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Effect of the dietary level of cull pinto beans (*Phaseolus vulgaris*) on ruminal fermentation, kinetics, and digestibility of hair lambs

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ABSTRACT - The objective was to evaluate the effect of three levels of cull pinto beans (CPB; *Phaseolus vulgaris*) on ruminal fermentation, kinetics, and nutrient digestibility in hair lambs. Six cannulated lambs averaging 56.6±3.8 kg were used and were randomly assigned to one of three treatments. Treatments were: 0.0 kg kg^{-1} of CPB in the supplement (control); 0.25 kg kg^{-1} of CPB in the supplement (CB25); and 0.40 kg kg^{-1} of CPB in the supplement (CB40). Dry matter intake, ruminal pH, NH_3, and volatile fatty acid (VFA) concentration, methane production, Kp (passage rate), MRT (mean retention time), and digestibility of dry matter, crude protein, and neutral detergent fiber were evaluated. Data were analyzed in a Latin square design, repeated in line, by MIXED procedure of SAS. Estimates used for Kp and MRT were obtained by a non-linear regression model (PROC NLIN). Dry matter intake was reduced by supplementation of CPB. No differences were found in ruminal pH or ruminal NH_3. During the trial, differences were found for ruminal VFA concentration (mM), which were greater for the CB25 group. The propionate:acetate ratio was greater for the CB40 treatment. Methane production (mM/m) differed among treatments, but it was the greatest for the CB40 group. Passage rate (kg kg^{-1}/h) and MRT (h) were similar among treatments and the digestibility (kg kg^{-1}) of dry matter, crude protein, and neutral detergent fiber was not different among treatments. The inclusion of 0.25 kg kg^{-1} of CPB in the diet of hair lambs allows for appropriate nutrient digestion without affecting Kp and MRT and increases the molar proportion of the ability of VFA to maintain acetate:propionate ratio without increasing methane production.

Key Words: ammonia, legumes, methane, ruminal pH, sheep, volatile fatty acids

Introduction

In the last decade, the price of cereal grains and proteic ingredients has increased due to ethanol production. Substitutes for these types of feeds have become a priority for producers (Ramos et al., 2009) and agricultural byproducts represent an alternative in animal feeding. Cull pinto beans (CPB; *Phaseolus vulgaris*) are an ingredient used for ovine feeding in Mexico when they do not meet the quality standards necessary for human consumption. Cull pinto beans represent a good source of protein, vitamins, minerals, and complex carbohydrates. Their effect on animal performance has also been well studied. Villalobos et al. (2010a) reported that, as dietary intake of CPB increased, average daily gain (ADG) decreased. In another study, Castillo et al. (2011) reported a reduction in dry matter intake (DMI) when CPB was present as a supplement for pregnant-lactating hair ewes. However, its effect on ruminal fermentation and kinetics has not previously been studied. Because of its nutritive characteristics, high oligosaccharide content (0.004 kg kg^{-1} of raffinose, 0.0323 kg kg^{-1} of stachyose, and 0.0012 kg kg^{-1} of verbascose of DM (Serrano and Goñi, 2004)), and the presence of anti-nutritional factors (lectins, protease inhibitor factor, tannins, saponins, etc.) (Mejía et al., 2003), it was hypothesized that its inclusion in the diet of lambs will affect the ruminal fermentation and kinetics of hair lambs.

The objective of this study was to evaluate the effect of three levels of CPB on feed intake, ruminal fermentation, nutrient digestibility, and ruminal kinetics in hair lambs. The present investigation contributes to the knowledge and development of effective feeding strategies for sheep producers with CPB and will help advance the understanding of how this ingredient affects fermentation patterns and ruminal kinetics in ovine.

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Material and Methods

All procedures involving animals followed the local official techniques for animal care and were approved by the relative authorities (NOM-051-ZOO-1995: Humanitarian care of animals during mobilization of animals; NOM-024-ZOO-1995: Animal health stipulations and characteristics during transportation of animals). This study was conducted in Chihuahua City, Chihuahua, Mexico (28º35'09"N and 106º06'27"W).

Six crossbred (Dorper × Pelibuey and Charolais × Pelibuey), rumen-fistulated wethers, averaging 56.6±3.8 kg, were used. At the start of the experiment, all wethers were identified, vaccinated with a three-way clostridial vaccine (Bacterina triple bovina, Bio- ZOO S. A. de C. V., Zapopan, Jalisco, Mexico), treated for external and internal parasites with ivermectin (Iverfull, Aranda Salud Animal, Querétaro, Querétaro, México), and given ADE vitamins. During the experiment, wethers were separated in individual cages and fed *ad libitum* (08.00 and 18.00 h). Diets were mixed once and consisted of 0.50 kg kg^{-1} alfalfa hay and 0.50 kg kg^{-1} concentrate based on ground sorghum grain and were formulated to contain 0.218 kg kg^{-1} crude protein (CP) and 2.8 Mcal of metabolizable energy (ME)/kg of dry matter (DM) (Table 1). Animals were allowed free access to water. Wethers were randomly assigned to one of the three treatments. Within second treatment, animals were randomly assigned to one of six cages. Treatments consisted of (DM basis): no CPB (control); 0.25 kg kg^{-1} CPB (CB25); and 0.40 kg kg^{-1} CPB (CB40) in the supplement, with CPB replacing ground sorghum grain, cottonseed meal, and corn dried distillers' grains. During each period, animals received nine days to adapt to their diets.

Table 1 - Ingredients and chemical composition (dry matter basis) of concentrate diets for hair lambs fed cull pinto beans

Item	Treaatment[1]		
	Control	CB25	CB40
Ingredient (kg kg^{-1})			
Cull pinto beans	0.0	0.266	0.436
Ground sorghum	0.347	0.176	0.283
Corn dried distillers' grains	0.472	0.443	0.062
Cottonseed meal	0.157	0.091	0.195
Salt	0.012	0.012	0.012
Microfos 12:10[2]	0.012	0.012	0.012
Calculated chemical composition (kg kg^{-1} dry matter basis)			
Crude protein	0.218	0.218	0.218
Metabolizable energy (Mcal/kg)	2.8	2.8	2.8
Ca	0.0025	0.0023	0.0022
P	0.0065	0.0051	0.0051

[1] Control - 0.0 kg kg^{-1} of cull pinto beans of the supplement; CB25 - 0.25 kg kg^{-1} of cull pinto beans of the supplement; CB40 - 0.40 kg kg^{-1} of cull pinto beans of the supplement.

[2] Microfos 12:10: P, 0.12 kg kg^{-1}; Ca, 0.115 kg kg^{-1}; Mg, 0.006 kg kg^{-1}; Mn, 2160 mg kg^{-1}; Zn, 2850 mg kg^{-1}; Fe, 580 mg kg^{-1}; Cu, 1100 mg kg^{-1}; I, 102 mg kg^{-1}; Co, 13 mg kg^{-1}; Se, 9 mg kg^{-1}; vitamin A, 22,000 IU kg^{-1}; vitamin E, 24,500 IU kg^{-1}.

Dry matter intake was evaluated daily during the sampling period (seven days). During the first day of the sampling period (day 10 of each period), a ruminal fluid sample (200 mL) was obtained (0, 1, 2, 4, 8, 12, 18, 24 h after morning feeding). In the ruminal fluid sample, ruminal pH was measured immediately (UltraBASIC pH/mV Meter; Denver Instrument) and four subsamples (15 mL) of strained fluid were taken and acidified with 0.2 mL of sulfuric acid (50 kg kg^{-1}), then frozen until laboratory analysis. Samples were later thawed in the refrigerator (12 h at 4 °C), then centrifuged at 13800 × g for 20 min, and supernatant fraction was analyzed for NH_3N (Broderick and Kang, 1980). Volatile fatty acid (VFA; acetate, propionate, and butyrate) samples were prepared by centrifuging the sample at 10,000 × g for 10 min at 4 °C. Supernatant fraction was filtered twice and three subsamples were prepared with meta-phosphoric acid (5 mL of sample and 1 mL of meta-phosphoric acid), according to Galyean (1980). Volatile fatty acids were analyzed with a Varian capillary column CP-wax 58 (FFAP) (15 m × 0.53 mm, 0.5 μm) by gas chromatography. Methane production was estimated according to the method proposed by Wolin (1960).

To analyze ruminal kinetics, 1 g of chromic oxide (0.99 kg kg^{-1} of purity) was administered to each lamb in the second day (08.00 h) of the sampling period to evaluate passage rate (Kp) and mean retention time (MRT). Fecal samples (30 g) were obtained during six days (at 0, 8, 12, 16, 20, 24, 28, 32, 36, 42, 48, 54, 60, 72, 84, 96, 108, 120, 132, 144 h) and frozen until laboratory analysis. Samples were dried in a forced-air oven (60 °C) for five days, then were ground in a Wiley mill through a 1-mm screen (Wiley mill model 4, Thomas Scientific, Swedesboro, NJ), and then incinerated (8 h at 600 °C). After that, samples were processed according to the method described by Williams et al. (1962) and Cr concentration was obtained by atomic absorption spectrophotometry (Atomic Absorption Spectrophotometer, Analyst 200, Perkin Elmer Instruments; Lumina Lamp, Perkin Elmer).

For digestibility of dry matter, crude protein, and neutral detergent fiber (NDF), fecal samples were taken directly from the rectum of the animals four times daily as follows: day 1, 08.00, 10.00, 12.00, and 14.00 h; day 2, 16.00, 18.00, 20.00, and 22.00 h; and day 3, 00.00, 02.00, 04.00, and 06.00; in the first three days of the sampling period (days 10, 11, and 12 of each period). Individual fecal samples consisted of approximately 50 g (wet basis). Samples for each animal were composited for analysis and stored at –20ºC. Composited fecal samples were dried in a forced-air oven (60 ºC) for five days. Feed, orts, and fecal samples were ground in a Wiley mill through a 1-mm screen and analyzed for CP (method 976.05; AOAC, 2003). Acid detergent fiber (ADF) and NDF were determined sequentially according to Van Soest et al. (1991) using an Ankom 200 fiber analyzer (Ankom Technology, Fairport, NY). Feed and fecal samples were incubated (DAISY[II] system; Ankom Technology Corp. Fairport, NY) during five days (Mabjeesh et al., 2000). After incubation, bags were washed four times with cold water for 5 min and then dried (60 ºC) for 24 h. Concentration of ADF was determined in the bag residue to calculate the percentage of indigestible ADF (Penning and Johnson, 1983).

Apparent dry matter digestibility was predicted using insoluble ADF according to the following formula (Schneider and Flatt, 1975):

$$DMD\ kg\ kg^{-1} = (100 - (100 \times (kg\ kg^{-1}\ IADF\ in\ feed/kg\ kg^{-1}\ IADF\ in\ feces)))$$

Apparent digestibility of CP and NDF were calculated using the following formula: ND = (100 – (100 × ((kg kg^{-1} IADF in feed/kg kg^{-1} IADF in feces) × (kg kg^{-1} of nutrient in feces/kg kg^{-1} of nutrient in feed)))), in which DMD = digestibility of dry matter; IADF = indigestible acid detergent fiber; and ND = nutrient digestibility.

Data for DMI, ruminal pH, ammonia, and VFA concentration; methane production, and digestibility of DM (DMD), CP (CPD), and NDF (NDFD) were analyzed with the MIXED procedure of SAS (Statistical Analysis System, version 9.1.3) in a 3 × 3 Latin square design, repeated in line. Model statement included the effect of treatment, period, animal, and hour (except for DMI, DMD, CPD, and NDFD) and the interaction treatment × hour.

The mathematical model used was:

$$y_{ijklm} = \mu + \tau_i + \rho_j + \sigma_k + o_l + \Theta_{il} + a_m(\sigma_k) + e_{ijklm},$$

in which y_{ijklm} = observed value of the variable that received the level of CPB i, period j, repetition k, hour l, and animal m; μ = overall mean; τ_i = dietary level of CPB effect; ρ_j = period effect; σ_k = Latin square repetition effect; o_l = hour effect; Θ_{il} = interaction between level of CPB and hour; $a_m(\sigma_k)$ = animal within Latin square repetition; and e_{ijklm} = random error associated with each observation.

To evaluate the concentration of Cr in feces, a non-linear regression model proposed by Pond et al. (1987) was used: Cr concentration = ((K0×L1×T)×(EXP(–L1T)))/0.59635, in which K0 = concentration of marker if instantaneously mixed in the compartment; L1 = age-dependent rate parameter; and T = time after marker administration.

Data were analyzed by NLIN procedure of SAS to obtain the non-linear model estimates for each animal within treatment. The data obtained were analyzed by ANOVA to understand the effect of CPB inclusion level on Kp and MRT in a 3 × 3 Latin square design, repeated in line. Data were analyzed by MIXED procedure of SAS. When significant (P<0.05), F-statistics were noted and the means separated using linear and quadratic contrast.

The mathematical model used was:

$$y_{ijkl} = \mu + \tau_i + \rho_j + \sigma_k + a_l(\sigma_k) + e_{ijkl},$$

in which y_{ijkl} = observed value of the variable that received the level of CPB i, period j, repetition k, animal l; μ = overall mean; τ_i = dietary level of CPB effect; ρ_j = period effect; σ_k = Latin square repetition effect; $a_l(\sigma_k)$ = animal within Latin square repetition; and e_{ijkl} = random error associated with each observation.

Results and Discussion

Dry matter intake was greater (P>0.05) for the control group (control: 1.77±0.08; CB25: 1.62±0.08; CB40: 1.62±0.08). Data for ruminal pH and ruminal NH_3N concentration did not differ (P>0.05) among treatments (Table 2). Treatment × hour interaction was found (P<0.05) for VFA, acetic, propionic, and butyric acid concentrations (Table 3). When feed was offered (0 h), total VFA concentration was greater (P<0.01) for CB25 group than for control and CB40 groups. However, in the first hour post feeding, VFA concentration of CB25 group increased and was greater (P<0.05) than VFA concentration of control and CB40 groups. During the trial, it was observed that total VFA concentration was greater (P<0.05) for CB25 group and acetic:propionic ratio was greater (P<0.01) for CB40 group. Acetic acid concentration was different (P≤0.01) when lambs were fed CPB and was greater for CB25 group. At 2 and 18 h post feeding, ruminal concentration of acetic acid was greater (P<0.05) for CB40 group. Average of acetic acid concentration during the complete period was greater (P<0.01) for those treatments that included CPB (Table 3). Data for propionic acid concentration showed

Table 2 - Effects of levels of cull pinto beans on ruminal pH and NH_3N concentration

Item	Treatment[1,2]			SE	P-value
	Control	CB25	CB40		
Ruminal pH					
Average	6.19	6.23	6.19	0.07	0.3602
Lowest	5.94	6.08	6.05	0.09	0.1673
Highest	6.5	6.4	6.44	0.09	0.2455
NH_3N (mg/100 mL)	27.23	26.55	27.43	1.67	0.8324

[1] Control - 0.0 kg kg^{-1} of cull pinto beans in the supplement; CB25 - 0.25 kg kg^{-1} of cull pinto beans in the supplement; CB40 - 0.40 kg kg^{-1} of cull pinto beans in the supplement.
[2] No treatment × hour interaction was observed (P>0.05).
SE - standard error.

that differences were found (P<0.05) at 0, 12, and 18 h and were greater for CB25 group during the test (Table 3). Butyric acid concentration was different (P<0.05) among treatments at 0, 1, 4, 8, and 18 h, and during the trial (Table 3). Differences (P<0.05) for methane production were found during the trial and were greater for CB40 group (Table 4). On the other hand, data for Kp (control: 3.6±1.9; CB25: 2.9±1.9; C40: 5.9±1.9) and MRT (control: 36.26±13.7; CB25: 57.68±13.7; C40: 36.24±13.7) were not different (P>0.05) among treatments. Similarly to Kp and MRT, dry matter, crude protein, and neutral detergent fiber digestibilities (P>0.05) were not different among treatments (Table 5).

The effect of CPB inclusion on DMI varied because of different factors, such as age of the animal, physiological state, nutrient requirements, and quantity of CPB in the diet. Because of *ad libitum* feeding, as DMI increased, CPB intake increased. Paduano et al. (1995) reported that ovine can tolerate low anti-nutritional factor intake without having negative effects, while higher intake can decrease DMI, which is assumed to have occurred in the present study. Similar results to this study were found when feedlot

Table 3 - Least square means for volatile fatty acids (mM/L; ±SE) of hair lambs supplemented with three levels of cull pinto beans

Post-feeding time (h)	Treatment[1]			SE	P-value
	Control	CB25	CB40		
Acetic					
0	34.43a	44.49b	41.55ab	1.05	0.0128
1	37.09a	43.97a	37.81a	2.82	0.0857
2	36.75a	37.76a	48.59b	2.82	0.0036
4	33.45a	40.16a	40.61a	2.82	0.0742
8	36.19a	33.78a	41.35a	2.82	0.0594
12	43.41a	39.16a	43.83a	2.82	0.2418
18	40.13a	44.62ab	49.25b	2.82	0.0237
24	32.95a	34.01a	35.63a	2.82	0.5001
Average	36.79a	39.75b	42.33b	2.82	0.001
Propionic					
0	12.01a	23.21b	13.14a	1.56	0.0450
1	18.07a	24.93a	14.44a	3.95	0.0600
2	16.21a	20.19a	15.24a	3.95	0.3712
4	14.17a	18.31a	15.02a	3.95	0.4544
8	14.41a	14.17a	13.79a	3.95	0.9117
12	27.05ab	30.87a	17.22b	3.95	0.0151
18	20.59ab	30.31a	17.87b	3.95	0.0265
24	17.31a	12.91a	9.38a	3.95	0.1534
Average	17.48a	21.86b	14.51a	3.95	0.0016
Butyric					
0	5.99a	8.09b	7.64b	0.33	0.0077
1	6.57a	8.15b	6.56a	0.61	0.0418
2	6.47a	6.63a	7.14a	0.61	0.3884
4	5.27a	6.85b	7.29b	0.61	0.0101
8	5.71a	5.63a	7.82b	0.61	0.0058
12	7.25a	5.97a	7.19a	0.61	0.1163
18	6.19a	7.37a	8.92b	0.61	0.0007
24	5.33a	5.75a	6.52a	0.61	0.1271
Average	6.09a	6.8b	7.39c	0.61	0.0001
Total concentration of VFA[2]					
0	52.43b	75.8a	62.73ab	5.8	0.0057
1	61.73ab	77.06a	58.8b	5.8	0.0296
2	59.43a	64.58a	70.97a	5.8	0.1655
4	52.89a	65.32a	62.93a	5.8	0.1355
8	56.31a	53.58a	62.97a	5.8	0.2585
12	77.71a	76a	68.25a	5.8	0.2550
18	66.91a	82.3a	76.05a	5.8	0.0654
24	55.59a	52.68a	51.53a	5.8	0.6243
Average	60.37b	68.42a	64.23ab	2.1	0.0294
Acetic:propionic ratio	2.34a	2.2a	2.9b	0.073	0.0001

[1] Control - 0.0 kg kg^{-1} of cull pinto beans in the supplement; CB25 - 0.25 kg kg^{-1} of cull pinto beans in the supplement; CB40 - 0.40 kg kg^{-1} of cull pinto beans in the supplement.
[2] Volatile fatty acids include acetic, propionic, and butyric acid.
Means in the same rows with different letters are significantly different (P<0.05).
SE - standard error.

Table 4 - Least squares means (±SE) for methane production (mM/mL) of hair lambs fed three different levels of cull pinto beans in the supplement during the day

Post-feeding time (h)	Treatment[1]			SE	P-value
	Control	CB25	CB40		
0	32.47ab	28.02b	34.24a	1.79	0.0139
1	27.91ab	25.88b	31.28a	1.79	0.0319
2	29.33ab	26.44b	33.19a	1.79	0.0077
4	29.59a	29.24a	31.78a	1.79	0.3084
8	30.41a	30.56a	33.32a	1.79	0.2438
12	25.21b	22.78b	30.92a	1.79	0.0015
18	28.37ab	25.42b	32.24a	1.79	0.0072
24	29.21b	32.38ab	35.99a	1.79	0.0074
Average	29.06b	27.59b	32.87a	0.75	0.0001

[1] Control - 0.0 kg kg^{-1} of cull pinto beans in the supplement; CB25 - 0.25 kg kg^{-1} of cull pinto beans in the supplement; CB40 - 0.40 kg kg^{-1} of cull pinto beans in the supplement.
Means in the same rows with different letters are significantly different (P<0.05).
SE - standard error.

Table 5 - Digestibility of nutrients (±SE) of hair lambs fed three different levels of cull pinto beans

Item	Treatment[1]			SE	P-value
	Control	CB25	CB40		
DMD (kg kg^{-1})	0.6897	0.6954	0.6938	0.0145	0.7617
CPD (kg kg^{-1})	0.7365	0.7381	0.7183	0.0158	0.3029
NDFD (kg kg^{-1})	0.4815	0.4863	0.4456	0.0207	0.2577

[1] Control - 0.0 kg kg^{-1} of cull pinto beans in the supplement; CB25 - 0.25 kg kg^{-1} of cull pinto beans in the supplement; CB40 - 0.40 kg kg^{-1} of cull pinto beans in the supplement.
DMD - dry matter digestibility; CPD - crude protein digestibility; NDFD - neutral detergent fiber digestibility; SE - standard error.

lambs were fed different levels of CPB. Villalobos et al. (2010a) reported a quadratic effect with the greatest DMI in the control group. Among other factors affecting DMI, there was the chemical composition of the diet and digestibility; in this study, CB40 had the lowest NDF digestibility. Ruminants fed concentrates that contain legume grains can have an effect on DMI. However, Lardy et al. (2009) found different results in three experiments with finishing steers and heifers fed different levels of field peas in their diet. Such inconsistencies to when legume grains are fed to ruminants can be due to differences in the intake of anti-nutritional factors. As such, other authors have reported similar results (Encinias et al., 2000; Soto-Navarro et al., 2004) when field peas were fed to beef cattle.

For pH, similar results were reported by Ramos et al. (2009), when 0.70 kg kg^{-1} of concentrate in the diet did not decrease ruminal pH from 6. A reduction in ruminal pH, however, can decrease fiber digestibility (Shriver et al., 1986) and, thus, a variation of ruminal pH during the day can negatively affect nutrient digestibility (Cerrato-Sánchez et al., 2007). The results of this study showed that ruminal pH did not affect DMD and NDF because it was similar across the day. Gilbery et al. (2007) reported that inclusion of field peas, lentil screenings, and chickpeas in feedlot cattle receiving diets did not affect ruminal pH, with an average pH of 6.35 across the treatments that included these legume grains.

Performance of ammonia concentration across the day showed that crude protein degradation rate and solubility of N sources were not affected by CPB inclusion. Preliminary research (Castillo, 2011) has demonstrated that CPB is an ingredient that contains a desirable crude protein content (0.21 kg kg^{-1}). The concentration of ammonia in the rumen is a function of both the rate of ruminal N degradation and the concentration of rumen degradable protein (RDP) above microbial needs and the amount of dietary energy available to the ruminal microorganisms (Hirstov et al., 2004). Increasing crude protein concentration or RDP percentage usually results in an increase of ruminal ammonia concentration (Armentano et al., 1993; Davidson et al., 2003). In the present study, supplements were isonitrogenous. Thus, it can be assumed that cull pinto bean ruminal degradation of the proteic fraction and RDP percentage are similar to those presented by the ingredients that were substituted by CPB. Similar results were presented by Singh et al. (2006), when cowpea grains were used in the diet of lambs. They found that the inclusion of this ingredient does not affect ruminal ammonia concentration; nonetheless, the results in this experiment are greater (0.281 kg kg^{-1}) than those presented by those authors.

Volatile fatty acids are the main products of ruminal fermentation; thus, CPB inclusion allowed for improved ruminal fermentation in hair lambs. Because of the greater DMI of the control group, a greater ruminal VFA concentration was expected in this group; however, DMI did not affect the total VFA concentration. Similar results were found in the study of Reed et al. (2004), in which ruminal VFA concentration was not affected by the substitution of corn grain with field peas. Soto-Navarro et al. (2004) did not report differences on fermentative parameters when field pea levels increased. In another study, in which different legume grains were included in diets of feedlot beef cattle, it was reported that the presence of these ingredients decreased ruminal VFA concentration (Gilbery et al., 2007). Reed et al. (2004), however, reported that inclusion of field peas linearly decrease acetate molar proportion and do not have an effect on propionate and butyrate molar proportions. Contrary to the results presented in the present study, Gilbery et al. (2007) found that inclusion of legume grains decrease acetate concentration. Volatile fatty acid concentration is regulated by the balance between production and absorption. Van Soest (1994) found that proportions of VFA can vary with diet and that acetate is the major fatty acid produced in most of the conditions, supporting the findings of the present experiment.

Methanogenesis is an important terminal step in anaerobic fermentation within the rumen. Carbohydrates are the major energy source for microbes of the rumen and the production of methane is related to their fermentation. Currently, little information is available for methane production by ruminants fed agro-industrial byproducts and feeding strategies for ruminants, which achieve significant methane suppression, remain limited (Moss et al., 2000). Pelchen and Peters (1998) have reported that methane production is similar among ovine fed diets containing from 60 to 0.80 kg kg^{-1} of dry matter digestibility, although, when DMD was less than 0.60 kg kg^{-1}, differences were found. In the present study, DMD in the diets averaged 0.69 kg kg^{-1} and did not differ significantly among treatments; nonetheless, differences for methane production were found during the trial and were greater for the CB40 group (Table 4). These differences could be due to the high level of CPB present, which, in turn, produced greater changes in ruminal fermentation compared with the ingredients already present in the concentrate because of the high oligosaccharide content of CPB. The rate of methane production is related to the production of hydrogen. Machmüller et al. (2003) found that methane production is similar throughout the day, it increases just after feeding, and is closely related to the fermentation rate. In the present study, methane production was similar during the day and it was associated with the *ad libitum* offer of feed.

The amount of feed consumed was probably the most important variable associated with retention time of digesta in the gastrointestinal tract of ruminant animals (Colucci et al., 1990). In ruminants, an increase in the amount of feed entering the gastrointestinal tract must be coupled with either an expansion of the organ or a faster rate of disappearance, which is related with digestibility or passage. During this experiment, it was noted that digestibility of dry matter was not different among treatments and DMI was similar between groups CB25 and CB40. Kp and MRT could be affected directly by both DMI and DMD; these processes depend directly on different factors such as density, particle size, starch content, protein content, and structural carbohydrates. The results of Kp and MRT could be associated with the physical and chemical characteristics of the feed, which, in the present study, were similar among treatments. Van Soest (1994) found that ruminal fermentation is a factor that can affect Kp and MRT. In this study, the similar ruminal fermentation (pH and NH_3N) observed, can partially be explained by the fact that there were no differences in retention time of digesta in the gastrointestinal tract based on the level of CPB, leading to similar digestibility of nutrients among treatments.

For dry matter digestibility, differences were expected among the treatments, owing to the presence of anti-nutritional factors present in CPB. Villalobos et al. (2010b) reported different results when feedlot lambs were fed cull pinto beans. These differences could be due to the type of animal and the concentrate:forage ratio being different among experiments. The results in this experiment are supported by those of Williams et al. (1984) who fed pinto beans to steers. Nonetheless, other authors (Stanford et al., 1999; Singh et al., 2006) found that DMD decreased when the level of legume grains increased in the diets of lambs. The most important anti-nutritional factors in legume grains are usually lectins and protease-inhibitor factors (Paduano et al., 1995). These factors directly affect the digestion and absorption of nutrients that reduce animal performance. Williams et al. (1984) reported negative effects due to the presence of those anti-nutritional factors and Soto-Navarro et al. (2004) found that the inclusion of field peas in the diet of steers reduce forage *in situ* CP degradability. In the present study, it was assumed that physiological maturity of wethers allowed them to tolerate the presence of anti-nutritional factors. Preliminary research in ewes supports this. Castillo et al. (2011) demonstrated that mature ewes can tolerate higher levels of CPB than primiparous (averaging 11 months old), which developed diarrhea when 0.50 kg kg^{-1} of CPB was included in their supplement, while multiparous ewes (averaging 36 months old) did not develop this metabolic disorder. Other studies have reported similar results when different legume grains were fed to ruminants (Surra et al., 1992; Singh et al., 2006; Gilbery et al., 2007). Patterson et al. (1999) found that the inclusion of cull beans in the diets of steers decreased NDF degradability; these results are in contrast with those reported in this study. Fiber digestibility can be associated with different factors, such as ruminal pH and passage rate. It has also been well established that ruminal pH can affect fiber and protein digestibility (Hoover, 1986; Shriver et al., 1986). In the present study, it was observed that ruminal pH was similar among treatments and the passage rate was not different among treatments. These results, thus, explain the equal digestibility of NDF among treatments.

Conclusions

Cull pinto beans reduce dry matter intake, although they do not affect ruminal pH and NH_3N production. The inclusion of 0.25 kg kg^{-1} of cull pinto beans in the diet of hair lambs allows for appropriate nutrient digestibility without affecting passage rate and mean retention time and this increases the molar proportion of volatile fatty acids and maintains acetate to propionate ratio that also keeps methane production low. Our research indicates that cull pinto beans is a suitable substitute for a combination of ground sorghum, dry corn distiller grain, and cottonseed meal in concentrate diets of hair lambs.

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