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# Effects of concentrate replacement by feed blocks on ruminal fermentation and microbial growth in goats and single-flow continuous-culture fermenters<sup>1</sup>

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**ABSTRACT:** The effect of replacing concentrate with 2 different feed blocks (FB) on ruminal fermentation and microbial growth was evaluated in goats and in single-flow continuous-culture fermenters. Diets consisted of alfalfa hay plus concentrate and alfalfa hay plus concentrate with 1 of the 2 studied FB. Three trials were carried out with 6 rumen-fistulated Granadina goats and 3 incubation runs in 6 single-flow continuous-culture fermenters. Experimental treatments were assigned randomly within each run, with 2 repetitions for each diet. At the end of each in vivo trial, the rumen contents were obtained for inoculating the fermenters. For each incubation run, the fermenters were inoculated with ruminal fluid from goats fed the same diet supplied to the corresponding fermenter flask. The average pH values, total and individual VFA, and NH<sub>3</sub>-N concentrations, and acetate:propionate ratios in the rumen of goats were not affected ( $P \geq 0.10$ ) by diet, whereas the microbial N flow (MNF) and efficiency were affected ( $P \leq 0.001$ ), with the greatest values observed for the diet without FB. In fermenters, the diet affected pH ( $P < 0.001$ ), propionate concentrations ( $P = 0.01$ ), acetate:propionate ratio ( $P = 0.03$ ), carbohydrate di-

gestibility ( $P = 0.05$ ), and total ( $P = 0.02$ ), NH<sub>3</sub> ( $P = 0.005$ ), and non-NH<sub>3</sub> ( $P = 0.02$ ) N flows, whereas the efficiency of VFA production was not affected ( $P = 0.75$ ). The effect of diet on MNF and efficiency depended on the bacterial pellet used as a reference. An effect ( $P < 0.05$ ) of diet on the composition of solid- and liquid-associated bacteria was observed. The compositions of liquid-associated bacteria in the fermenter contents and effluent were similar ( $P \geq 0.05$ ). Differences ( $P < 0.001$ ) between in vivo and in vitro values for most fermentation variables and bacterial pellet compositions were found. Partial replacement of the concentrate with FB did not greatly compromise carbohydrate fermentation in unproductive goats. However, this was not the case for MNF and efficiency. Differences between the results obtained in vivo and in vitro indicate a need to identify conditions in fermenters that allow better simulation of fermentation, microbial growth, and bacterial pellet composition in vivo. Reduced feeding cost could be achieved with the inclusion of FB in the diets of unproductive goats without altering rumen fermentation.

**Key words:** bacterial pellet, feed block, goat, microbial growth, rumen fermentation, single-flow continuous-culture fermenter

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## INTRODUCTION

Livestock production in Mediterranean countries is constrained by the scarcity and high price of feedstuffs such as cereals and soybean meal. Feed blocks (FB) based on local resources and by-products (Ben Salem and Nefzaoui, 2003) might be used to overcome this situation. Because olive trees and the derived industries are of great importance in Mediterranean countries, their by-products are of economic and environmental interest. The most important by-product is the crude 2-stage olive cake (CTSOC), composed of olive pulp, skin, and stones as well as water (Hermoso et al., 1995).

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Production of CTSOC accounts for 2,000,000 t/yr (Molina-Alcaide and Yáñez-Ruiz, 2008) with high pollutant potential, in part because of its high moisture content. Use of CTSOC has never been studied for ruminant feeding. Feed blocks including local resources (Ben Salem and Nefzaoui, 2003) and by-products could allow for the substitution of part of the concentrate in goat diets without compromising rumen fermentation. This could decrease the amount of cereals required in ruminant diets. Local legume seeds or by-products, such as sunflower meal, could be alternatives to soybean meal. Additionally, FB utilization could decrease the feeding cost. The effect on ruminal fermentation and microbial growth of partial substitution of a concentrate, composed of 800 g/kg of cereal and 160 g/kg of soybean meal, with FB including only 320 g/kg of barley grain and local sources of N (180 g/kg of sunflower meal or 400 g/kg of fava beans) as well as by-products such as CTSOC and beet molasses, was estimated by using different bacterial pellets as a reference in goats and in single-flow continuous-culture fermenters. Direct comparisons of rumen fermentation and bacterial pellet composition *in vivo* vs. *in vitro* are limited, and most have been conducted in cows (Hannah et al., 1986; Mansfield et al., 1995). We compared the results obtained in goats with those from single-flow continuous-culture fermenters.

## MATERIALS AND METHODS

All management and experimental procedures involving animals were carried out by trained personnel in strict accordance with the Spanish guidelines (Act No. 1201/2005 of October 10, 2005) for experimental animal protection.

### *Animals and Diets*

Six adult, dry, nonpregnant, rumen-fistulated Granadina goats ( $46.9 \pm 2.2$  kg of BW) were used. The animals were placed in individual boxes and had free access to water. Three experimental diets were formulated with alfalfa hay plus concentrate (diet **AC**) and alfalfa hay plus concentrate with FB (diets **ACBI** and **ACBII**). Concentrate and FB were handmade and the relative cost of ingredients was estimated; the composition of the ingredients is shown in Table 1. In both cases, solid ingredients were ground (2 mm) and mixed in a horizontal mixer (model C, P. Prat, Sabadell, Spain). The concentrate was then granulated in a rotating pelletizer (Vector CPM Europe, Andrijftechniek B.V., Rotterdam, the Netherlands) by spraying it with water. Block manufacturing followed the protocol of Ben Salem and Nefzaoui (2003), with modifications. Solid ingredients were mixed with a liquid mixture composed of water quicklime, salt, sugarcane molasses, urea, and CTSOC, and then heavily packed in aluminum molds.

Compacted blocks were taken from the molds, air-dried at room temperature, and stored until use.

### *In Vivo Ruminal Fermentation and N Flow*

Animals were previously adapted to the experimental diets for 25 d in individual boxes to achieve adequate intake of FB, as defined by Ben Salem and Nefzaoui (2003). Animals were then adapted to metabolic cages for 3 d, and three 10-d trials were conducted. Experimental treatments were assigned randomly within each experimental trial, with 2 repetitions for each diet. Animals were fed once a day with 600 g of alfalfa hay plus 400 g of concentrate (diet **AC**), or 600 g of alfalfa hay plus 200 g of concentrate and FB I (diet **ACBI**) or FB II (diet **ACBII**). Feed blocks were supplied for ad libitum intake, resulting in average intakes of  $180 \pm 80$  and  $291 \pm 61$  g/d for FB I and II, respectively. No refusals were observed for hay or concentrate. From d 1 through 5, total urine was collected daily in the morning from each animal in buckets with 10% H<sub>2</sub>SO<sub>4</sub> to maintain acidic pH and avoid degradation of purine derivatives (**PD**) by microorganisms. The urine was then weighed, and 100-mL aliquots were taken for PD analysis. On d 6 and 7, rumen content samples (500 mL) were obtained from each animal 2 h after feeding and pooled for isolation of solid- (**SAB**) and liquid (**LAB**)-associated bacteria. On d 8 and 9, rumen content samples (100 mL) were obtained from each animal 0, 2, 4, and 6 h after feeding and strained through 2 layers of cheesecloth. The pH was then measured, and aliquots were taken for NH<sub>3</sub>-N and VFA analyses. On d 10, rumen contents were obtained for single-flow continuous-culture fermenter inoculation.

### *In Vitro Ruminal Fermentation and Microbial Growth*

Six single-flow continuous-culture fermenters (Miettinen and Setälä, 1989) were used. Fermenters were inoculated with rumen fluid from rumen-fistulated Granadina goats used for *in vivo* trials. Rumen contents were collected from each animal 2 h after feeding, immediately transported to the laboratory in thermal bottles, and strained through 2 layers of cheesecloth; 700 mL of filtrate was inoculated into each fermenter within 30 min after rumen content collection. Three identical 11-d incubation runs were carried out. Experimental treatments were assigned randomly within each incubation run so that each dietary treatment was applied to 6 fermenters by the end of 3 trials. Diets consisted of 45 g of alfalfa hay and 30 g of concentrate (diet **AC**), 45 g of alfalfa hay, 15 g of concentrate, and 15 g of FB I (diet **ACBI**) or FB II (diet **ACBII**), on a fresh matter basis, supplied in 2 equal portions at 0900 and 1600 h. Flow-through fermenters were maintained by continuous infusion of McDougall (1948) artificial

**Table 1.** Composition of diet ingredients

Item	Alfalfa hay	Concentrate	Feed block I	Feed block II
Ingredient, g/kg as fed				
Oat	—	350	—	—
Corn grain	—	350	—	—
Crude 2-stage olive cake	—	—	120	100
Fava beans	—	—	—	400
Barley grain	—	100	320	200
Soybean meal	—	160	—	—
Beet molasses	—	—	210	110
Sunflower meal	—	—	180	—
Quicklime	—	—	70	90
NaCl	—	10	60	60
Urea	—	—	10	10
Vitamin-mineral mixture <sup>1</sup>	—	30	30	30
Nutrient, g/kg of DM				
DM, g/kg of fresh matter	864	860	790	760
OM	894	901	731	740
CP	175	159	168	172
Ether extract	13.6	3.9	3.4	2.6
NDF	491	214	167	127
ADF	331	59	80	24
ADL	84.1	6.8	13.0	1.9
GE, MJ/kg of DM	16.9	16.7	12.5	13.3
Purine bases, $\mu\text{mol/g}$ of DM				
Adenine	7.8	10.0	15.4	14.5
Guanine	9.1	4.0	3.9	3.6
Total	16.9	14.0	19.3	18.1

<sup>1</sup>Contained (per kilogram): NaCl, 277 g; ash from the 2-stage dried olive cake, 270 g;  $\text{CaH}_4(\text{PO}_4)_2$ , 250 g;  $\text{MgSO}_4$ , 200 g;  $\text{CuO}$ , 184 mg;  $\text{I}$ , 25 mg;  $\text{CoO}$ , 8.5 mg;  $\text{Se}$ , 4 mg;  $\text{ZnO}$ , 2.28 mg; 83,500 IU of vitamin A; and 16,700 IU of vitamin D.

saliva at a rate of 40 mL/h (5.33% dilution rate), and  $\text{CO}_2$  was continuously infused to maintain anaerobic conditions. The effluent from each fermenter was collected into a vessel maintained at 3°C to prevent microbial growth.

Each incubation run included 7 d for adaptation and 4 d for sampling. Twice a day, the pH was measured in the vessels immediately before feeding. On d 8, the total effluent from each fermenter was collected and subjected to vigorous mechanical pummeling between 2 metal plates (Masticator, IUL Instruments GmbH, Königswinter, Germany) for 5 min to detach bacteria from the feed particles (Michalet-Doreau and Ould-Bah, 1992), and the effluent was strained through 4 layers of cheesecloth. The solid fraction was washed with cold (4°C) NaCl solution (9 g/L) and strained through 4 layers of cheesecloth. The final filtrate was centrifuged at  $800 \times g$  for 10 min at 4°C to remove feed particles. The supernatant fraction was centrifuged at  $27,800 \times g$  for 10 min at 4°C to isolate effluent bacteria (**EB**). On d 9, 10, and 11, the total effluent from each fermenter was collected and the weight and volume were recorded before storage at -20°C. At the end of the trial, pooled subsamples from each fermenter and sampling day were made. Aliquots were taken for  $\text{NH}_3\text{-N}$  and VFA concentration analyses. The remainder was freeze-dried and analyzed for DM, OM, total N, and purine bases (**PB**). Fermenter contents collected on the last sampling day were used for SAB and LAB isolation.

For SAB and LAB isolation, rumen and fermenter contents were strained through 4 layers of cheesecloth. The solid residue was washed with cold (4°C) NaCl solution (9 g/L) and strained again through 4 layers of cheesecloth. The final filtrate was used to isolate LAB by differential centrifugation following the procedure described previously for the EB pellet. The solid fraction was resuspended in cold (4°C) NaCl solution (2.5 mL/g of solid), subjected to vigorous mechanical pummeling as described above, and strained through 4 layers of cheesecloth. The solid residue was washed with cold (4°C) NaCl solution (9 g/L) and strained, and the filtrate was added to that obtained previously. The final mixed filtrate was differentially centrifuged as described for EB to isolate the SAB pellet. All bacterial pellets were lyophilized and ground to a fine power with a mortar and pestle before analysis for N, OM, and PB.

### Chemical Analyses

Dry matter (method 934.01), ash (method 942.05), and N (method 984.13) were determined in samples of diet ingredients, effluents, and bacterial pellets according to the AOAC (1999). Samples of diet ingredients were analyzed for NDF, ADF, and ADL according to the methods of Van Soest et al. (1991) by using an Ankom<sup>220</sup> Fiber Analyzer unit (Ankom Technology Corporation, Macedon, NY). No amylase was used for

analysis of NDF, and both NDF and ADF were analyzed by excluding residual ash. The ADL was determined by solubilization of cellulose with sulfuric acid. The ADIN was determined by Kjeldahl analysis of ADF residues. Free, protein-bound, and fiber-bound condensed tannins were sequentially extracted following the procedure described by Pérez-Maldonado and Norton (1996). Condensed tannins from quebracho powder (Roy Wilson Dickson Ltd., Mold, UK) were used as a standard. The GE content was determined in an adiabatic calorimeter (model 1356, Parr Instrument Co., Moline, IL). Ether extract (method 7.045; AOAC, 1999) was analyzed in the diet ingredient and effluent samples. Rumen content and effluent samples were also analyzed for total and individual VFA by gas chromatography following the method described by Isac et al. (1994). The  $\text{NH}_3\text{-N}$  concentration of these samples was determined by a colorimetric method (Weatherburn, 1967). The PD in urine samples and the PB in the effluents and bacterial pellets were determined following the method described by Balcells et al. (1992).

### Calculations and Statistical Analyses

Total carbohydrate content of the diets was calculated as the OM content minus the content of both CP and ether extract. The apparent digestibility of total carbohydrates in vitro was calculated from the intake and output of carbohydrates. Carbohydrate content in the effluents was calculated as described for the diets but was corrected for the amount of OM in the VFA. The daily flow of microbial N was estimated for each fermenter by multiplying total non- $\text{NH}_3\text{-N}$  (NAN) production by the PB:N ratio of the effluent, EB, LAB, or SAB, or the average of LAB and SAB. In goats, the daily flow of microbial N was estimated from the urinary PD concentration (Belenguer et al., 2002) and from the PB:N ratio of bacterial pellets (LAB, SAB, or the average of both).

To compare the effect of diet on fermentation, digestibility, and N metabolism (including PB:N) within animals or fermenters, data were analyzed by 1-factor ANOVA (SPSS Inc., Chicago, IL). Diet was considered a fixed effect. The experimental model in fermenters allowed the study of diet (D) and pellet (P) effects and their interaction ( $D \times P$ ), which was given by  $Y = \mu + D_i + P_j + (D \times P)_{ij} + \varepsilon$ , where  $\mu$  is the overall mean and  $\varepsilon$  is the model error. For comparing in vivo and in vitro systems, the experimental model accounted for origin (O, animal or fermenters) and diet (D) effects and their interaction ( $D \times O$ ), which was given by  $Y = \mu + D_i + O_j + (D \times O)_{ij} + \varepsilon$ , where  $\mu$  is the overall mean and  $\varepsilon$  the model error. In both cases, the data were analyzed with the GLM procedure by using univariate ANOVA with 2 fixed factors (SPSS Inc.). The period, animal, and fermenter vessel were considered random effects. When significant effects of diet, bacterial pellet, origin, or their interactions were found,

post hoc comparisons of means were made by using the Bonferroni test. Differences were considered significant at  $P < 0.05$ .

## RESULTS

### *In Vivo Ruminant Fermentation and N Flow*

Average pH, total and individual VFA,  $\text{NH}_3\text{-N}$  concentrations, and the acetate:propionate ratio in the rumen of goats were not affected ( $P \geq 0.10$ ) by diet (Table 2). Acetate was the primary VFA. At 2 h after feeding, the least pH values and the greatest VFA and  $\text{NH}_3\text{-N}$  concentrations were observed (data not shown). The PB:N ratios for SAB ( $P = 0.01$ ) and for the average of SAB and LAB ( $P = 0.03$ ) were affected by diet. In contrast, the PB:N ratio for LAB was not affected ( $P = 0.23$ ) by diet. The PB:N ratio in SAB for animals fed diets including FB I were greater ( $P = 0.01$ ) than those for animals fed diet AC, but were similar ( $P \geq 0.05$ ) to values found in animals fed diet ACBII. When the PB:N ratio for SAB or the average of SAB and LAB was used, microbial N flow (MNF,  $P = 0.004$  and  $0.001$ , respectively) and efficiency ( $P = 0.008$  and  $P < 0.001$ , when expressed as g of microbial N/kg of OM ingested, and  $P = 0.005$  and  $P < 0.001$ , when expressed as g of microbial N/kg of digestible OM) were greater in animals fed diet AC compared with those fed diets including either FB, which were similar to each other ( $P \geq 0.05$ ). Microbial N flow values and efficiencies obtained with LAB were not affected by diet ( $P \geq 0.10$ ).

### *In Vitro Ruminant Fermentation, Microbial Growth, and Bacterial Pellet Composition*

In fermenters (Table 3), an effect of diet on average pH values ( $P < 0.001$ ) was observed. The greatest value was found for fermenters given diet ACBII, but values were similar ( $P \geq 0.05$ ) in fermenters given diets AC and ACBI. Production of total VFA was not affected by diet ( $P = 0.86$ ). An effect of diet was observed for propionate ( $P = 0.01$ ) and butyrate ( $P = 0.01$ ) molar proportions and for the acetate:propionate ratio ( $P = 0.03$ ). Propionate was greater only for diet AC compared with ACBII. Butyrate was less for diet ACBI in comparison with diets AC and ACBII, which showed similar ( $P \geq 0.05$ ) values. The acetate:propionate ratios were least for diet AC in comparison with diets including either FB, which were similar ( $P \geq 0.05$ ). Carbohydrate digestibility was greater in fermenters fed ACBII than in those fed AC, but efficiency of VFA production was not affected by diet ( $P = 0.75$ ). The PB:N ratio was affected by diet ( $P \leq 0.04$ ). The greatest PB:N ratio for both SAB and EB were observed in fermenters given diet ACBI, whereas for LAB and the average SAB and LAB, the greatest values were found in fermenters fed diets including FB compared with diet AC. True dietary OM digestibility was affected ( $P = 0.01$ )

**Table 2.** Average pH, VFA, and NH<sub>3</sub>-N concentrations, and microbial growth and efficiency, using different bacterial pellets, in the rumen of goats fed the experimental diets<sup>1</sup>

Item <sup>2</sup>	Diet			SEM	P-value
	AC	ACBI	ACBII		
pH	6.35	6.45	6.51	0.030	0.10
VFA, mmol/L	80.5	88.8	95.7	4.3	0.35
Acetate	59.1	64.1	69.1	3.2	0.45
Propionate	13.3	16.3	15.5	0.73	0.24
Isobutyrate	0.38	0.62	0.53	0.052	0.18
Butyrate	7.0	6.9	9.2	0.61	0.23
Isovalerate	0.60	0.54	0.67	0.063	0.68
Valerate	0.74	0.89	1.05	0.071	0.23
Acetate:propionate	4.44	3.93	4.46	0.23	0.38
NH <sub>3</sub> -N, mg/100 mL	10.9	11.4	14.5	0.93	0.25
Purine bases:N, µmol/mg					
SAB	0.50 <sup>a</sup>	0.71 <sup>b</sup>	0.59 <sup>ab</sup>	0.04	0.01
LAB	0.66	0.92	0.75	0.10	0.23
SAB and LAB	0.58 <sup>a</sup>	0.82 <sup>b</sup>	0.67 <sup>ab</sup>	0.06	0.03
Microbial N flow, g/d					
SAB	19.4 <sup>b</sup>	10.9 <sup>a</sup>	10.9 <sup>a</sup>	1.6	0.004
LAB	14.5	8.74	11.6	2.4	0.23
SAB and LAB	17.0 <sup>b</sup>	9.8 <sup>a</sup>	11.3 <sup>a</sup>	1.1	0.001
Microbial N/OM intake, g/kg					
SAB	25.2 <sup>b</sup>	15.1 <sup>a</sup>	13.3 <sup>a</sup>	1.3	0.008
LAB	18.6	11.7	14.6	2.6	0.21
SAB and LAB	21.9 <sup>b</sup>	13.4 <sup>a</sup>	14.0 <sup>a</sup>	1.8	<0.001
Microbial N/digestible OM intake, g/kg					
SAB	36.0 <sup>b</sup>	20.5 <sup>a</sup>	17.6 <sup>a</sup>	1.4	0.005
LAB	26.7	15.9	18.4	3.4	0.10
SAB and LAB	31.4 <sup>b</sup>	18.2 <sup>a</sup>	18.0 <sup>a</sup>	1.4	<0.001

<sup>a,b</sup>Within a row means not bearing a common superscript letter differ ( $P < 0.05$ ).

<sup>1</sup>AC = 600 g of alfalfa hay and 400 g of concentrate; ACBI = 600 g of alfalfa hay, 200 g of concentrate, and 180 ± 80 g of feed block I; ACBII = 600 g of alfalfa hay, 200 g of concentrate, and 291 ± 61 g of feed block II (fresh matter basis).

<sup>2</sup>SAB = solid-associated bacteria; LAB = liquid-associated bacteria; SAB and LAB = solid and liquid associated bacteria.

only by diet when it was estimated by using EB as the reference pellet. Differences were found between diets AC and ACBII.

Total N ( $P = 0.02$ ), NH<sub>3</sub>-N ( $P = 0.005$ ), and NAN flows ( $P = 0.02$ ) were affected by diet (Table 4). The least total N and NAN values were observed for fermenters administered diet ACBI when compared with the AC and ACBII diets. A significant effect of diet on MNF was found regardless of the bacterial pellet used as the reference for estimations ( $P = 0.01$  for SAB,  $P = 0.03$  for LAB,  $P < 0.001$  for EB, and  $P = 0.04$  for the average SAB and LAB value). With SAB and the average of SAB and LAB, greater values were found for diet ACBII in comparison with ACBI, whereas diets AC and ACBI promoted similar ( $P \geq 0.05$ ) MNF values. With EB, the MNF values for diets AC and ACBI were similar and were less ( $P < 0.001$ ) than those for diet ACBII. When comparing diets including FB (ACBI and ACBII), greater ( $P < 0.05$ ) MNF was found for diet ACBII regardless of which bacterial pellet was used as the reference. Dietary N degradation, calculated from the MNF estimated by using different bacterial pellets, was not affected ( $P \geq 0.06$ ) by diet. The efficiency of microbial growth, expressed as milligrams of microbial N per gram of digested carbohydrate, was only affected by diet ( $P = 0.01$ ) when MNF was estimated by using

EB as a reference. The greatest values were observed for diet ACBII compared with those observed for AC and ACBI, which were similar. However, when efficiency was expressed as milligrams of microbial N per kilogram of OM intake, the effect of diet was detected not only in estimations made by using EB as the reference ( $P < 0.001$ ), but also in those obtained by using SAB ( $P = 0.004$ ) and the average of SAB and LAB ( $P = 0.02$ ). Values for fermenters given diet ACBII were greater than for those given diets AC and ACBI, which were similar ( $P \geq 0.05$ ) except when values were estimated from the average of SAB and LAB. When microbial growth efficiency was expressed as milligrams of microbial N per kilogram of true digested OM diet, the only affected ( $P = 0.01$ ) values were those estimated from SAB, with smaller values found in fermenters fed diet ACBI compared with ACBII. However, the values obtained with diet AC were similar ( $P \geq 0.05$ ) to those obtained with diets ACBI and ACBII.

The DM, OM, and N contents in bacterial pellets (Table 5) were affected ( $P < 0.001$ ) by diet. Diet also affected adenine ( $P = 0.008$ ), guanine ( $P = 0.02$ ), and total PB ( $P = 0.01$ ) contents as well as PB:N ratios ( $P < 0.001$ ). Adenine, guanine, and total PB values were similar for diets ACBI and ACBII, which were greater than those found in pellets from fermenters fed diet

**Table 3.** Average values of pH, VFA production, molar proportions of VFA, carbohydrate digestibility, and efficiency of VFA production in single-flow continuous-culture fermenters fed the experimental diets<sup>1</sup>

Item <sup>2</sup>	Diet			SEM	P-value
	AC	ACBI	ACBII		
pH	5.96 <sup>a</sup>	5.78 <sup>a</sup>	6.29 <sup>b</sup>	0.038	<0.001
Total VFA, mmol/d	89.1	94.6	88.8	4.0	0.86
Molar proportion, mol/100 mol					
Acetate	51.5	57.2	54.8	1.3	0.22
Propionate	29.0 <sup>b</sup>	25.6 <sup>ab</sup>	21.9 <sup>a</sup>	0.87	0.01
Isobutyrate	0.172	0.218	0.328	0.028	0.08
Butyrate	17.0	14.6	19.9	0.97	0.01
Isovalerate	0.581	0.603	0.865	0.082	0.30
Valerate	1.81	1.82	2.09	0.13	0.61
Acetate:propionate	1.74 <sup>a</sup>	2.23 <sup>ab</sup>	2.50 <sup>b</sup>	0.11	0.03
Carbohydrate digestibility, g/g	0.469 <sup>a</sup>	0.483 <sup>ab</sup>	0.517 <sup>b</sup>	0.021	0.05
VFA, mmol/g digested carbohydrates	4.22	4.60	3.95	0.15	0.75
Purine bases:N, µmol/mg					
SAB	0.60 <sup>a</sup>	0.79 <sup>c</sup>	0.69 <sup>b</sup>	0.02	<0.001
LAB	0.89 <sup>a</sup>	1.19 <sup>b</sup>	1.02 <sup>ab</sup>	0.08	0.04
EB	1.08 <sup>a</sup>	1.26 <sup>b</sup>	1.05 <sup>a</sup>	0.04	0.002
SAB and LAB	0.74 <sup>a</sup>	0.99 <sup>b</sup>	0.86 <sup>ab</sup>	0.04	0.002
True dietary OM digestibility, g/g					
SAB	0.48	0.49	0.52	0.018	0.71
LAB	0.38	0.38	0.44	0.019	0.42
EB	0.34 <sup>a</sup>	0.37 <sup>ab</sup>	0.45 <sup>b</sup>	0.028	0.01
SAB and LAB	0.42	0.43	0.47	0.018	0.50

<sup>a,b</sup>Within a row means not bearing a common superscript letter differ ( $P < 0.05$ ).

<sup>1</sup>AC = 45 g of alfalfa hay and 30 g of concentrate; ACBI = 45 g of alfalfa hay, 15 g of concentrate, and 15 g of feed block I; ACBII = 45 g of alfalfa hay, 15 g of concentrate, and 15 g of feed block II (fresh matter basis).

<sup>2</sup>SAB = solid associated bacteria; LAB = liquid associated bacteria; EB = effluent bacteria; SAB and LAB = average value of SAB and LAB.

AC. With the exception of N ( $P = 0.11$ ), composition was affected ( $P < 0.001$ ) by the type of pellet. A diet  $\times$  pellet interaction was detected for DM ( $P < 0.001$ ), OM ( $P < 0.001$ ), and N ( $P = 0.008$ ) contents. The DM contents of both SAB and LAB were similar for all the experimental diets, whereas EB showed similar DM values for diets AC and ACBI, which were less compared with diet ACBII. The greatest OM content in SAB was found for diet AC, then for ACBI, and finally for diet ACBII. In LAB, the OM content was similar for diets AC and ACBII, with greater values than those found for diet ACBI. The OM content of EB was the least for diet ACBI. The N content of SAB was the greatest for diet AC, then for ACBI, and finally for ACBII. In LAB, the N content was similar for diets AC and ACBII, with greater values than those found for diet ACBI. The N in EB was greater for diet AC than for diets ACBI and ACBII, which showed similar values. With all the diets, the least values for adenine, guanine, and the PB:N ratio were found in SAB.

### *In Vivo-In Vitro Comparisons*

When comparing fermentation values obtained in the rumen of goats and in fermenters (Table 6), an origin effect ( $P < 0.001$ ) was observed, with the exception of total VFA concentration ( $P = 0.07$ ) and isovalerate molar proportion ( $P = 0.67$ ). Some values were greater in the rumen than in fermenters, but propionate, butyrate,

and valerate molar proportions and the efficiency of microbial growth showed greater values in fermenters than in the rumen. Total VFA concentration and isovalerate molar proportion were similar ( $P \geq 0.07$ ) in the rumen and in fermenters. The type of diet affected pH ( $P < 0.001$ ), propionate and butyrate molar proportions ( $P = 0.02$ ), and microbial growth efficiency ( $P \leq 0.03$ ) regardless of the pellet used as reference. The greatest values were for diet ACBII, with the exception of acetate, propionate, and isobutyrate molar proportions, for which the greatest values were observed for diet ACBI. An origin  $\times$  diet interaction was detected for pH ( $P = 0.003$ ), propionate molar proportion ( $P = 0.02$ ), and efficiency of microbial growth ( $P < 0.001$ ). The pH values were similar ( $P \geq 0.05$ ) for diets AC and ACBI, regardless of the origin, unlike diet ACBII, for which values were similar to those of only diets AC and ACBI in the rumen. The propionate molar proportions were similar ( $P \geq 0.05$ ) in the rumen of goats for all the diets, whereas in fermenters, values for diets AC and ACBII were different. The efficiency of microbial growth showed the greatest values for diet AC in the rumen, whereas in fermenters, the greatest values were found for diet ACBII.

In Table 7, the SAB composition in both rumen and fermenters is compared. An effect of origin ( $P < 0.001$ ) was observed, with the exception of concentrations of guanine ( $P = 0.87$ ) and total PB ( $P = 0.06$ ). Values found in fermenters were greater than in the rumen,

**Table 4.** Nitrogen metabolism in single-flow continuous-culture fermenters fed the experimental diets<sup>1</sup>

Item <sup>2</sup>	Diet			SEM	P-value
	AC	ACBI	ACBII		
Flow, mg/d					
Total N	1,817 <sup>b</sup>	1,545 <sup>a</sup>	1,817 <sup>b</sup>	32	0.02
NH <sub>3</sub> -N	49.5 <sup>b</sup>	37.1 <sup>ab</sup>	28.7 <sup>a</sup>	1.8	0.005
Non-NH <sub>3</sub> -N	1,767 <sup>b</sup>	1,508 <sup>a</sup>	1,788 <sup>b</sup>	32	0.02
Microbial N					
SAB	1,423 <sup>ab</sup>	1,274 <sup>a</sup>	1,711 <sup>b</sup>	97	0.01
LAB	1,026 <sup>b</sup>	863 <sup>a</sup>	1,181 <sup>b</sup>	34	0.03
EB	783 <sup>a</sup>	795 <sup>a</sup>	1,135 <sup>b</sup>	48	<0.001
SAB and LAB	1,171 <sup>ab</sup>	1,020 <sup>a</sup>	1,392 <sup>b</sup>	64	0.04
Dietary N degradation, g/g					
SAB	0.80	0.87	0.96	0.14	0.36
LAB	0.58	0.63	0.65	0.054	0.83
EB	0.44	0.59	0.62	0.031	0.06
SAB and LAB	0.66	0.729	0.77	0.049	0.61
Efficiency of microbial growth, g of microbial N/kg of digested carbohydrates					
SAB	70.2	62.0	79.5	4.5	0.13
LAB	51.8	41.9	54.9	4.0	0.25
EB	38.2 <sup>a</sup>	39.0 <sup>a</sup>	52.5 <sup>b</sup>	2.5	0.01
SAB and LAB	58.6	49.6	64.7	4.0	0.15
Efficiency of microbial growth, g of microbial N/kg of OM intake					
SAB	24.5 <sup>a</sup>	23.2 <sup>a</sup>	31.2 <sup>b</sup>	0.95	0.004
LAB	17.6	15.7	21.5	1.16	0.11
EB	13.5 <sup>a</sup>	14.4 <sup>a</sup>	20.7 <sup>b</sup>	0.52	<0.001
SAB and LAB	20.2 <sup>ab</sup>	18.5 <sup>a</sup>	25.4 <sup>b</sup>	0.96	0.02
Efficiency of microbial growth, g of microbial N/kg of true digested OM					
SAB	51.0 <sup>ab</sup>	47.8 <sup>a</sup>	59.9 <sup>b</sup>	0.84	0.01
LAB	46.0	41.8	48.8	0.91	0.64
EB	40.1	40.0	46.1	0.66	0.09
SAB and LAB	48.0	44.5	53.8	0.86	0.74

<sup>a,b</sup>Within a row means not bearing a common superscript letter differ ( $P < 0.05$ ).

<sup>1</sup>AC = 45 g of alfalfa hay and 30 g of concentrate; ACBI = 45 g of alfalfa hay, 15 g of concentrate, and 15 g of feed block I; ACBII = 45 g of alfalfa hay, 15 g of concentrate, and 15 g of feed block II (fresh matter basis).

<sup>2</sup>SAB = solid associated bacteria; LAB = liquid associated bacteria; EB = effluent bacteria; SAB and LAB = average value of SAB and LAB.

with the exception of guanine, which was similar in rumen and fermenters. Diet affected ( $P \leq 0.03$ ) SAB composition. The greatest DM, OM, and N contents were observed for diet AC, whereas SAB from fermenters fed diet ACBI showed the greatest adenine, guanine, and total PB contents and PB:N ratios. An origin  $\times$  diet interaction was detected for OM ( $P < 0.001$ ) and N ( $P = 0.01$ ). The OM content was greater for fermenters than in the rumen with diets AC and ACBI, but not with diet ACBII. The N content was greater for fermenters than in the rumen with diets AC and ACBII, but not with diet ACBI.

The composition of LAB in the rumen and in fermenters is shown in Table 8. There was an effect ( $P < 0.001$ ) of bacterial pellet origin, with the exception of DM ( $P = 0.93$ ). When significant differences were found, the values were greater for pellets isolated from fermenter contents in comparison with those from the rumen, with the exception of OM, which showed greater ( $P < 0.001$ ) values for pellets from the rumen. Diet affected ( $P \leq 0.03$ ) LAB composition, with the exception of DM ( $P = 0.41$ ) and N ( $P = 0.29$ ). Adenine concentration in LAB showed the greatest values for diet ACBI, whereas the greatest guanine value was for

diet ACBII. The total PB content was similar for diets ACBI and ACBII, with greater values than for diet AC. The greatest PB:N ratio of LAB was for diet ACBI. An origin  $\times$  diet interaction was detected for DM and OM ( $P < 0.001$ ) and for N ( $P = 0.02$ ). The DM content was similar ( $P \geq 0.05$ ) in LAB from rumen and fermenters given diets AC and ACBI, but not when given diet ACBII ( $P < 0.05$ ). The OM content in the rumen was similar ( $P \geq 0.05$ ) for diets AC and ACBI, whereas in fermenters, it was similar ( $P \geq 0.05$ ) for diets AC and ACBII. The least values for N content in LAB from the rumen were found for diet ACBII, whereas in LAB from fermenters, the least values were observed with diet ACBI.

## DISCUSSION

The shortage of cereals for animal feeding obligates animal nutritionists to look for locally available alternative sources of nutrients. Feed blocks may allow for decreased use of concentrate and cereals in animal nutrition. The use of FB may also allow for the integration of by-products, especially those with high moisture, thereby permitting recycling and decreased

**Table 5.** Chemical composition of solid-associated bacteria (SAB) and liquid-associated bacteria (LAB) isolated from the fermenter contents and from effluents (EB) in single-flow continuous-culture fermenters fed the experimental diets<sup>1</sup>

Item	Diet												P-value
	AC			ACBI			ACBII			D × P			
	SAB	LAB	EB	SAB	LAB	EB	SAB	LAB	EB	SEM	Diet (D)	Pellet (P)	
DM, g/g of fresh matter	0.92 <sup>c</sup>	0.86 <sup>ab</sup>	0.83 <sup>a</sup>	0.91 <sup>c</sup>	0.86 <sup>ab</sup>	0.83 <sup>a</sup>	0.91 <sup>c</sup>	0.88 <sup>bc</sup>	0.87 <sup>bc</sup>	0.002	<0.001	<0.001	0.001
OM, g/g of DM	0.87 <sup>c</sup>	0.75 <sup>ab</sup>	0.70 <sup>ab</sup>	0.79 <sup>bc</sup>	0.66 <sup>a</sup>	0.67 <sup>a</sup>	0.64 <sup>a</sup>	0.74 <sup>ab</sup>	0.74 <sup>ab</sup>	0.004	<0.001	<0.001	<0.001
Total N, mg/g of DM	78.9 <sup>b</sup>	76.9 <sup>ab</sup>	75.0 <sup>ab</sup>	70.9 <sup>ab</sup>	70.4 <sup>ab</sup>	71.9 <sup>ab</sup>	69.3 <sup>a</sup>	77.6 <sup>ab</sup>	70.3 <sup>ab</sup>	0.59	<0.001	0.11	0.008
Purine bases, µmol/g of DM													
Adenine	16.8 <sup>a</sup>	30.1 <sup>bcd</sup>	35.4 <sup>d</sup>	22.3 <sup>abc</sup>	37.0 <sup>d</sup>	40.5 <sup>d</sup>	19.9 <sup>ab</sup>	36.4 <sup>d</sup>	32.3 <sup>cd</sup>	2.3	0.008	<0.001	0.36
Guanine	21.6 <sup>a</sup>	38.4 <sup>bc</sup>	45.5 <sup>c</sup>	27.6 <sup>ab</sup>	45.7 <sup>c</sup>	49.5 <sup>c</sup>	25.8 <sup>a</sup>	46.4 <sup>c</sup>	41.3 <sup>c</sup>	2.5	0.02	<0.001	0.22
Total	38.4 <sup>a</sup>	68.5 <sup>bc</sup>	81.0 <sup>c</sup>	50.0 <sup>ab</sup>	82.7 <sup>c</sup>	89.8 <sup>c</sup>	45.7 <sup>a</sup>	82.8 <sup>c</sup>	73.6 <sup>c</sup>	4.7	0.01	<0.001	0.26
Purine bases:N, µmol/mg	0.60 <sup>a</sup>	0.89 <sup>bcd</sup>	1.08 <sup>cd</sup>	0.79 <sup>abc</sup>	1.19 <sup>e</sup>	1.26 <sup>e</sup>	0.69 <sup>ab</sup>	1.02 <sup>cde</sup>	1.05 <sup>de</sup>	0.028	<0.001	<0.001	0.42

<sup>a-c</sup>Within a row means not bearing a common superscript letter differ ( $P < 0.05$ ).

<sup>1</sup>AC = 45 g of alfalfa hay and 30 g of concentrate; ACBI = 45 g of alfalfa hay, 15 g of concentrate, and 15 g of feed block I; ACBII = 45 g of alfalfa hay, 15 g of concentrate, and 15 g of feed block II (fresh matter basis).

feeding cost. End users often ask what quantity of FB can be used to reduce feeding cost and increase income from sheep and goat products. Block intake by goats in the present study was  $180 \pm 80$  and  $291 \pm 61$  g/d for diets ACBI and ACBII, respectively. These intake values were greater than those found by Gasmi-Boubaker et al. (2006) using FB with grazing goats. The concentrate cost for diet AC was approximately 0.10 euros/d and the cost for concentrate plus FB in diets ACBI and ACBII was 0.09 and 0.15 euros/d, respectively, representing a 10% decrease in the cost for diet ACBI in comparison with diet AC. Houmani and Tisserand (1999) reported that the cost decreased from 12 to 23% on replacement of 75% of the concentrate with molasses-free or molasses-containing blocks, with similar growth rates observed in animals fed the full amount of concentrate and blocks. In lambs fed barley straw supplemented with blocks based on 3-stage olive cake, Ben Salem and Nefzaoui (2003) reported that the daily BW gain cost decreased by 18% compared with animals given barley straw and 500 g of concentrate. El Hag et al. (2002) found that it was economically viable to use FB-containing by-products and urea for sheep and goats because the cost per kilogram of grain was reduced by 38% as compared with the control diet (grass hay and concentrate).

The inclusion of high-moisture by-products such as CTSOC in ruminant diets is challenging, but might help to prevent environmental pollution. The CTSOC could offer an additional advantage based on the presence of polyphenols (37.3 g of condensed tannins/kg of DM) and fat (100 g of ether extract/kg of DM) rich in fatty acids, such as oleic (70.1%) and linoleic (9.5%) acids. Biohydrogenation of these fatty acids in the rumen produces CLA (Jenkins et al., 2008), which are considered healthy for consumers (Pariza, 2004). Inclusion of this by-product in animal feed might thus enhance animal product quality.

Almost no information is available regarding the effect of FB on rumen fermentation. Feed blocks have been used as a supplement to improve poor roughage utilization in goats (Samanta et al., 2003) without affecting VFA, in agreement with our observations both in vivo and in vitro. The absence of differences in VFA concentration among animals or fermenters given the different experimental diets, together with the absence of effects of diet on in vitro VFA production, suggest that partial substitution of the concentrate with either of the 2 studied FB does not compromise ruminal carbohydrate fermentation. Rumen concentrations of VFA in the present study with all the experimental diets were similar to those reported by Lu et al. (1990) in lactating dairy goats fed a diet containing soybean meal. Varying results have been reported for VFA production efficiency. Dann et al. (2006) reported 11.8 mol of VFA/kg of digested carbohydrate; this value is much greater than ours.

Complete FB including roughage have been used by Bhatta et al. (2005), who found decreased  $\text{NH}_3$  con-

**Table 6.** Average values of pH, VFA concentration, acetate:propionate ratio, NH<sub>3</sub>-N concentration, and efficiency of microbial N synthesis in the rumen of goats and in single-flow continuous-culture fermenters fed the experimental diets<sup>1</sup>

Item <sup>2</sup>	Origin						SEM	<i>P</i> -value		
	Rumen			Fermenter				Origin (O)	Diet (D)	O × D
	AC	ACBI	ACBII	AC	ACBI	ACBII				
pH	6.35 <sup>b</sup>	6.45 <sup>b</sup>	6.51 <sup>b</sup>	5.96 <sup>a</sup>	5.78 <sup>a</sup>	6.29 <sup>b</sup>	0.024	<0.001	<0.001	0.003
Total VFA, mmol/L	80.5	88.8	95.7	89.1	94.6	88.8	2.8	0.07	0.34	0.73
Molar proportion, mol/100 mol										
Acetate	73.4 <sup>b</sup>	72.2 <sup>b</sup>	72.2 <sup>b</sup>	51.5 <sup>a</sup>	57.2 <sup>a</sup>	54.8 <sup>a</sup>	0.70	<0.001	0.43	0.14
Propionate	16.5 <sup>a</sup>	18.4 <sup>a</sup>	16.2 <sup>a</sup>	29.0 <sup>c</sup>	25.6 <sup>bc</sup>	21.9 <sup>ab</sup>	0.53	<0.001	0.02	0.02
Isobutyrate	0.47 <sup>bcd</sup>	0.70 <sup>d</sup>	0.56 <sup>cd</sup>	0.17 <sup>a</sup>	0.22 <sup>ab</sup>	0.33 <sup>abc</sup>	0.024	<0.001	0.07	0.20
Butyrate	8.73 <sup>ab</sup>	7.76 <sup>a</sup>	9.66 <sup>ab</sup>	17.0 <sup>cd</sup>	14.6 <sup>bc</sup>	19.9 <sup>d</sup>	0.51	<0.001	0.02	0.26
Isovalerate	0.75	0.61	0.70	0.58	0.60	0.87	0.055	0.67	0.28	0.31
Valerate	0.92 <sup>a</sup>	1.00 <sup>ab</sup>	1.09 <sup>ab</sup>	1.81 <sup>bc</sup>	1.82 <sup>bc</sup>	2.09 <sup>c</sup>	0.074	<0.001	0.38	0.87
Acetate:propionate	4.44 <sup>b</sup>	3.93 <sup>b</sup>	4.46 <sup>b</sup>	1.78 <sup>a</sup>	2.23 <sup>a</sup>	2.50 <sup>a</sup>	0.095	<0.001	0.17	0.10
NH <sub>3</sub> N, mg/100 mL	10.9 <sup>b</sup>	11.4 <sup>b</sup>	14.5 <sup>b</sup>	5.1 <sup>a</sup>	3.8 <sup>a</sup>	3.1 <sup>a</sup>	0.42	<0.001	0.44	0.05
Efficiency of microbial growth, g of microbial N/kg of OM intake										
SAB	25.2 <sup>b</sup>	15.1 <sup>a</sup>	13.3 <sup>a</sup>	24.5 <sup>b</sup>	23.2 <sup>b</sup>	31.2 <sup>c</sup>	0.54	<0.001	<0.001	<0.001
LAB	18.6 <sup>bc</sup>	11.7 <sup>a</sup>	14.6 <sup>ab</sup>	17.6 <sup>c</sup>	15.7 <sup>abc</sup>	21.5 <sup>c</sup>	0.64	<0.001	0.03	<0.001
SAB and LAB	21.9 <sup>b</sup>	12.5 <sup>a</sup>	13.8 <sup>a</sup>	20.2 <sup>bc</sup>	18.5 <sup>b</sup>	25.4 <sup>c</sup>	0.53	<0.001	0.02	<0.001

<sup>a-d</sup>Within a row means not bearing a common superscript letter differ (*P* < 0.05).

<sup>1</sup>AC = 600 g of alfalfa hay and 400 g of concentrate; ACBI = 600 g of alfalfa hay, 200 g of concentrate, and 180 ± 80 g of feed block I; ACBII = 600 g of alfalfa hay, 200 g of concentrate, and 291 ± 61 g of feed block II (fresh matter basis for in vivo experiments), and AC = 45 g of alfalfa hay and 30 g of concentrate; ACBI = 45 g of alfalfa hay, 15 g of concentrate, and 15 g of feed block I; ACBII = 45 g of alfalfa hay, 15 g of concentrate, and 15 g of feed block II (fresh matter basis for in vitro experiments).

<sup>2</sup>SAB = solid-associated bacteria; LAB = liquid-associated bacteria; SAB and LAB = average value from both pellets.

centrations in the rumen than we found in the present experiments (5.4 vs. 7.9 mg/dL). The NH<sub>3</sub>-N concentrations in the present work were similar to those reported by Lu et al. (1990) in lactating dairy goats fed a diet containing soybean meal. The in vivo and in vitro results in this study were different; no effect of diet on NH<sub>3</sub> was observed in vivo, whereas in vitro NH<sub>3</sub> production in fermenters given diet ACBII was less than that in fermenters given diets AC and ACBI. Although no differences were found for NH<sub>3</sub> concentration in the goat rumen, both MNF and efficiency were significantly

greater in animals fed diet AC as compared with those fed diets ACBI and ACBII when using SAB or an average of SAB and LAB as a reference. This suggests that partial substitution of concentrate with blocks might compromise rumen N metabolism. Because blocks I and II produced the same results but the price was less for block I, its use may be more highly recommended. However, this pattern did not appear in vitro because the MNF in fermenters given diet ACBII was not less than that in fermenters receiving diet AC, regardless of which bacterial pellet was used as the reference.

**Table 7.** Composition of solid-associated bacteria (SAB) isolated from the rumen of goats and from the content of single-flow continuous-culture fermenters fed the experimental diets<sup>1</sup>

Item	Origin						SEM	<i>P</i> -value		
	Rumen			Fermenter				Origin (O)	Diet (D)	O × D
	AC	ACBI	ACBII	AC	ACBI	ACBII				
DM, g/g of fresh matter	0.89 <sup>ab</sup>	0.87 <sup>a</sup>	0.88 <sup>a</sup>	0.92 <sup>c</sup>	0.91 <sup>bc</sup>	0.91 <sup>bc</sup>	0.0025	<0.001	0.03	0.64
OM, g/g of DM	0.75 <sup>c</sup>	0.70 <sup>b</sup>	0.70 <sup>b</sup>	0.87 <sup>d</sup>	0.79 <sup>c</sup>	0.64 <sup>a</sup>	0.0035	<0.001	<0.001	<0.001
Total N, mg/g of DM	67.5 <sup>b</sup>	67.0 <sup>b</sup>	59.9 <sup>a</sup>	78.9 <sup>c</sup>	70.9 <sup>b</sup>	69.3 <sup>b</sup>	0.52	<0.001	<0.001	0.01
Purine bases, μmol/g of DM										
Adenine	11.9 <sup>a</sup>	17.4 <sup>ab</sup>	11.7 <sup>a</sup>	16.8 <sup>ab</sup>	22.3 <sup>b</sup>	19.9 <sup>b</sup>	0.67	<0.001	0.005	0.51
Guanine	21.2 <sup>a</sup>	31.5 <sup>b</sup>	23.0 <sup>a</sup>	21.6 <sup>a</sup>	27.6 <sup>ab</sup>	25.8 <sup>ab</sup>	0.80	0.87	0.001	0.24
Total	33.1 <sup>a</sup>	48.9 <sup>b</sup>	34.8 <sup>a</sup>	38.4 <sup>ab</sup>	49.9 <sup>b</sup>	45.7 <sup>ab</sup>	1.5	0.06	0.002	0.38
Purine bases:N, μmol/mg of N	0.49 <sup>a</sup>	0.73 <sup>c</sup>	0.58 <sup>ab</sup>	0.60 <sup>ab</sup>	0.79 <sup>c</sup>	0.69 <sup>bc</sup>	0.025	<0.001	<0.001	0.58

<sup>a-d</sup>Within a row means not bearing a common superscript letter differ (*P* < 0.05).

<sup>1</sup>AC = 600 g of alfalfa hay and 400 g of concentrate; ACBI = 600 g of alfalfa hay, 200 g of concentrate, and 180 ± 79.7 g of feed block I; ACBII = 600 g of alfalfa hay, 200 g of concentrate, and 291 ± 60.9 g of feed block II (fresh matter basis for in vivo experiments), and AC = 45 g of alfalfa hay and 30 g of concentrate; ACBI = 45 g of alfalfa hay, 15 g of concentrate, and 15 g of feed block I; ACBII = 45 g of alfalfa hay, 15 g of concentrate, and 15 g of feed block II (fresh matter basis for in vitro experiments).

**Table 8.** Composition of liquid-associated bacteria (LAB) isolated from the rumen of goats and from fermenters content of single-flow continuous-culture fermenters fed the experimental diets<sup>1</sup>

Item	Origin						SEM	<i>P</i> -value		
	Rumen			Fermenter				Origin (O)	Diet (D)	O × D
	AC	ACBI	ACBII	AC	ACBI	ACBII				
DM, g/g of fresh matter	0.88 <sup>b</sup>	0.88 <sup>b</sup>	0.84 <sup>a</sup>	0.86 <sup>ab</sup>	0.86 <sup>ab</sup>	0.88 <sup>b</sup>	0.003	0.93	0.41	<0.001
OM, g/g of DM	0.82 <sup>d</sup>	0.83 <sup>d</sup>	0.79 <sup>c</sup>	0.75 <sup>b</sup>	0.66 <sup>a</sup>	0.74 <sup>b</sup>	0.005	<0.001	0.03	<0.001
Total N, mg/g of DM	68.6 <sup>ab</sup>	70.7 <sup>abc</sup>	68.0 <sup>a</sup>	76.9 <sup>bc</sup>	70.4 <sup>abc</sup>	77.6 <sup>c</sup>	0.78	<0.001	0.29	0.02
Purine bases, μmol/g of DM										
Adenine	16.6 <sup>a</sup>	21.6 <sup>ab</sup>	20.8 <sup>ab</sup>	30.1 <sup>bc</sup>	37.0 <sup>c</sup>	36.4 <sup>c</sup>	0.92	<0.001	0.03	0.90
Guanine	28.4 <sup>a</sup>	38.0 <sup>ab</sup>	38.9 <sup>ab</sup>	38.4 <sup>ab</sup>	45.7 <sup>b</sup>	46.4 <sup>b</sup>	1.1	0.001	0.003	0.78
Total	45.1 <sup>a</sup>	59.6 <sup>ab</sup>	59.7 <sup>ab</sup>	68.5 <sup>bc</sup>	82.7 <sup>c</sup>	82.8 <sup>c</sup>	1.9	<0.001	0.006	0.96
Purine bases:N, μmol/mg of N	0.66 <sup>a</sup>	0.83 <sup>ab</sup>	0.90 <sup>ab</sup>	0.89 <sup>ab</sup>	1.19 <sup>c</sup>	1.02 <sup>bc</sup>	0.025	<0.001	0.001	0.16

<sup>a-d</sup>Within a row means not bearing a common superscript letter differ ( $P < 0.05$ ).

<sup>1</sup>AC = 600 g of alfalfa hay and 400 g of concentrate; ACBI = 600 g of alfalfa hay, 200 g of concentrate and 180 ± 80 g of feed block I; ACBII = 600 g of alfalfa hay, 200 g of concentrate and 291 ± 61 g of feed block II (fresh matter basis), and AC = 45 g of alfalfa hay and 30 g of concentrate; ACBI = 45 g of alfalfa hay, 15 g of concentrate and 15 g of feed block I; ACBII = 45 g of alfalfa hay, 15 g of concentrate and 15 g of feed block II (fresh matter basis, for in vitro experiments).

The greater efficiency for MNF in goats fed diet AC in comparison with those fed diets including blocks may indicate a better supply of available carbohydrates in diet AC, as previously suggested by Lee et al. (2003). It may also indicate increased efficiency of N use (Merry et al., 2006) in animals fed diet AC as compared with those receiving diets ACBI and ACBII. Although the most important factors influencing microbial growth are rumen outflow and energy available to microbes, growth may also be influenced by the substrate and type of microbes growing in the rumen (Van Soest et al., 1988). Dietary energy supply and synchronization between N and OM release from diets have been positively associated with the efficiency of microbial growth (Henning et al., 1993; Shabi et al., 1998). Some of our observed values for MNF and efficiency were within the range observed for ewes fed diets including soybean meal (84.0 to 92.2 g of microbial protein/d and 10.8 to 11.4 g of microbial protein/100 g of OM truly degraded in the rumen) by Karsli et al. (2006). The reduced MNF and microbial growth efficiency in animals fed diets including FB may limit the benefits of this feeding strategy. However, the high degree of variability found in the available literature concerning MNF has to be taken into account. Richardson et al. (2003) found that MNF varied from 8.2 to 14.2 g/d in lambs. Yáñez-Ruiz et al. (2004) found that the microbial protein supply to the duodenum varied from 0.50 to 1.53 in goats and from 0.49 to 0.85 g/kg<sup>0.75</sup> in sheep when both animal species were fed different diets. Cantalapiedra-Híjar et al. (2008) also found varying results (12.1 to 28.9 g/d) in goats fed diets with different forage types and forage:concentrate ratios. Dann et al. (2006) found microbial N efficiency values varying from 40.9 to 46.8 g/kg of digested OM and from 46.4 to 56.3/kg of total carbohydrate; this was similar to our results.

In agreement with other studies (Martín-Orue et al., 1998; Carro and Miller, 2002; Molina-Alcaide et al., 2008), LAB were found to contain greater concentra-

tions of both adenine and guanine, in addition to exhibiting a greater PB:N ratio than SAB, both in vivo and in vitro. Although Rodríguez-Prado et al. (2004) also found less PB in SAB than in LAB, they did not find in vitro differences in PB:N ratios between SAB and LAB. Smaller values for SAB isolated from the rumen of steers in comparison with LAB have also been reported by Cecava et al. (1990). Similar observations have been made in vitro by Molina-Alcaide et al. (2008), who used fermenters given diets composed of alfalfa hay and a concentrate including dry and extracted 2-stage olive cake. Rodríguez et al. (2000) found greater purine concentrations and PB:N ratios with LAB as compared with SAB in sheep rumen, and associated the increased amount of purines in bacteria with increased ruminal outflow. Because changes in PB concentration have been associated with the presence of different bacterial species with different growth rates (Obispo and Dehority, 1999), the decreased content of PB in SAB might be due to the decreased growth rate of the microbial population in this pellet as compared with LAB. The PB:N ratio in EB was close to that in LAB with all diets and was always greater than in SAB. This difference might be due to the effect of differences in processing (solid for isolation of SAB and total effluent for EB) on the effectiveness of detachment. As a consequence of the different marker:N ratios in SAB and LAB, the estimated MNF has been reported to be greater for SAB than for LAB (Pérez et al., 1998; Rodríguez et al., 2000; Carro and Miller, 2002; Molina-Alcaide et al., 2008).

Our in vivo and in vitro results showed smaller values for MNF and its efficiency when using LAB as compared with SAB (data not shown). The values obtained in vitro with EB were the least (data not shown) of the 3. This is difficult to explain because EB should represent a mixture of LAB and SAB in single-flow continuous-culture fermenters. The PB:N ratio of pellets did not change across diets (diet × pellet interaction);

therefore, different MNF values across diets cannot be attributed to differences in the microbial marker. Regardless of the bacterial pellet used, the MNF values of diets including FB II were always greater than those of diets including FB I. These results do not agree with those reported by Rodríguez-Prado et al. (2004), who did not find differences in bacterial N flow and efficiency for LAB and SAB isolated from fermenters inoculated with rumen liquor from cows.

Of interest for ruminant nutritionists is the possibility of simulating ruminal fermentation with in vitro techniques, thereby reducing the need for animals, especially fistulated animals. Studies comparing in vivo and in vitro results are scarce, especially with small ruminants (Carro et al., 2005, 2008). It is of interest not only to simulate rumen fermentation variables, but also to compare the chemical composition of bacterial pellets from in vivo and in vitro experiments and of pellets isolated from fermenter contents and effluents. Because EB isolated from the effluent collected over a 24-h period represents a mixed bacterial pellet, its use as a reference pellet could simplify the estimation of MNF, which requires LAB and SAB isolation. On the other hand, García et al. (2006) used  $^{15}\text{N}$  as a microbial marker for single-flow continuous-culture fermenters given 2 different diets, and reported no differences in MNF estimation between EB and mixed pellets isolated from fermenter flask contents immediately before feeding.

Our finding of greater pH values in the rumen than in fermenters agrees with the observations by Carro et al. (2008), who compared single-flow continuous-culture fermenters with the rumen of sheep. Those authors also found less  $\text{NH}_3\text{-N}$  in fermenters than in sheep rumen. The similarity of the VFA concentration between the goat rumen and fermenters agrees with the observations of Carro et al. (2008) comparing sheep rumen and fermenters. However, our comparison of goats and fermenters provides different results concerning the molar proportions of VFA. In contrast to the findings of Carro et al. (2008) with sheep, we found that acetate:propionate ratios were greater in the goat rumen than in fermenters. The buffering capacity of saliva added to the fermenters, together with differences in input diet between fermenters and goats, might explain the differences in pH and VFA concentration, although the lack of absorption in fermenters might result in the expectation of greater values in fermenters. Comparison with other results is difficult because of the different input methods used in fermenters. Changes in microbial species might be derived from changes in pH and might explain the differences in VFA when comparing in vivo and in vitro results. Almost no information is available on microbial communities in the goat rumen and in fermenters. Results obtained by our group (Cantalapiedra-Híjar et al., 2007) using denaturing gradient gel electrophoresis suggest less diversity in fermenters than in goats fed diets including alfalfa hay and concentrate at different ratios. Soto et al. (In

press) applied that methodology and real-time PCR to show that fermenters can support most rumen microorganisms, but they concluded that in vitro conditions seem to alter the quantity and diversity of bacterial communities, although these results depended on the experimental diet.

Although similar values for some of the fermentation variables have been obtained in the rumen of goats and in fermenters, the observed differences could limit the potential for simulation of the in vivo conditions used in the present study. A comparison of the composition of bacterial pellets isolated in vivo and in vitro (SAB and LAB) showed differences depending on the origin and significant origin  $\times$  diet interactions for many of the analyzed variables. However, PB:N values did not demonstrate origin  $\times$  diet interactions; this is crucial for evaluating diet effects on MNF as well. These findings suggest the potential of single-flow continuous-culture fermenters to rank diets similarly to in vivo results in terms of MNF.

Partial substitution of concentrate with FB for unproductive goats seems to be possible because notable differences were not observed in ruminal carbohydrate fermentation either in vivo or in vitro. However, in vivo differences found for MNF and its efficiency that indicate better microbial fermentation for diet AC in comparison with ACBI and ACBII may limit the potential of this feeding strategy. The use of these experimental FB and other formulae that allow optimal synchronization of energy and N should be investigated further. The promotion of better N metabolism by diet AC is not as clear in vitro. Differences between in vivo and in vitro results suggest that fermenter conditions allowing better simulation of in vivo fermentation should be investigated further.

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