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Research article

Effect of blocks with calcium propionate on lamb productive performance and *in vitro* GHG

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ABSTRACT

Objective. Evaluate multinutritional blocks with and without calcium propionate (Ca-Pr) on digestibility and live weight changes of lambs and in the green house emission *in vitro*. **Materials and methods**. Twelve lambs were used (20.17±2.35 Katahdin x criollo) in three treatments: Basal diet (BD 70% oat straw, 30% concentrate), BD+ Block without Ca-Pr and BD+ Block with 1.5% Ca-Pr in 50 days assay. *In vitro* gas production (GP) and kinetic parameters were estimated (Vmax, S, Lag). *In vitro* and *in vivo,* CH_4, CO_2 and digestibility were estimated. **Results.** The dry matter intake was the lowest ($p<0.0001$) in lambs without block (753 g/d) compared lambs supplemented with block without (839 g) or with Ca-Pr (828 g); including blocks increased methane (16.16 and 16.18 g/d; 0 and 1.5% Ca-Pr respectively) than BD (13.93 g/d). The GP *in vitro* was higher ($p=0.0001$) with BD (380.76, ml) than with blocks without differences among blocks (335.76 and 341.13 ml, 0 and 1.5% Ca-Pr respectively), and the BD had higher ($p=0.0001$) production of CH_4 (47.16 mol) and CO_2 (200.04 mol) than with blocks (42.25 and 41.58 mol CH_4; 179.21 and 176.39 mol CO_2; 0 and 1.5% Ca-Pr respectively). **Conclusions.** Block supplementation improved dry matter intake. Blocks reduced *in vitro* gas production and increased digestibility by reducing CH_4 and CO_2.

Keywords: Additive, greenhouse gas, ruminant, supplement (*Source: CAB*).

RESUMEN

Objetivo. Evaluar bloques multinutricionales con y sin propionato de calcio (Pr-Ca) en la digestibilidad y cambios de peso de corderos y en la emisión de gases *in vitro*. **Materiales y métodos.** Se utilizaron doce borregos (20.17±2.35 Katahdin x criollo) en tres tratamientos: Dieta basal (DB 70% paja de avena; 30% concentrado), DB+ Bloque sin Pr-Ca y DB + Bloque con 1.5% de Pr-Ca, por 50 días. Se midió producción de gas (PG) *in vitro* y parámetros de cinética (Vmax, S, Lag). Se estimó la digestibilidad*,* CH_4 y CO_2 *in vitro* e *in vivo*. **Resultados.** El consumo de materia seca fue menor ($p<0.0001$) en borregos sin bloque (753 g/d) en comparación con bloque sin (839 g) o con Pr-Ca (828 g) al incluir bloques aumentó metano (16.16 y 16.18 g/d; 0 y 1.5% Pr-Ca respectivamente) que con DB (13.93 g/d). La PG *in vitro* fue mayor ($p=0.0001$) con la DB (380.76 ml) sin diferencias entre bloques (335.76 y 341.13 ml, 0 y 1.5% Pr-Ca respectivamente), y la DB tuvo mayor ($p=0.0001$) producción de CH_4 (47.16 mol) y CO_2 (200.04 mol) que con bloques (42.25 y 41.58 mol CH_4; 179.21 y 176.39 moles CO_2; 0 y 1.5% Ca-Pr respectivamente). **Conclusiones.** La suplementación con bloques mejora el consumo de materia seca. Los bloques disminuyeron la producción de gas *in vitro* e incrementaron la digestibilidad reduciendo CH_4 y CO_2.

Palabras clave: Aditivo, gas efecto invernadero, rumiante, suplemento (*Fuente:CAB*).

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INTRODUCTION

Studies have been conducted to evaluate the contribution of ruminants to greenhouse gas (GHG) emissions and alternatives to mitigate this problem (1), which represent up to 12% of the energy consumed (2). To reduce these losses and make ruminant production more efficient, the use of additives has been evaluated (3,4), were sometimes have not improved yield (5) or fermentation (6) and the most efficient ones, such as Ionophores have been banned because are antibiotics. Calcium propionate (Ca-Pr) as an unconventional food ingredient has been used in lambs to reduce the use of grains, increasing ruminal propionate (7). Its potential to reduce methane is explained by the fact that during its dissociation captures a hydrogen ion, reducing its availability to form methane (8). The GHG emissions can be reduced if digestibility is improved, which is achieved by meeting nutritional requirements, particularly in ruminants fed diets low quality forages (9).

One option to an option to complement the deficiencies is to supplement with multinutritional blocks (MB) that have been evaluated worldwide (10), however, animal response has not been constant because for many years FAO promoted a block formula for all the conditions (11) when the nutrient requirements are different for each physiological stage with a diversity of basal diets. Therefore, the objective of this experiment was to evaluate multinutritional blocks formulated to improve lambs growth fed a basal diet with low nutritional value, with or without Ca-Pr, evaluating the impact on lamb growth, digestibility and in GHG emissions *in vivo* and *in vitro*.

MATERIALS AND METHODS

Location. This work was carried out in the facilities of the UAEM University Center in the Zootechnical Post of the Autonomous University of the State of Mexico, Amecameca, State of Mexico located in the south eastern zone of the State of Mexico.

Weather conditions. Subhumid temperate climate with an annual average temperature of 14.7°C.

Animals. Twelve Katahdin x creole lambs (20.17±2.35 initial weight) housed in individual cages with access to feed and clean water ad libitum were used. This work was carried out under the guidelines of the Academic Committee of the Department of Animal Science, in accordance with the regulations established by the Animal Protection Law of the State of Mexico, Mexico.

Treatments. The experiment lasted 50 days and lambs were distributed in a Completely Randomized Design (n=4 lambs) in three treatments: basal diet without supplement (BD: 70% corn stover, 30% concentrate, Table 1), BD with access a multinutritional block with or without 1.5% of Ca-Pr (Alimentaria Mexicana Bekarem, Mexico City) (Table 2).

Basal diet and blocks were offered *ad libitum*. The basal diet was designed to simulate those offered in family-type production units (11% protein, 2.7 Mcal/kg DM ME). The multinutritional blocks (MB) were formulated so that when were supplemented fulfilled the nutritional requirements for lambs according to the NRC (12) for a weight gain of 150 g/d estimating an intake of 100 g/d block. The basal diet and blocks were analyzed to determine: dry matter (DM), organic matter (OM), crude protein (CP) (13), neutral detergent fiber (NDF) and acid detergent fiber (ADF) (14) (Table 3). The lambs were weighed with a 12-hour fast. On day 24, fecal samples were collected for four consecutive days to determine total tract DM digestibility using insoluble acid ash as an internal marker (15).

Table 1. Composition of the basal diet (dry matter) concentrated forage (70:30).

Ingredients	Inclusion, %
Corn stover	70.00
Corn grain	9.22
Soybean meal	11.00
Sorghum grain	8.80
Salt	1.00
Total	100.00

Table 2. Formulation of multi-nutritional blocks.

	Calcium Propionate 0%	Calcium propionate 1.5%
Molasses	31.81	31.91
Urea	9.09	9.11
Calcium propionate	0	1.50
Soybean meal	4.54	4.55
Canola meal	2.27	2.27
Fish meal	2.27	2.27
Salt	4.54	4.55
Mineral premix[a]	4.54	4.55
Calcium oxide	4.54	4.55
Cement	4.54	4.55
Mineral premix organic[b]	0.90	0.91
Wheat bran	9.09	9.11
Soybean hulls	4.54	4.55
Corn grain	8.18	6.38
Wheat grain	9.09	9.11

[a] Vitasal Engorda Ovinos Plus contained: Ca 270 g, P 30 g, Mg 7.5 g, Na 65.6 g, Cl 100 g, K 0.5 g, S 42 mg, Fe 978 mg, Zn 3000 mg, Se 20 mg, Co 15 mg, vitamin A 35000 IU, vitamin D 1500000 IU and vitamin E 150 IU.
[b] Ovy ways 3 contained: Selenium 590 mg, Chromium 990 mg, Copper 1500 mg, Iron 3000 mg, Zinc 3000 mg, Manganese 3000 mg, Living yeast cells.

Table 3. Chemical composition of the basal diet and multi nutritional blocks.

	Basal ration	Multinutritional Blocks Ca-Pr 0%	Multinutritional Blocks Ca-Pr 1.5%
DM, %	89.28	89.32	89.26
Ashes, %	4.47	7.63	7.73
OM, %	95.53	92.36	92.25
CP, %	11.05	11.53	11.76
NDF, %	40.28	36.71	36.50
ADF, %	14.85	13.77	13.57
EE, %	3.13	2.72	2.72

Ca-Pr:Block calcium propionate; DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; EE: ether extract.

CH_4 and CO_2 estimation *in vivo*. The IPCC equations were used (16) to estimate the ruminal CH_4 using the annual emission factor (EF) per lamb, where the Ym or fraction for gross energy of the feed transformed to CH_4 was calculated using the digestibility of each treatment with lamb's equations (17). Carbon dioxide emissions were estimated from the intake of digestible carbohydrates (18), which were used to estimate the moles of hexose fermented in the rumen using the molecular weight of anhydrous glucose (19). The fermentation pattern was from the forage: concentrated ratio and the produced moles of CO_2 were calculated from the stoichiometric equations of Wolin (20).

Kinetics of *in vitro* gas. The gas production derived from ruminal fermentation was determined by the *in vitro* gas technique (21). The BD was used as substrate incubated with each MB in a proportion of 5% of the basal diet. Prior to incubation, the substrates were dried at 55 °C for 48 h in an oven and milled (<2 mm). In 500 ml amber flasks, 500 mg of each treatment were placed. Flasks were then incubated in anaerobic conditions with 90 ml of a diluted inoculum (1:10) of rumen bacteria obtained from two fasted lambs. The flasks were hermetically sealed and incubated at 39°C for 72 h in a water bath. The volume of gas produced was recorded at 2, 4, 6, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60 and 72 h and the pressure values transformed to gas volume with the equation of linear regression, used to estimate the parameters of the gas production kinetics: maximum gas volume (Vm; mL g^{-1} DM of the substrate), gas production rate (S; h-1) and the lag time of the fermentation (L; h), with the model: Vo=Vm/(1+ e (2-4 * s * (t-L))) (21). At the end of the fermentation the residual dry matter (DM) was obtained to calculate *in vitro* dry matter digestibility (DIVDM) at 72 h of incubation; each treatment was incubated in triplicate.

CH_4 and CO_2 estimation *in vitro.* Those gases were estimated from the maximum gas volume; short chain fatty acids were calculated with the Getachew equation (22) and the proportion of CH_4 and carbon dioxide with the stoichiometric factors 0.538 mmol for CO_2 and 0.348 mmol for CH_4 that have been described in other *in vitro* studies (23).

Experimental design. The data from each experiment were analyzed according to a Completely Randomized Design with a generalized linear model using each lamb as an experimental unit in the *in vivo* experiment or the test parameters obtained from the *in vitro* incubations, considering the treatments as fixed effects and random errors associated with each observation. The means of the treatments were compared using the Tukey test (p=0.05). For the *in vivo* experiment the initial weight was analyzed as a covariate using the JMP software (24). *In vitro* gas kinetics parameters were estimated for Vo=Vm/(1+ e (2-4 * s * (t-L))) using non-linear models of the JMP. A simple correlation between GHG results *in vivo* and *in vitro* was estimated.

RESULTS

The multinutritional blocks supplementation increased dry matter intake (p<0.01) by 10%, however, no differences were found in other variables (Table 4). Block supplementation did not reduce daily methane and CO_2 emissions due to increased feed intake. The lambs that consumed blocks with Ca-Pr did not reduce intake nor had an effect on the estimated GHG emissions.

Table 4. Lamb performance and emissions of methane and carbon dioxide from lambs supplemented with blocks with or without calcium propionate.

		Multinutritional Blocks			
	Control	Ca-Pr 0%	Ca-Pr 1.5%	SEM	p-value
Initial BW, kg	21.23	19.70	19.60	0.40	0.59
Final BW, kg	23.98	23.25	23.52	0.74	0.94
DM Intake, g	753^c	839^b	828^a	0.01	0.0001
Intake block, g	0.0^b	82^a	86^a	6.48	0.0001
ADG, g	0.098	0.126	0.140	0.34	0.36
FC	9.94	6.98	6.39	0.37	0.51
DM Digestibility,%	76.87	80.04	81.22	0.28	0.32
CH_4, g/d	13.93^b	16.16^a	16.18^a	0.003	0.004
CO_2, g/d	55.29^b	64.15^a	64.24^a	0.003	0.004

Ca-Pr: block calcium propionate; SEM: standard error of the mean; DM intake: dry matter intake; ADG: daily weight gain; FC: feed conversion, DM: dry matter; CH_4: methane; CO_2: carbon dioxide.
[ab] Means with different superscripts are different (p<0.05).

Table 5 shows the *in vitro* gas parameters. The basal diet resulted with a high volume of gas (p<0.001) and as a consequence, more moles of CH_4 and CO_2 were produced (p<0.0001). There were no differences between the blocks due to the inclusion of Ca-Pr. *In vitro* digestibility was not affected by block supplementation. *In vitro* digestibility values were correlated with those observed *in vivo* (r=0.9964, p=0.054), and *in vivo* digestibility was associated with daily weight gain (r=0.997, p=0.0434). The CH_4 and CO_2 emissions were positively correlated with the dry matter consumption (r=0.9921, p=0.07, r=0.9920, p=0.08). CH_4 and CO_2 *in vivo* and *in vitro* showed a high negative correlation (r=-0.99, p=0.07, r=-0.99, p=0.07).

Table 5. Parameters of *in vitro* gas production, methane and carbon dioxide, of basal diet incubated plus blocks with or without calcium propionate

		Multinutritional Blocks			
	Control	Ca-Pr 0%	Ca-Pr 1.5%	SEM	p-value
Vmax, ml	380.76^a	335.76^b	341.13^b	0.567	0.0044
S, h^{-1}	0.030^b	0.033^a	0.032^a	0.108	0.0005
Lag, h	2.87	3.10	2.79	0.059	0.1283
DIVDM, %	60.96	63.89	64.58	0.622	0.0734
CH_4, mol	47.16^a	41.58^b	42.25^b	0.568	0.0044
CO_2, mol	200.04^a	176.39^b	179.21^b	0.567	0.0044

Ca-Pr: block calcium propionate; SEM, standard error of the mean; Vmax: maximum volume, S: gas production rate, Lag: delay time, DIVDM: in vitro digestibility of dry matter.
[ab] Means with different superscripts are different (p<0.05).

DISCUSSION

As observed in this experiment, it has been reported that MB stimulate intake (25) but there are studies where blocks had no effect (26). The composition of the blocks can modify intake (10); there are interactions between nutrient in the block and in the basal diet. In those studies, where intake was improved, generally a greater daily gains or final weight have been observed and in some cases, this is associated with greater digestibility (9) and nutrient consumption. In this study, the gain was improved by 35% but the low number of repetitions and the variation did not allow to detect differences. The type of block can have different effects on intake and digestion, by modifying the energy source, it was affected the block consumption without affecting digestibility (25).

The incorporation of Ca-Pr in the MB did not improve lamb performance or carbon dioxide emissions. If the values of CH_4 and CO_2 *in vivo* were expressed per kg of DM consumed, similar values would result (BD 1.84, 1.95 and 1.92 per MB with 0 or 1.5% Ca-Pr), indicating that the consumption of blocks and their additives were insufficient to modify the ruminal fermentation. In other evaluations, Ca-Pr did not affect the intake or lambs performance, 1% MS (7) and above 5.5% DM (27) the amount consumed in the MB was below of those studies. *In vitro* gas results indicate that MBs would reduce CH_4, but *in vivo* values contradict this as they increase intake. Caution should be exercised in extrapolating the results of *in vitro* gas studies where no *in vivo* data are presented (28). With respect to other parameters of *in vitro* gas, it has been reported that 1% Ca-Pr increases the Lag phase but does not affect the fermentation pattern or CH_4 losses (29).

In another *in vitro* study with 10% of Ca-Pr, the volume of gas increased, which was attributed to the effects on pH and osmotic pressure (8). In an *in vivo* evaluation with bulls receiving 20 g/d of Ca-Pr, did not affect fermentation or the microbial population (30), but the dose was very low.

The use of MB could reduce the lamb's time to reach the final weight by 44 or 60 days by supplementing without or with Ca-Pr respectively, reducing daily emissions, and could be an alternative to reduce global GHG emissions. Most studies focus on daily data, but it is important to consider the effects and their impact on global warming in terms of time (21).

In conclusion, supplementation with multinutritional blocks in low quality diets improved intake. *In vitro* the blocks reduced gas production and increased digestibility, so they could potentially reduce methane and carbon dioxide emissions.

Conflict of interests.

The authors declare no conflict of interests.

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