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Effects of dietary calcium propionate on growth performance and carcass characteristics of finishing lambs

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Abstract. The objective of this study was to evaluate the effects of the addition of two levels of calcium propionate on lamb performance and some carcass characteristics. Twenty-one male Creole lambs with an initial weight of 25.3 ± 3.3 kg were randomly assigned to one of the following treatments: 0, 10, and 20 g of calcium propionate/kg of diet (dry matter basis). Intake, daily gain, feed conversion, carcass weight, and rib eye area were not affected ($P < 0.05$) by calcium propionate addition. Ruminal fermentation was not altered (rumen pH, volatile fatty acids concentration, and fermentation pattern), and ruminal ammonia-N presented a quadratic response ($P < 0.05$). In fat from the longissimus dorsi muscle, oleic acid showed a linear decrease ($P < 0.05$) and α-linolenic presented a linear increment ($P < 0.05$). The addition of 10 or 20 g of calcium propionate in diets containing 350 g/kg grain and 100 g/kg molasses did not modify the productive performance of lambs or ruminal fermentation, and minor changes were detected in long-chain fatty acid in intramuscular fat.

Additional keywords: long-chain fatty acid, sheep.

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Introduction

Grain prices are rising worldwide, thus the use of unconventional energy such as glycerol, propylene glycol, calcium propionate (Ferraro *et al*. 2009), or sodium propionate (Bas *et al*. 2000) may be an alternative to partially replace grains. Glucose precursors such as propylene glycol and calcium propionate have been used in dairy cattle to correct metabolic problems; however, as propylene glycol may be metabolised in toxic sulfur compounds (Trabue *et al*. 2007) only calcium or sodium propionate could be used as an ingredient of rations (Bas *et al*. 2000; Lee-Rangel *et al*. 2012).

Propionate supplementation affects glucose flux (van Houtert *et al*. 1993), fat deposition, and muscle growth in lambs (Moloney 1998). An increase in propionate absorption can be brought about by a direct intraruminal infusion of propionate or after a diet manipulated in favour of a propionic fermentation profile (Savary-Auzeloux *et al*. 2003). A higher availability of glucose could increase the potential for marbling (Smith and Crouse 1984). Lee-Rangel *et al*. (2012) observed that daily gain in finishing lambs was not affected by reducing the grain level (from 650 to 550 g/kg) or by including 10 g/kg of calcium propionate in finishing rations. Bas *et al*. (2000) included 5 g/kg of sodium propionate in rations with 46 g/kg grain and observed a significant increase in the proportion of odd-numbered fatty acids adipose tissue sampled, showing the propionate role as a precursor of those fatty acids. In the experiment conducted by Lee-Rangel *et al*. (2012) carcass yield and fatty acid composition were not measured, whereas in the experiment from Bas *et al*. (2000) lamb performance data was not reported. Therefore, the objective of the present study was to evaluate the effects of the addition of two levels of calcium propionate in finishing rations for lambs on productive performance, ruminal fermentation, and long-chain fatty acid deposition in the carcass.

Materials and methods

The Animal Care and Use Committee of the Veterinary and Animal Science Faculties from the Universidad Autonoma de San Luis Potosí approved all procedures. The experiment was conducted at the Universidad Autonoma de San Luis Potosí Research experimental station at the Facultad de Agronomia y Veterinaria.

Animals and diets

Twenty-one male Creole sheep (initial weight 25.3 ± 3.3 kg) were randomly assigned to one of three experimental diets: 0, 10, and 20 g calcium propionate (Alimentaria Mexicana Bekarem SA de CV México, D.F.) per kg dietary dry matter (DM, Table 1). Diet was offered as a total mixed ration, and was composed by ground corn stover (2 mm), ground corn grain, ground sorghum grain, whole sorghum grain, whole corn grain, ground soybean meal, and cane molasses to give consistency and reduce dustiness to food. The lambs were housed in individual cages equipped with feed and water bowls. Feed was provided at 0800 hours and 1500 hours. Lambs were adapted to their diets for 10 days, and the study lasted 42 days (27 February–9 April 2011). All lambs had free access to feed to ensure 100 g of orts per kg of the amount fed daily.

Feed analyses

Daily samples of feed and orts were collected. DM and total nitrogen (N) in the diets were analysed according to the AOAC (1999) (Table 1). Neutral detergent fibre and acid detergent fibre analyses were carried out according to Van Soest *et al.* (1991) using sodium sulfite and heat-stable amylase to determine neutral detergent fibre.

Growth assay

The study lasted 42 days. Food intake was recorded daily, and the lambs were weighed at the beginning and at the end of the experiment after an adaptation period to estimate average daily gain. Feed conversion was expressed as the ratio of feed intake to average daily gain. Chop area was assessed 1 day before slaughter by ultrasonography (Silva *et al.* 2005). Once the growth performance trial period (42-day period) was concluded, the bodyweight was immediately recorded before slaughter. Lambs were slaughtered by standard commercial procedures. Hot carcass weight was recorded at slaughter, and warm carcasses were refrigerated at 4°C.

Table 1. Experimental diets and chemical composition
CaPr, calcium propionate; DM, dry matter

	Calcium propionate (g/kg DM)		
	0	10	20
Ingredient (%, *as-fed basis*)			
Corn grain	17.5	17.5	17.5
Sorghum grain	17.5	17.5	17.5
Soybean meal	10.0	10.0	10.0
Cane molasses	10.0	10.0	10.0
Corn stover	43.0	42.0	41.0
Mineral and vitamin premix[A]	1.0	1.0	1.0
Urea	1.0	1.0	1.0
CaPr[B]	0	1.0	2.0
Nutrient composition (*DM basis*)			
Crude protein (%)	15.2	14.9	15.1
Neutral detergent fibre (%)	44.1	43.6	44.4
Acid detergent fibre (%)	22.5	22.5	23.3

[A]Ca 240 g, P 30 g, Mg 20 g, Na 80 g, Cl 120 g, K 5 g, S 5 g, lasalocid 2000 mg, Mn 4000 mg, Fe 2000 mg, Zn 5000 mg, Se 30 mg, Co 60 mg, vitamin A 500 000 IU, vitamin D 300 000 IU, and vitamin E 1000 IU.
[B]Propionic acid 780 g and Ca 220 g.

Rumen fermentation

Rumen fluid (50 mL) was extracted with an esophageal tube on the last day of the growth performance at 0700 hours (fasted for 16 h) of the trial, and pH was measured using a pH meter (Benchtop Cole Parmer 05669–20, Vernon Hills, IL, USA). Then, ruminal fluid was acidified with 1 mL of sulfuric acid (300 g/L) and stored in a freezer (−20°C) for further analyses. Volatile fatty acids (VFA) were measured by gas chromatography in samples prepared with metaphosporic acid (Erwin *et al.* 1961). Ammonia concentration in ruminal fluid was analysed by the phenol hypochlorite method (NH_3N; McCullough 1967).

Fatty acid composition of intramuscular fat

Lipids for fatty acid analysis were extracted from 500 mg of muscle and analysed in a sample obtained from the area between the 11th and 12th ribs (2.5 cm^2) using 2 : 1 (vol/vol) chloroform-metanol (Folch *et al.* 1957). A total of 10–20 mg of extracted lipid was derivatised using 1 : 4 (vol/vol) tetramethylguadine and methanol (Shantha *et al.* 1993) after including heptadecanoic acid (17 : 0) as an internal standard. Fatty acid profiles were determined by chromatography on a Supelco-2560, 100 m × 0.25 mm × 0.20-μm column (Sigma Aldrich Canada, Oakville, ON, Canada) installed in a gas chromatograph (Agilent 6890, Agilent United States, Santa Clara, CA, USA) by flame ionisation detection and splitless injection. Fatty acids from the muscle samples were identified by comparison with retention times of known standards (Sigma Aldrich Canada).

Statistical analyses

The results were analysed according to a completely randomised design using each lamb as an experimental unit (Steel *et al.* 1997). Orthogonal polynomial contrasts were used to verify linear or quadratic effects for calcium propionate level on lamb performance, ruminal fermentation and long-chain fatty acid in intramuscular fat. The *P*-value of 0.05 was selected as the significance level.

Results

Dry matter intake, daily gain, and feed conversion were not modified by calcium propionate level in the diet (Table 2). The addition of calcium propionate had no effects on hot carcass weight and chop area (Table 2). There were no differences in rumen pH and VFA among treatments (Table 3). However, ammonia-N showed a quadratic response ($P < 0.05$; Table 3). Oleic acid showed a linear decrease ($P < 0.05$) and α-linolenic acid presented a linear increase ($P < 0.05$) in fat from the longissimus dorsi muscle (Table 4).

Table 2. Performance of lambs fed different levels of calcium propionate
DM, dry matter; s.e.m., standard error of the mean

	Calcium propionate (g/kg)				*P*-value	
	0	10	20	s.e.m.	Linear	Quadratic
Initial weight (kg)	26.4	24.1	25	2.3	–	–
Final weight (kg)	34.8	33.5	34.3	2.4	–	–
DM intake (g/day)	1174	1173	1293	59.52	0.17	0.41
Daily liveweight gain (g/day)	200	223	221	51.01	0.20	0.38
Feed conversion	5.87	5.26	5.85	1.39	0.77	0.18
Hot carcass weight (kg)	16.7	16.8	16.9	0.61	0.82	0.95
Rib eye area (mm^2)	880	877	909	36.18	0.53	0.68

Table 3. Characteristics of rumen fluid samples taken on Day 42 from lambs fed different levels of calcium propionate
s.e.m., standard error of the mean; VFA, volatile fatty acid

	Calcium propionate (g/kg)				*P*-value	
	0	10	20	s.e.m.	Linear	Quadratic
pH	6.8	6.64	6.57	0.17	0.38	0.82
Total VFA (mmol/L)	36.55	39.73	40.78	10.6	0.76	0.93
Acetate (mol/100 mol of total VFA)	68.59	67.22	69.64	7.41	0.68	0.97
Propionate (mol/100 mol of total VFA)	19.19	19.88	18.12	1.92	0.99	0.82
Butyrate (mol/100 mol of total VFA)	12.16	12.86	12.21	1.41	0.92	0.90
Ammonia-N (mg/dL)	3.98	4.65	4.60	0.72	0.52	0.05

Table 4. Fatty acid composition of muscle lipids from lambs fed two levels of calcium propionate
s.e.m., standard error of the mean

	Calcium propionate (g/kg)				*P*-value	
	0	10	20	s.e.m.	Linear	Quadratic
	Muscle fatty acid profile (g/100 g fatty acid)					
C14 : 0	2.4	2.9	2.5	0.35	0.60	0.26
C16 : 0	24.3	23.9	26.0	0.67	0.09	0.73
C16 : 1	3.0	2.7	2.7	0.33	0.21	0.98
C18 : 0	19.2	20.0	24.0	1.94	0.43	0.95
C18 : 1	47.3	45.9	39.6	1.37	0.05	0.17
C18 : 2n-6	3.1	3.7	4.1	0.25	0.13	0.34
C18 : 3n-3	0.7	0.9	1.1	0.19	0.05	0.84

Discussion

Bas *et al*. (2000) estimated that metabolised energy (ME) in the ration increased only by 2.5% with 50 g/kg of sodium propionate, but our results indicate that the energetic value of the rations increased by 10% in both levels of inclusion. From the results of Sheperd and Combs (1998), Oba and Allen (2003) estimated an ME of 4.956 Mcal/kg for propionic acid. Considering that the efficiency of utilisation of calcium propionate may be similar to the propionic acid and a gross energy of 3.965 Mcal/kg, the ME estimated is 3.766 Mcal/kg, and it would be expected a greater daily gain. Yet gain was not statistically different and perhaps is an overestimated value. Other estimations can be deduced from the experiment of Berthelot *et al*. (2001) by algebraic substitution, but the value is very low (1.2 Mcal/kg). Results from Sheperd and Combs (1998) showed an increment in milk production of 5% when the energy input increased 10% with ruminal infusions of propionic acid, which can be explained by the better efficiency of energy utilisation for lactation than for tissue deposition.

The molar concentration estimated in this study was 65.13 and 130.27 mmol for the two levels of calcium propionate, and no hypophagic effect of propionate was observed. Similar results were reported by Lee-Rangel *et al*. (2012), who fed an equivalent to 64.3 mmol/day of propionate in finishing lambs. In contrast, Bradford and Allen (2007) found a decrease in DM intake by ruminal infusion of 19 mol/day of sodium propionate in the portal vein in lactating cows. Leuvenink *et al*. (1997) showed that propionate infusion at 2 mmol/min decreased feed intake, but not at 1 mmol/min. There is a study in dairy cattle where calcium propionate was included in the diet in doses equivalent to 0.96, 1.24, 1.60, and 1.85 mol/day and no changes were observed in feed intake (DeFrain *et al*. 2005); however, McNamara and Valdez (2005) reported a reduction in feed intake with doses of 12.61, 16.57, and 17.18 mol/day. Comparing the molar

concentration of those studies allows us to hypothesise that the threshold to cause hypophagic effect of propionate effect may be around 12 moles per day.

As VFA pattern was not affected, gain was similar among treatments. It has been shown that if there are changes in propionate concentrations weight gain can be improved in steers (Whitney *et al*. 2000). The response could be modified if forage concentrate ratio alters the molar concentration of propionate (Liu *et al*. 2010).

Lee-Rangel *et al*. (2012) reported that sheep supplemented with calcium propionate had no differences in carcass yield. Fluharty *et al*. (1999) found that lambs fed diets with high amounts of energy showed higher carcass yields than animals fed *Medicago sativa*. The differences in energy consumption in the present study were not sufficient to affect this variable, which could be attributed to similar final bodyweight among treatments.

Loerch *et al*. (1983) observed that ruminal pH decreased as grains increased in the diet. In this study, ruminal pH was similar among treatments, even though a change was expected with the increase in VFA concentrations (Mendoza *et al*. 1993). Fermentation parameters are similar to those reported with diets high in forage for steers (Viswanathan *et al*. 2007). The molar proportions of propionate are lower than those reported by Lee-Rangel *et al*. (2012), which can be attributed to the amount of forage in diets.

It is not clear why ruminal ammonia-N increased quadratically with calcium propionate. Nozière *et al*. (2003) observed a reduction in ammonia-N after 7 days of propionate infusion in ruminally cannulated sheep. The effects of long-term exposure to propionate may be different because the rumen ciliates can incorporate propionate (Emmanuel 1974). The presence of rumen protozoa increases ruminal ammonia concentrations (Belanche *et al*. 2011); however, ciliates were not counted in this experiment.

Regarding the changes in oleic and α-linoleic acids observed, He *et al*. (2012) found no differences in the amount of C18 : 1, although they reported differences in conjugated linoleic acid and α-linolenic acid. Some studies indicate that some rumen bacteria in pure cultures (Emmanuel 1978) and rumen protozoa (Emmanuel 1974) can incorporate propionate into odd-numbered long-chain fatty acids. Changes in the ratio of acetate to propionate may affect *de novo* fatty acid synthesis, the accumulation of palmitic acid, its elongation, and dehydrogenation products, which are the saturated and monounsaturated C16–18 fatty acids. A low concentrate to forage ratio increases the amount of α-linoleic acid in meat (French *et al*. 2000).

It is concluded that the addition of 10 or 20 g of calcium propionate in finishing diets did not modify the productive performance of lambs or ruminal fermentation, and minor changes were detected in long-chain fatty acid in intramuscular fat.

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