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Effects of forage:concentrate ratio and forage type on apparent digestibility, ruminal fermentation, and microbial growth in goats¹

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ABSTRACT: The effects of forage type and forage:concentrate ratio (F:C) on apparent nutrient digestibility, ruminal fermentation, and microbial growth were investigated in goats. A comparison between liquid (LAB) and solid (SAB)-associated bacteria to estimate microbial N flow (MNF) from urinary purine derivative excretion was also examined. Treatments were a 2 × 2 factorial arrangement of forage type (grass hay vs. alfalfa hay) and high vs. low F:C (70:30 and 30:70, respectively). Four ruminally cannulated goats were fed, at maintenance intake, 4 experimental diets according to a 4 × 4 Latin square design. High-concentrate diets resulted in greater ($P < 0.001$) nutrient digestibility except for ADF. However, CP digestibility increased ($P < 0.001$) only for the high-concentrate diets including grass hay. Likewise, N retention, ruminal $\text{NH}_3\text{-N}$ concentration, and urinary excretion of purine derivatives increased ($P < 0.05$) with increasing concentrate in animals fed diets based on grass hay (0.23 vs. 0.13 g of retained N/g of digested N, 30.1 vs. 12.9 mg of $\text{NH}_3\text{-N}/100$ mL, and 11.5 vs. 8.40 mmol/d, respec-

tively), but not ($P > 0.05$) when diets included alfalfa hay. Total protozoa numbers and holotricha proportion were greater and less ($P < 0.001$), respectively, in high than in low-concentrate diets. The F:C affected ($P < 0.001$) ruminal pH but not total VFA concentration ($P = 0.12$). Ammonia-N concentration was similar ($P = 0.13$) over time, whereas pH, VFA concentration, and protozoa numbers differed ($P < 0.001$) among diets. Estimated MNF was strongly influenced by using either the purine bases:N ratio obtained in our experimental conditions or values reported in the literature for small ruminants. There was a F:C effect ($P = 0.006$) on MNF estimated from LAB but not from SAB. The effect of F:C shifting from 70:30 to 30:70 in goat diets depends on the type of forage used. The MNF measured in goats fed different diets was influenced by the bacterial pellet (LAB or SAB). In addition, the purine bases:N ratio values found were different from those reported in the literature, which underlines the need for these variables to be analyzed directly in pellets isolated from specific animals and experimental conditions.

Key words: bacterial pellet, goat, microbial growth, purine derivative, rumen fermentation

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INTRODUCTION

The demand for animal products is increasing in developing countries (Delgado et al., 1999), and, as a consequence, the demand for cereals for animal feed competes with that for human consumption. In addi-

tion, there is growing use of cereals in biofuel production, which has pushed the prices of most cereals to record levels (FAO, 2008). These facts highlight the need to design diverse strategies in animal nutrition to utilize alternative sources of nutrients and decrease cereal use.

The incorporation of concentrates into ruminant diets is intended to increase dietary energy, proteins, minerals, and vitamins and to optimize the efficiency of feed utilization (Morand-Fehr and Sauvant, 1987). However, grain supplementation may decrease digestibility of forage-containing diets for cattle and sheep (Archimède et al., 1995). The degree to which concentrates affect fiber digestion may depend on the nature and proportion of the concentrate as well as the quality of the forage (Matejovsky and Sanson, 1995).

The goat production systems traditionally developed in the Mediterranean Basin were based on graz-

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ing. However, there is a tendency toward intensification (Castel et al., 2003) and, hence, to the use of increasing amounts of concentrate in diets. The world goat population has been steadily increasing (39% increase from 1986 to 2000; FAO, 2008), but research into digestibility, ruminal fermentation, or microbial protein synthesis (Cerrillo et al., 1999; Yáñez-Ruiz et al., 2004) in goats fed different quality forages is scarce compared with that carried out with other animal species.

The objective of this study was to evaluate the effects of high vs. low concentrate levels using alfalfa (**A**) vs. grass (**G**) hay diets for goats on apparent nutrient digestibility, ruminal fermentation, and microbial protein synthesis. An additional aim was to compare the use of liquid (**LAB**) vs. solid (**SAB**)-associated rumen bacteria to estimate microbial N flow (**MNF**) from the rumen.

MATERIALS AND METHODS

Animal management and experimental procedures were conducted by trained personnel in strict accordance with the Spanish research guidelines for animal protection.

Animals and Diets

Four adult dry nonpregnant Granadina goats (46.3 ± 3.0 kg of BW) fitted with permanent ruminal cannulas were used in a 4×4 Latin square design experiment, with a 2×2 factorial arrangement of treatments. The main effects were forage type (G vs. A) and level (70 vs. 30%) of concentrate. Forages G and A differed mainly in CP and NDF content (95 and 192 g of CP/kg of DM, and 506 and 428 g of NDF/kg of DM, respectively). Ingredient composition of concentrate (g/kg of fresh matter) was as follows: 215 barley, 204 corn gluten meal, 200 wheat bran, 135 soybean, 115 palm meal, 50 wheat, 50 corn, 10 calcium carbonate, 10 white salt, 5 vitamin-mineral premix, 4 calcium soap, 4 dicalcium phosphate, and 3 sodium bicarbonate. Four complete diets were formulated and supplied to meet 1.2 times the energy maintenance requirements of the goats (Prieto et al., 1990). Treatments, on a DM basis, were: 70% G and 30% concentrate (**GF**), 30% G and 70% concentrate (**GC**), 70% A and 30% concentrate (**AF**), and 30% A and 70% concentrate (**AC**).

Apparent Nutrient Digestibility and Energy and N Utilization

Animals were kept in individual metabolism crates where feces and urine were separately collected using a collector device equipped with a plastic net including an angled ramp. Animals had free access to water throughout the experiment. Concentrate and forage were manually mixed before feeding twice daily, at 0800 and 1400 h. A 5-L bucket containing 50 mL of 10% H_2SO_4 to keep the final pH below 3 was placed under

each crate for urine collection. Each of the 4 experimental periods consisted of 20 d: 10 d for diet adaptation, 7 d for digestibility determination, and 3 d in which microbial protein flow was estimated and rumen fermentation variables were analyzed. At the beginning of each trial, the animals were weighed to determine their food requirements, and feed samples were collected and kept at -20°C for analysis. To calculate the apparent digestibility of diets, from d 11 through 17, total feces, urine, and refusals were daily weighed. Fecal, urine, and refusal samples were taken (20, 10, and 30% of the total wet weight, respectively) each sampling day, composited within animal and period, and stored at -20°C before analysis.

Microbial N Flow

From d 15 through 17, a 100-mL subsample of urine was collected daily and stored at -20°C for purine derivative (**PD**) analysis, after measuring its density. On d 20, a 700-mL sample of rumen contents from each animal was collected 2 h after feeding for LAB and SAB isolation, following the procedure described by Martín-Orúe et al. (1998). The bacterial pellets obtained were freeze-dried before analysis.

Ruminal Fermentation

On d 18, a 100-mL sample of rumen contents was individually collected at 0, 2, 4, 6, 8, 10, and 12 h after the morning feeding and squeezed through 4 layers of cheesecloth under continuous flushing with CO_2 . The pH was measured immediately, and 3 different aliquots were taken at each time. Then, 5 mL of strained fluid was acidified with 5 mL of 0.5 M HCl and frozen for NH_3 analysis. For VFA analysis, 800 μL of a solution made up of orthophosphoric and crotonic acids (as internal standard) diluted in 0.5 M HCl was added to 800 μL of strained rumen liquid and then frozen. A 5-mL aliquot was prepared for protozoa counting, following the procedure described by Dehority (1984). Twenty fields per aliquot were counted, and total protozoa numbers and holotricha proportion were recorded.

Chemical Analyses

Once thawed, feed, refusals, and feces were dried in a forced-air oven at 60°C for 48 h and ground through a 1-mm sieve before being analyzed. The DM (method 934.01), ash (method 942.05), and N (method 984.13) contents were determined according to the AOAC (1999). The NDF and ADF contents were analyzed according to Van Soest et al. (1991) using an ANKOM Model 220 Fiber Analyzer (Macedon, NY). The α -amylase was used for NDF analysis in concentrate and feces samples. Both NDF and ADF were expressed exclusive of residual ash. Samples of feed, refusals, feces, and urine were also analyzed for GE content in an adiabatic calorimeter (model 1356, Parr Instrument

Table 1. Chemical composition (g/kg of DM) and in vitro digestibility (g/g) of diet ingredients

Item	Grass hay	Alfalfa hay	Concentrate
DM, g/kg of fresh matter	904	895	899
OM	933	893	905
CP	94.7	192	199
Ether extract	16.1	14.9	33.1
NDF	506	428	329
ADF	273	320	131
ADL	39.2	73.0	27.0
GE, MJ/kg of DM	18.7	18.3	18.2
IVDMD	0.60	0.66	0.78
In vitro OM digestibility	0.60	0.65	0.78
In vitro CP digestibility	0.68	0.79	0.82

Co., Moline, IL). In vitro DM and OM digestibilities of diet ingredients were determined according to the procedure described by Tilley and Terry (1963), using an ANKOM Model Daisy Incubator.

Urinary PD, including allantoin, hypoxanthine, uric acid, and xanthine, as well as creatinine and bacterial pellets purine base (PB) content, were determined following the procedures described by Balcells et al. (1992), using HPLC analysis. Aliquots of strained rumen content collected at different times after feeding were thawed in the refrigerator overnight, centrifuged at 4°C and 20,000 × *g* for 20 min, and the supernatant was analyzed for VFA and NH₃-N. Individual VFA were analyzed (Isac et al., 1994) by gas chromatography. The NH₃-N concentration was determined by a colorimetric method (Weatherburn, 1967).

Calculations and Statistical Analysis

Apparent digestibility was calculated as 1 minus the quotient between fecal output and diet intake. Microbial N flow was calculated as described by Belenguer et al. (2002) for goats: MNF (g/d) = (PD excretion/0.76)/(0.92 × PB:N), where 0.76 is the incremental recovery of PD, 0.92 is the true digestibility of duodenal PB (Chen et al., 1990), and PB:N is the ratio between PB and N content recorded in LAB, SAB, or an average value from both bacterial pellets.

Data were analyzed as a 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments, using the GLM procedure (SPSS v.15, SPSS Inc., Chicago, IL). The linear model used for each dependent variable accounted for the effects of forage type (F), F:concentrate ratio (F:C), F × F:C interaction, and period as fixed effects. Animal was considered a random effect. Effects were considered significant at *P* < 0.05. When significant differences were detected, differences among means were tested using Tukey's comparison test. Data corresponding to ruminal content samples, obtained at different times after feeding, were analyzed taking into account the correlation among repeated measures from the same animal, and accordingly, a linear mixed model procedure (Littell et al., 1998) was used rath-

er than GLM. The linear mixed model contained the same fixed effects described for GLM model, except that time after feeding and its interaction with main factors were included (Rotger et al., 2006). Time after feeding was considered as the repeated factor and each animal within treatment as the subject. For each variable analyzed, data were subjected to 3 different covariance structures: variance components, compound symmetry, and unstructured. The covariance structure that yielded the greatest Schwarz's Bayesian was considered to be the most desirable for analysis (Littell et al., 1998).

RESULTS AND DISCUSSION

In this work, we studied the effect of increasing the level of concentrate in diets based on 2 forages (G vs. A) of different quality that are commonly used in Mediterranean goat farming. The significance of the F × F:C interaction is used to evaluate the differential response of both forages to shifts in F:C from 70:30 to 30:70.

Composition, Apparent Nutrient Digestibility, and Energy and N Utilization

Composition and in vitro digestibility of diet ingredients are shown in Table 1. Grass hay was less in CP and greater in NDF than A and concentrate. In vitro CP digestibility values for concentrate, A, and G were 0.82, 0.79, and 0.68, respectively. Differences between both forages in composition and nutrient availability can explain the different effect of increasing concentrate level (F × F:C interaction) on digestibility variables.

Although offered DM was the same for all diets, nutrient intakes (Table 2), except for OM and NDF, were greater (*P* ≤ 0.04) for diets based on A than G, which may be due to a greater palatability of A and differences in chemical composition and in vitro digestibility between both forages. Moreover, intake of legume forages seems to be greater than G due to less cell wall content, greater ruminal degradation, and faster digestion and passage rates (Pinos-Rodríguez et al., 2002) in legumes than in grass. The NDF and ADF intake were greater (*P* ≤ 0.004) for low- than high-concentrate diets, owing to the greater cell wall content of forages compared with concentrate, as has been reported previously for goats (Ramanzin et al., 1997; Kawas et al., 1999). The effect of increasing the level of concentrate on CP and NDF intake depended on the forage fed (F × F:C interaction; *P* ≤ 0.05). The greater similarity in CP and NDF content between forage A and concentrate compared with forage G can explain these results. Increasing concentrate proportions in diets based on A did not result in greater N supply, whereas it increased it in diets based on G by almost 50%.

Increasing concentrate in diets increased (*P* < 0.001) apparent nutrient digestibilities except for ADF (*P* = 0.13). In general, it is accepted that adding concentrate to ruminant diets increases DM and OM digestibility

Table 2. Effect of forage (F) and forage:concentrate ratio (F:C) on intake and in vivo apparent digestibility of diets by goats

Item	Diet ¹				SEM	P-value		
	GF	GC	AF	AC		F	F:C	F × F:C
Intake, g/kg of BW ^{0.75}								
DM	46.4	45.8	49.3	51.3	0.80	0.04	0.67	0.45
OM	42.9	42.4	44.1	45.5	1.1	0.08	0.19	0.87
CP	5.65 ^a	7.30 ^b	9.48 ^c	9.85 ^c	0.12	<0.001	0.006	0.04
NDF	22.0 ^b	16.6 ^a	20.0 ^{ab}	18.4 ^{ab}	0.4	0.89	0.004	0.05
ADF	10.3 ^{ab}	7.61 ^a	13.0 ^b	9.50 ^{ab}	0.16	<0.001	<0.001	0.23
In vivo apparent digestibility, g/g								
DM	0.67 ^a	0.78 ^b	0.67 ^a	0.74 ^b	0.0058	0.11	<0.001	0.10
OM	0.68 ^a	0.81 ^b	0.69 ^a	0.77 ^b	0.0053	0.09	<0.001	0.07
CP	0.63 ^a	0.82 ^b	0.80 ^b	0.79 ^b	0.0052	<0.001	<0.001	<0.001
NDF	0.64 ^b	0.72 ^c	0.55 ^a	0.67 ^b	0.0039	<0.001	<0.001	0.07
ADF	0.58 ^a	0.71 ^b	0.56 ^a	0.54 ^a	0.015	0.02	0.13	0.04
GE	0.66 ^a	0.76 ^b	0.68 ^a	0.74 ^b	0.025	0.91	<0.001	0.04

^{a-c}Within a row, means without a common superscript letter differ ($P < 0.05$).

¹GF = 70% grass hay and 30% concentrate, DM basis; GC = 30% grass hay and 70% concentrate, DM basis; AF = 70% alfalfa hay and 30% concentrate, DM basis; AC = 30% alfalfa hay and 70% concentrate, DM basis.

(Molina-Alcaide et al., 2000; Fimbres et al., 2002). However, there is some controversy regarding the digestibility of other nutrients when forage is supplemented with concentrate in the diet of the goat, which is likely to be due to the type of ingredients used in each experiment and also to the pH in the rumen. Molina-Alcaide et al. (2000) reported increased digestibility of all nutrients except for CP in Granadina goats when diets based on A were supplemented with sugar beet pulp and oat grain. Likewise, Cerrillo et al. (1999) supplemented a diet based on hay for goats with grain, in proportions varying from 0 to 50%, and found increased DM digestibility, but no effect on CP digestibility. Ramanzin et al. (1997) observed increased OM and N apparent digestibility with decreasing F:C in goats, whereas the digestibility of fibrous fraction decreased. Similarly, decreased ruminal fiber digestion has been observed in goats fed A when concentrate was incorporated at a 1:1 ratio (Antoniou and Hadjipanayiotou, 1985). The decrease in ruminal fiber digestion is believed to be caused by the inhibited growth of cellulolytic bacteria when ruminal pH decreases below 6.2 (Grant and Mertens, 1992). In this experiment, the addition of high levels of concentrate did not decrease either NDF or ADF apparent digestibility ($P > 0.05$), perhaps because ruminal pH was not low enough to negatively affect fiber digestion. Increased CP digestibility with high level of concentrate was observed only in diets including G (F × F:C interaction; $P < 0.001$). Because A had greater in vitro CP digestibility than G and was more similar to that of concentrate, the effect of increasing concentrate proportions had only a significant effect on diets based on G. On the other hand, diets based on G had greater ADF digestibility ($P = 0.02$) compared with those based on A, because rumen retention time is shorter with legumes than G (Pinos-Rodríguez et al., 2002). In our study, this is confirmed in diets based on G, but only when high level of concentrate was added (F ×

F:C interaction; $P = 0.04$), likely because the greater microbial growth and fiber digestion promoted by high level of concentrate allowed the differences in ADF digestibility between the forages to be observed more clearly. The GE digestibility increased with high levels of concentrate ($P < 0.001$) for both forages studied, but the different magnitude of increase led to a significant F × F:C interaction effect ($P = 0.04$). This supports once more that the effect of changing F:C from 70:30 to 30:70 on nutrient digestibility was more beneficial for diets based on G than those based on A.

Energy losses in feces (Table 3) were less for high- than low-concentrate diets ($P < 0.01$), as reported by Molina-Alcaide et al. (2000) in goats. More N (g/g of N intake) was retained in goats fed diets based on G when the concentrate level increased ($P > 0.05$), whereas N retention remained the same in diets based on A regardless of concentrate proportion (F × F:C interaction; $P = 0.05$). As mentioned above, similar in vitro N digestibility and N content in A and concentrate may explain the different response observed between diets based on A and G when concentrate level increased. High level of concentrate in diets based on A did not result in a more efficient use of N. Because A is one of the most common preserved forages used for goat feeding, care must be taken by goat producers and nutritionists when supplementing diets based on A to avoid N waste and save money.

Ruminal Fermentation

Average pH values were unaffected ($P = 0.63$) by F (Table 4), but a F:C effect was evident ($P < 0.001$) as reported in previous studies with goats (Hadjipanayiotou and Antoniou, 1983; Cerrillo et al., 1999). Greater amounts of starch in high-concentrate diets may yield greater lactic acid concentration (Slyter, 1976), and hence less ruminal pH, compared with low-concentrate

Table 3. Effect of forage (F) and forage:concentrate ratio (F:C) on energy and N metabolism in goats fed the experimental diets

Item	Diet ¹				SEM	P-value		
	GF	GC	AF	AC		F	F:C	F × F:C
Energy metabolism								
Intake, kJ/kg of BW ^{0.75}	861	839	907	936	13	0.03	0.91	0.36
Energy in feces, kJ/kg of BW ^{0.75}	297 ^b	202 ^a	293 ^b	246 ^{ab}	6.0	0.15	<0.001	0.10
Energy in urine, kJ/kg of BW ^{0.75}	36.7	38.4	46.8	45.0	1.6	0.04	0.98	0.60
N metabolism								
N intake, g/kg of BW ^{0.75}	0.90 ^a	1.17 ^b	1.52 ^c	1.58 ^c	0.052	<0.001	0.006	0.04
N in feces, g/kg of BW ^{0.75}	0.33 ^b	0.21 ^a	0.30 ^b	0.33 ^b	0.0092	<0.001	0.56	0.09
N in urine, g/kg of BW ^{0.75}	0.50 ^a	0.74 ^b	0.92 ^c	0.98 ^c	0.021	<0.001	0.008	0.05
Retained N								
g/kg of DMI	1.52 ^a	4.77 ^{ab}	6.08 ^b	5.25 ^{ab}	0.24	0.03	0.22	0.06
g/g of N intake	0.09 ^a	0.19 ^b	0.20 ^b	0.17 ^b	0.0082	0.16	0.15	0.05
g/g of digested N	0.13 ^a	0.23 ^b	0.25 ^b	0.22 ^b	0.017	0.18	0.29	0.01

^{a-c}Within a row, means without a common superscript letter differ ($P < 0.05$).

¹GF = 70% grass hay and 30% concentrate, DM basis; GC = 30% grass hay and 70% concentrate, DM basis; AF = 70% alfalfa hay and 30% concentrate, DM basis; AC = 30% alfalfa hay and 70% concentrate, DM basis.

diets. The pH response over time was different ($P < 0.001$) among diets with greater decreases in pH after feeding (Figure 1A) in goats receiving diets containing A than in those including G. This might be explained by the slightly greater degradability and the greater DMI ($P = 0.04$) of diets based on A. These factors may also contribute to the greater ($P = 0.001$) total VFA concentration in the rumen of goats fed diets including A in comparison with those receiving diets based on G.

Total VFA concentration in the rumen of goats fed diets based on A was within the range previously reported for Granadina and Saanen goats fed similar diets (Molina-Alcaide et al., 2000; Fujita et al., 2006). The expected increase in VFA concentration with high level of concentrate, and hence more digestible OM, was not observed ($P = 0.12$). High fiber content in the concentrate (329 g of NDF/kg of DM) may promote

similar VFA regardless of the concentrate proportion in the diet. Likewise, Archimède et al. (1996) and Cerrillo et al. (1999) found no effect of concentrate content on total VFA concentration in the rumen of goats. Because of greater fluctuations over time for diets based on A (Figure 1B) in comparison with G, a time after feeding × diet interaction ($P < 0.001$) was detected for postprandial changes in VFA concentration. The molar proportion of acetate decreased with concentrate level, whereas the proportions of propionate and butyrate increased ($P < 0.001$), as reported by others in goats (Archimède et al. 1996). Diets based on A promoted greater molar proportions of propionate and valerate ($P \leq 0.04$) and less ($P = 0.02$) proportions for butyrate compared with diets based on G.

The ruminal NH₃-N concentration varied ($P < 0.001$), being greater for high- than low-concentrate diets, and for diets based on A compared with those based on G

Table 4. Effect of forage (F) and forage:concentrate ratio (F:C) on ruminal fermentation, total protozoa concentration, and holotricha proportion in goats consuming the experimental diets

Item	Diet ¹				SEM	P-value		
	GF	GC	AF	AC		F	F:C	F × F:C
pH	6.43	6.21	6.43	6.26	0.055	0.63	<0.001	0.71
NH ₃ -N, mg/100 mL	12.9 ^a	30.1 ^b	22.9 ^b	31.0 ^b	1.9	<0.001	<0.001	0.002
Total VFA, mmol/L	68.8 ^a	74.7 ^a	93.0 ^b	75.9 ^{ab}	4.2	<0.001	0.12	0.002
VFA, mol/100 mol								
Acetate	70.2 ^b	65.4 ^a	68.3 ^{ab}	66.2 ^a	0.64	0.17	<0.001	0.05
Propionate	16.0 ^a	18.4 ^b	17.4 ^{ab}	19.3 ^b	0.52	0.04	<0.001	0.60
Butyrate	10.8 ^a	12.9 ^b	10.4 ^a	11.1 ^{ab}	0.41	0.02	<0.001	0.08
Isobutyrate	0.79	0.86	1.0	0.88	0.078	0.10	0.66	0.35
Valerate	1.02 ^a	1.29 ^a	1.91 ^b	1.76 ^b	0.086	<0.001	0.24	0.03
Isovalerate	1.07	1.02	1.05	0.97	0.12	0.81	0.72	0.96
Acetate: propionate	4.45 ^b	3.50 ^a	3.95 ^{ab}	3.47 ^a	0.11	0.04	<0.001	0.15
Total protozoa, ×10 ⁴ /mL	58.3 ^a	128.3 ^b	67.5 ^a	133.1 ^b	5.8	0.04	<0.001	0.47
Holotricha, %	6.40 ^b	1.35 ^a	6.53 ^b	2.58 ^a	0.42	0.28	<0.001	0.38

^{a,b}Within a row, means without a common superscript letter differ ($P < 0.05$).

¹GF = 70% grass hay and 30% concentrate, DM basis; GC = 30% grass hay and 70% concentrate, DM basis; AF = 70% alfalfa hay and 30% concentrate, DM basis; AC = 30% alfalfa hay and 70% concentrate, DM basis.

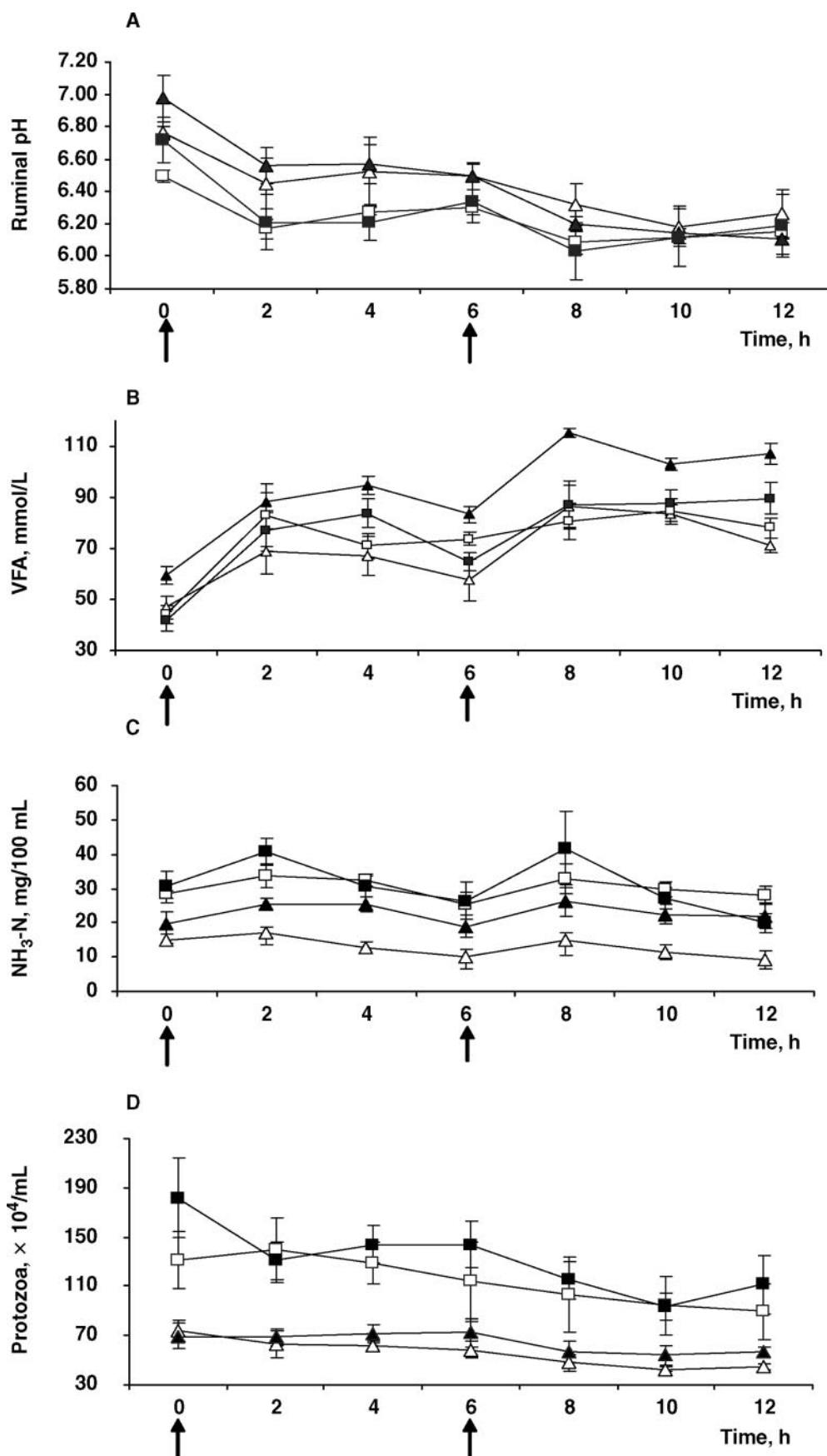


Figure 1. Ruminal pH (A), total VFA concentration (B), NH₃-N concentration (C), and total protozoa concentration (D) with time after feeding in the rumen of goats fed the experimental diets. Diets were 70% grass hay and 30% concentrate (GF = △), 30% grass hay and 70% concentrate (GC = □), 70% alfalfa hay and 30% concentrate (AF = ▲), and 30% alfalfa hay and 70% concentrate (AC = ■). Diet × time after feeding interaction values were significant ($P < 0.001$) for ruminal pH, VFA, and total protozoa concentration, but not ($P = 0.13$) for NH₃-N concentration. Arrows show feeding times.

($P < 0.001$). Because CP content and in vitro CP digestibility (both factors involved in ruminal $\text{NH}_3\text{-N}$ concentration) were similar in A and concentrate, the shift in the level of concentrate did not increase ruminal NH_3 concentration in animals fed diets based on A ($F \times F:C$ interaction; $P = 0.002$). Factors promoting greater NH_3 yield, such as the presence of A and a high proportion of concentrate in the diet, also seemed to be responsible for greater ($P \leq 0.03$) urinary PD excretion. A relationship between rumen $\text{NH}_3\text{-N}$ concentration above the minimum required to maximize microbial protein synthesis (3.3 to 8.5 mg/100 mL; Kang-Meznarich and Broderick, 1981) and urinary PD excretion was also found by Balcells et al. (1993). Ruminal $\text{NH}_3\text{-N}$ values were within the range of those found in previous studies with goats fed similar diets (Molina-Alcaide et al., 2000; Fujita et al., 2006) and less than those reported by Isac et al. (1994) and Yáñez-Ruiz et al. (2004). Concentration of NH_3 over time (Figure 1C) was similar among diets (time after feeding $\times F \times F:C$ interaction; $P = 0.13$), peaking about 2 h after feeding.

Total protozoa numbers were affected by $F:C$ ($P < 0.001$) and F ($P < 0.04$), and they also were different ($P < 0.001$) among diets across time (Figure 1D). Despite differences observed among ruminants for protozoa numbers (Santra et al., 1998; Yáñez-Ruiz et al., 2004) and the role of protozoa in fiber degradation and N turnover in the rumen (Eugene et al., 2004), information about diurnal variation of rumen protozoa numbers in goats fed different diets is very scarce in the literature. The progressive decrease of ciliate protozoa in the rumen after feeding has been widely described and ascribed to sequestration of Entodiniomorpha and also to the dilution effect of saliva influx and passage rate (Dehority, 2003). Total protozoa numbers were similar to values obtained by Yáñez-Ruiz et al. (2004) in Granadina goats fed A or A plus concentrate. Greater protozoa numbers were found with mixed diets than with those based on forage alone (Grubb and Dehority, 1975). Dennis et al. (1983) reported greater protozoa numbers in cattle as the proportion of concentrate in the diet increased from 30 to 70%, as found in the present study. Greater rapidly fermentable carbohydrates in diets including high levels of concentrate may explain the increase of protozoa numbers (Franzolin and Dehority, 1996). The proportion of holotricha was less ($P < 0.01$) for high- than low-concentrate diets, likely because the increase in the amount of starch (Dennis et al. 1983) and the lesser pH (Williams and Coleman, 1991) with high-concentrate diets may promote greater numbers of entodinia compared with holotricha.

Microbial N Flow

Creatinine excretion (Table 5) varied from 410 to 467 $\mu\text{mol/kg}$ of $\text{BW}^{0.75}$, which was within the range of values reported for goats and sheep fed at maintenance level (Chen et al., 1992; Yáñez-Ruiz et al., 2004) and greater than those obtained by Mota et al. (2008) with

similar diets fed to lactating goats (382 $\mu\text{mol/kg}$ of $\text{BW}^{0.75}$). Because BW of the experimental animals did not change over time and urinary excretion of creatinine is considered as an index of lean body mass (Van Niekerk et al., 1963), the similar levels of creatinine excretion among animals and across periods in the present experiment ensures that total urine production was indeed collected.

Total urinary excretion of PD in animals fed diets based on A was similar to that obtained by Belenguer et al. (2002) in Granadina goats fed A at maintenance level (701 μmol of PD/kg of $\text{BW}^{0.75}$). However, our values were less (792 vs. 982 $\mu\text{mol/kg}$ of $\text{BW}^{0.75}$) than those obtained by Mota et al. (2008) in lactating Granadina goats fed A and concentrate (75:25), which may be due to greater OM and N intakes in lactating than in dry goats; the contribution of milk PD to total excretion in lactating goats is very low (from 0.3 to 1%). Values obtained in animals fed diets based on G are slightly less than those found by Chen et al. (1992) and by Santoso et al. (2007) in goats fed a 70:30 diet based on G and concentrate (700 $\mu\text{mol/kg}$ of $\text{BW}^{0.75}$) and in sheep fed a diet based on timothy hay supplemented with rolled barley (649 $\mu\text{mol/kg}$ of $\text{BW}^{0.75}$), respectively. However, the intake was slightly greater in both studies than that of animals consuming diets based on G in the present experiment (66.0 and 56.6 g of DM/kg of $\text{BW}^{0.75}$, respectively, vs. 46.1 g of DM/kg of $\text{BW}^{0.75}$).

Urinary PD excretion (mmol/d) was greater ($P < 0.001$) in animals fed diets based on A than in those receiving diets including G. On the other hand, urinary PD excretion was greater ($P = 0.03$) with high- than low-concentrate diets. However, despite the fact that high-concentrate diets led to greater ($P = 0.002$) digestible OM intake (DOMI), the relationship between urinary PD excretion and DOMI previously reported (Chen et al., 1992; Pérez et al., 1998) was only significant ($P < 0.05$) in goats fed diets based on G ($F \times F:C$ interaction; $P = 0.08$). Ruminal microbial protein synthesis depends mainly on an adequate supply of carbohydrates as the energy source, which is believed to be the main factor limiting microbial growth. The supply of available N and the synchrony with which N and energy become available are also factors influencing microbial protein synthesis in the rumen (Bach et al., 2005). In this experiment, high levels of concentrate enhanced DOMI ($P = 0.002$) with both forages, whereas N intake only increased in goats receiving diets based on G ($F \times F:C$ interaction; $P = 0.01$). The PD excretion seemed to depend not only on DOMI but also on N intake, which should be also taken into account when interpreting urinary PD excretion results.

In general, estimation of microbial N supply from urinary PD excretion assumes a constant PB:N ratio in ruminal microbial pellets (Chen et al., 1992). However, this ratio may change with diet (Ranilla and Carro, 2003), time after feeding (Cecava et al., 1990), type of bacterial pellet (Firkins et al., 1987), or incomplete degradation of dietary purines in the rumen (Smith et

Table 5. Effect of forage source (F) and forage:concentrate ratio (F:C) on daily urinary purine derivatives excretion and microbial N flow in goats consuming the experimental diets

Item	Diet ¹				SEM	<i>P</i> -value		
	GF	GC	AF	AC		F	F:C	F × F:C
Digestible OM intake, g/kg of BW ^{0.75}	29.7 ^a	35.4 ^b	30.4 ^a	35.2 ^b	0.49	0.84	0.002	0.69
Digested N intake, g/kg of BW ^{0.75}	0.59 ^a	0.98 ^b	1.22 ^c	1.31 ^c	0.019	<0.001	<0.001	0.01
Purine derivatives in urine, µmol/kg of BW ^{0.75}								
Allantoin	422 ^a	593 ^b	692 ^c	718 ^c	19	0.005	0.10	0.23
Xanthine	4.49	7.00	14.6	13.5	1.7	0.05	0.83	0.61
Hypoxanthine	15.3	21.6	39.7	37.8	5.5	0.12	0.84	0.72
Uric acid	20.0	26.2	38.5	28.8	2.3	0.03	0.34	0.32
Total purine derivative excretion								
mmol/d	8.40 ^a	11.5 ^b	13.8 ^b	14.3 ^b	0.33	<0.001	0.03	0.08
µmol/kg of BW ^{0.75}	462 ^a	648 ^b	785 ^b	798 ^b	19	<0.001	0.04	0.08
mmol/kg of RDOM ²	24 ^a	28 ^{ab}	40 ^c	34 ^{bc}	0.89	<0.001	0.73	0.04
µmol/g of digested N	795 ^b	668 ^{ab}	641 ^{ab}	612 ^a	13	0.006	0.02	0.10
Creatinine excretion, µmol/kg of BW ^{0.75}	410	467	462	415	14	0.99	0.90	0.23
Purine bases:N ratio, µmol/mg								
Liquid-associated bacteria (LAB)	0.98	1.12	1.10	0.89	0.062	0.67	0.69	0.20
Solid-associated bacteria (SAB)	0.72	1.14	1.16	0.71	0.083	0.76	0.99	0.53
Average value LAB + SAB	0.85	1.13	1.13	0.80	0.061	0.95	0.91	0.36
Microbial N flow, ³ g/d								
LAB	12.1 ^a	14.7 ^{ab}	18.1 ^b	23.1 ^c	0.45	<0.001	0.006	0.23
SAB	16.3 ^a	14.4 ^a	17.0 ^a	28.9 ^b	0.63	<0.001	0.002	<0.001
Average value LAB + SAB	13.9 ^a	14.5 ^a	17.5 ^a	25.6 ^b	0.60	<0.001	0.003	0.009
Microbial synthesis efficiency, g/kg of RDOM								
LAB	35 ^a	36 ^a	51 ^b	56 ^b	1.2	<0.001	0.29	0.49
SAB	47 ^a	36 ^a	49 ^a	69 ^b	1.6	<0.001	0.13	<0.001
Average value LAB + SAB	40 ^{ab}	36 ^a	50 ^{bc}	59 ^c	1.5	<0.001	0.19	0.01

^{a-c}Within a row, means without a common superscript letter differ ($P < 0.05$).

¹GF = 70% grass hay and 30% concentrate, DM basis; GC = 30% grass hay and 70% concentrate, DM basis; AF = 70% alfalfa hay and 30% concentrate, DM basis; AC = 30% alfalfa hay and 70% concentrate, DM basis.

²RDOM = rumen-degradable OM, calculated as $0.65 \times$ digestible OM intake.

³Calculated as reported by Belenguer et al. (2002): Microbial N flow = (PD excretion/0.76)/(0.92 × PB:N ratio), where 0.76 is the incremental recovery of purine derivatives (PD), 0.92 is the true digestibility of duodenal purine bases (PB; Chen et al., 1990), and PB:N is the ratio between PB and N content recorded in LAB, SAB, or an average value from both bacterial pellets.

al., 1978). The isolation of bacterial pellets from the rumen liquid phase is unlikely to be representative of the whole microbial biomass reaching the duodenum, because it lacks both protozoa cells and, what is more important, SAB, which may account for up to 75% of the total microbial mass (Craig et al., 1987). Despite that protozoa are largely retained within the rumen, its contribution to the duodenal N flow may be as high as 40% of the total MNF (Yáñez-Ruiz et al., 2006). Thus, it seems necessary to take into account the composition of both protozoa and SAB when estimating the contribution of microbial N to the duodenal flow.

In the present study, despite the fact that similar PB:N ratio values were found in LAB vs. SAB across the diets ($P = 0.17$; data not shown), estimations of MNF to the duodenum ($P = 0.02$; data not shown) and the microbial N synthesis efficiency ($P = 0.01$; data not shown) depended on the bacterial pellet used, suggesting that much of the variation reported in the literature is associated with the bacterial pellet of choice and the method used to isolate it. The PB:N values found in LAB were less than those obtained by Belenguer et al. (2002) in goats fed A at maintenance level (1.92 µmol/mg) and by Chen et al. (1992) in sheep (1.66 µmol/mg).

However, our results agree with the assumption made by Chen et al. (1992) concerning the lack of diet effect on the PB:N ratio of mixed ruminal microbes, because there were no differences in the PB:N ratio in LAB, SAB, or an average value from SAB and LAB among treatments ($P > 0.05$). The use of LAB or SAB pellets to estimate MNF produced contradictory results; when LAB was used, MNF was greater ($P = 0.006$) for high- than for low-concentrate diets, whatever the forage used. When SAB or an average value from SAB and LAB were considered, differences ($P < 0.01$) were found between diets based on G and A. Microbial yield efficiency ranged from 35.2 to 69.2 g of N/kg of rumen-degradable OM (RDOM), depending on the bacterial pellet used for MNF estimation. With LAB, efficiency was greater ($P < 0.001$) for diets based on A than for those based on G, which might be due to less N intakes for the latter. High concentrate in diet did not modify ($P > 0.05$) microbial synthesis efficiency, likely because greater protozoa numbers ($P < 0.001$) result in more bacterial predation (Harrison et al., 1979). Most of the values obtained were above the mean value (32 g/kg of RDOM) established by the ARC (1984) for sheep fed different diets and much greater than values reported

by others (Chen et al., 1992; Pérez et al., 1996; Fujihara et al., 2005) despite similar levels of PD excretion, which may be due to the decreased PB:N ratio found in this experiment in comparison with others. Obispo and Dehority (1999) suggested that mixed ruminal bacteria may be contaminated with feed particles containing protein, which would result in an overestimation of MNF due to a decreased PB:N ratio. When the PB:N ratio proposed by Belenguer et al. (2002) was used in the present experiment, both MNF (g/d) and efficiency (g of N/kg of RDOM) would be within the range of those calculated by others (data not shown). This underlines the importance of using an adequate and representative bacterial pellet to calculate the MNF, and to determine the PB:N values in different pellets, animal species, and diets.

Our results show that the magnitude of the effect of shifting the F:C from 70:30 to 30:70 in goat diets depends on the forage type. With A, the benefit of increasing concentrate level in the diet, especially in terms of achieving greater urinary PD excretion and greater N retention efficiency, was less evident than in the case of G. This could be explained by the greater CP content and digestibility in legume vs. G forages. Therefore, low-concentrate diets (70:30, F:C) may not have detrimental effects on N metabolism if good quality forages such as A are used. The MNF estimated in goats fed different diets was influenced by the bacterial pellet of choice (LAB or SAB). In addition, the observed PB:N values were different from those reported in the literature, which underlines the need for these parameters to be analyzed directly in pellets isolated from specific animals and experimental conditions.

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