggests that these increases in EBW are attributable to the increase in energy availability offered by the lambs gluconeogenic precursors. Additionally, Carrillo-Muro et al. [9], when feeding CaPr for 42 d to finishing lambs, reported that EBW increased ($p < 0.03$), while with ZH supplementation in lambs, Rivera-Villegas et al. [2] noted that EBW increased by 4.6%.

The impact of CaPr and ZH on the visceral organ mass has garnered limited attention. However, Carrillo-Muro et al. [10] investigating fattening lambs, did not observe effects on organ weights ($p > 0.05$). In contrast, Cifuentes-López et al. [33] studying finishing lambs for 42 d and supplemented with CaPr, did observe reductions in perirenal fat ($p < 0.05$), coinciding that visceral organ mass remains unaffected ($p > 0.05$). Additionally, Carrillo-Muro et al. [9] fed CaPr for 42 d to finishing lambs. They reported that heart weight and small intestine weight increased ($p < 0.03$), with a tendency ($p < 0.07$) to increase liver mass, but without effects ($p \geq 0.43$) on the mass of other organs. Likewise, Macías-Cruz et al. [40] and Avendaño-Reyes et al. [41] did not detect effects of ZH on cattle organ weights. Nevertheless, Rivera-Villegas et al. [2] points out that ZH supplementation had a tendency ($p = 0.08$) of decreasing (8.9%) visceral fat, but with no effect on the rest of the organ mass. It's noteworthy that a decrease in hepatic weights has consistently been observed in feedlot lambs receiving ZH [29,30].

The supplementary energy provided by CaPr supplementation in the lamb diet contributes to the effectiveness of ZH, all while maintaining visceral organ mass; since Rivera-Villegas et al. [2] propose that the reductions in visceral organ mass are to contribute with the energy that is needed by the use of ZH. Also, Elam et al. [42], hypothesized that the increase in HCW and D% could be due to a change in visceral organ mass, or more substrate distributed in the carcass than in visceral organ mass. In this study, the lack of impact on visceral organ mass could be attributed to the extra substrate being supplied by the additional energy from CaPr.

4.4. Whole Cuts

Whole cuts are mainly affected by the nutritional level [43], resulting in varying rates of tissue growth and maturation, with higher levels seen in diets with abundant nutrition [44]. Similarly, Shadnoush et al. [45] indicate out that a high energy level (2.64 Mcal/kg ME) in finishing lambs increases shoulder ($p < 0.05$), while having no significant effect on the other whole cuts ($p > 0.05$). They also note that as FBW increases, the weights of all cuts increase except for the neck. Based on these observations, it can be infered that the changes observed in feedlot lambs are the result of the increase in FBW caused by the increase in productive performance (ADG, DMI and ADG:DMI ratio).

The results of this study reveal that the inclusion of CaPr when lambs are finished with ZH increased the values of breast IMPS209 (linear effect, $p < 0.05$) and shoulder IMPS207 (linear trend, $p = 0.09$) increased, while other whole cuts were unaffected ($p > 0.05$). Contrasting findings were reported by Carrillo-Muro et al. [10] who observed that in lambs supplemented with CaPr alone for varying durations, increased inclusion periods led to greater forequarter and neck weights at 28 d (quadratic effect, $p < 0.04$), and also the inclusion period more prolonged reduced the loin IMPS231 and increased the rack IMPS204 (linear effect, $p < 0.04$). In contrast, Gomes et al. [46] in finishing lambs supplemented with glycerin, did not observe any effect on whole cuts.

Overall, in the present study, the inclusion of CaPr from 14 to 28 d with ZH increased ($p < 0.05$) the rack IMPS204 19%, compared to ZH alone, and 38% in comparison to CTL. The effect of ZH supplementation in increasing whole cuts is very common in feedlot cattle [47], with a more pronounced impact observed in the hindquarters [13]. However, there is no previous information on the effect of CaPr in presence with ZH on whole cuts. Some studies indicate that ZH supplementation alone increases the loin IMPS231 and the leg IMPS233 [2,40]. Conversely, Avendaño-Reyes et al. [27] and Rojo-Rubio et al. [48] mention that ZH supplementation alone had no effect on the majority of whole cuts. The data from various authors exhibit considerable variability. As, such, Hilton et al. [49] suggest that the variation in the effect of ZH on whole cuts could depend on the presence or quantity of type II muscle fibers in each of these cuts, since the β2-adrenergic receptors are primarily located in these fibers.

4.5. Meat Characteristics

In CKL%, the maximum value was estimated at 26 d of inclusion of CaPr being lower for CTL and ZH. As the CaPr inclusion period increased, the PRL_{24}% and PRL_{48}% values rose, particularly with CaPr supplementation in conjunction with ZH. However, Carrillo-Muro et al. [10] in a study of fattening lambs supplemented with varying periods of CaPr alone, found that most of the meat characteristics variables were not affected. However, they noted that as the inclusion period increased, muscle pH also rose. However, Carrillo-Muro et al. [50] point out that ZH supplemented alone, compared to CTL, does not appreciably affect WHC%, PRL%, color and CKL%; but it led to a 36% increase in WBFS. Holmer et al. [15] mentions that with 30 d of ZH, 8% CKL% is increased in steers.

In the present study, the inclusion of CaPr (14 to 28 d) in lambs fed ZH did not exhibit an effect on meat color ($p > 0.05$). However, concerning feedlot cattle, according to our study, the influence of ZH supplementation alone on color has been negligible [42,51]. Contrary to this, Partida et al. [52] indicate that ZH supplementation alone in lambs either reduces or tends to reduce, a* (redness). This phenomenon might arise from the dilution in myoglobin concentration resulting from an increase in sarcomere size due to ZH supplementation [53].

5. Conclusions

The gluconeogenic compound CaPr can be used in finishing diets when lambs receive ZH during the final phase of fattening. At daily dosage of 10 g/lamb for 28 d improves the responses to ZH supplementation, enhancing daily gain and feed efficiency, carcass weight D%, as well as the proportion and weight of some whole cuts without affecting carcass fat. However, it does have a negative impact on CKL% and PRL% after 14 d of CaPr supplementation.

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Data Availability Statement: The information published in this study is available on request from the corresponding author.

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