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In vivo digestibility and *in vitro* gas production of diets supplemented with fibrolytic enzymes in dairy goats*

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ABSTRACT

Digestive effects of diet supplementation with an exogenous fibrolytic enzyme preparation (cellulase and xylanase activities) were evaluated under *in vivo* and *in vitro* conditions. Eight lactating Murciano-Granadina dairy goats (four per treatment) were used to measure the total tract digestibility of an *ad libitum* total mixed ration (forage, 65%; concentrate, 35%) to which the enzyme was, or was not, added to the concentrate. *In vitro* degradability and gas production were also studied using 2 goats (1 per dietary treatment) as ruminal liquor donors. Dry matter digestibility increased by effect of the enzyme supplemented goats, while digestibilities of organic matter, protein and fibre fractions did not vary. Differently, *in vitro* trial showed no differences either in dry matter or fibre degradabilities or in gas production. In conclusion, supplementing dairy goat concentrate with a fibrolytic enzyme preparation, under our conditions, enhanced *in vivo* dry and organic matter digestibilities although the effects were not observed *in vitro*.

KEY WORDS**:** dairy goat, fibrolytic enzyme, digestibility, gas production

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INTRODUCTION

Exogenous enzymes are commonly used to improve the nutrient value of feeds for non-ruminants and as silage additives. The use of fibrolytic enzymes in ruminant diets has received considerable research interest in dairy cattle, although performance responses were variable (Beauchemin et al., 1999, 2000; Yang et al., 1999; Kung et al., 2000). The same controversial results have been observed in goats (Titi and Lubbadeh, 2004; González et al., 2008).

Responses observed when enzymes are added to ruminant diets are not due to a single effect, rather, they are the result of a combination of pre- and post-feeding mechanisms (Colombatto et al., 2003). Exogenous enzymes can enhance fibre degradation both *in vitro* and *in vivo* (Feng et al., 1996; Yang et al., 1999), while the beneficial effects of fibrolytic enzymes appear to be related to improvements in rumen digestibility (Feng et al., 1996; Beauchemin et al., 1999; Yang et al., 1999).

Despite not being perfect for measuring fermentation by rumen microorganisms, the technique of *in vitro* gas production is convenient to use as a first approximation of diet digestibility, and it is particularly useful for comparative purposes; measuring the impact of enzymes on rumen fermentation is one of those applications for which it would be expected to be most valid (Wallace et al., 2001).

The objective of this study was to evaluate the effects of using an exogenous fibrolytic enzyme preparation, characterized by cellulase and xylanase activities, on the *in vivo* total tract digestibility and *in vitro* gas production and degradability of the diets previously tested in lactating Murciano-Granadina dairy goats (González et al., 2008).

MATERIAL AND METHODS

The experiment was conducted on the Experimental Farm of the Universitat Autònoma de Barcelona. Experimental and animal care procedures were approved by the Ethical Committee on Human and Animal Experimentation of the UAB.

Digestibility experiment

Eight lactating Murciano-Granadina dairy goats were used to measure the digestibility of an *ad libitum* total mixed ration (TMR), based on 65% forage and 35% concentrate, the enzyme being added or not added to the concentrate. Diets and feeding procedure (once daily at 130% previous day intake) were also similar to those used in the previously done lactation experiment. Goats (4 per treatment) with body weight (BW), 41.7±1.6 kg, milk yield, 1.52±0.05 l/d and milk composition

similar to the group average, were selected at the end of the previously done lactation experiment (wk 26) (González et al., 2008), and housed in individual collection crates from wk 27 to 30 of lactation (the last week being the collection period). During the collection week, offered and refused feed were recorded daily to determine voluntary intake. Samples of diet and orts were collected daily and composited for each animal. Dry matter (DM) contents of diets and orts were determined by oven drying at 105ºC for 24 h. Total daily faeces were collected and weighed and a 10% representative sample per goat was retained. The faecal samples were composited for each animal and stored at -20ºC until analysis.

In vitro gas production experiment

The ruminal fermentation pattern of experimental diets was studied by gas production and DM and neutral detergent fibre (NDF) disappearance during *in vitro* incubations, following the technique of Theodorou et al. (1994). Representative samples of the TMR were ground to pass through 1-mm stainless steel screen (Cyclotec 1093 Sample mill, Tecator, Sweden) and used as substrate for the incubations. In our experiment, substrate (0.8 g) was incubated by duplicate in sealed 110 ml bottles with 80 ml of incubation solution. Duplicate bottles of incubation solution without substrate were also included as blanks.

Two culled goats (1 goat adapted per each diet treatment) from the previous lactation experiment (González et al., 2008) were euthanized with sodium pentothal (4 ml i.v., Laboratorios Abbott, Madrid, Spain) before feeding (8 h); these goats were used as donors of ruminal liquor for the incubations. Total rumen content was removed and equal amounts of cheese-cloth filtered rumen liquor were collected and used for preparation of inoculum solutions at the final ruminal liquor to buffer proportion of 1:9. Incubations were made using the two inoculum solutions and the two substrates according to a 2×2 factorial design by duplicate. Bottles were incubated at 39ºC and the pressure of gas produced in each bottle was recorded by means of a manometer with a pressure gauge (Delta OHM, Padua, Italy) after 2, 4, 8, 20, 24, 32 and 48 h of incubation. Gas production was not recorded after 48 h because of the concentrate characteristics of the experimental diets according to Wallace et al. (2001). Pressure readings were converted into volume by using a linear regression, previously recorded in the same type of bottles with known air volumes. The pH of the incubated bottles was checked at 48 h to detect if their buffer capacity was surpassed.

Analytical procedures

Samples of TMR, orts and faeces and enzyme activities were analysed as described in Gonzalez et al. (2008).

Calculations and statistical analyses

Energy balance was determined for each goat during the digestibility trial. Net energy intake was calculated from OM digestibility according to INRA methodology (Jarrige, 1989). Milk energy was calculated as 4% energy corrected milk (l) × 3.12 (MJ/l). Net energy for maintenance was calculated by using the specific values calculated by Aguilera et al. (1990) in lactating Granadina dairy goats. Energy balance was calculated as the difference between energy input and output.

Analyses of variance for data of the *in vivo* digestibility experiment were performed using the GLM procedure of SAS v. 8.1 (SAS Inst. Inc., Cary, NC). The model included the effects of experimental treatment and the residual error.

Gas volume for each incubation time was expressed per unit of incubated substrate DM and obtained data was analysed according to Ørskov and McDonald (1979) and France et al. (1993), considering that the nonlinear model adapts to the gas production data. Incubation residues were filtered and analysed for DM and NDF disappearance.

In vitro gas production data were analysed by using the GLM procedure for repeated measurements of SAS, considering the effects of treatment, inoculum source, the interaction between treatment and inoculum source, and the residual error. Disappearance of DM and NDF were also analysed using the same procedure without repeated measures. For all cases, differences were tested using the PDIFF option of SAS and were declared significant at $P<0.05$; tendencies were discussed at $P<0.10$.

RESULTS AND DISCUSSION

Nutrient digestibility. Although no differences in total daily DM intake were observed in the digestibility experiment (Table 1), the enzyme supplemented goats had lower intake values ($P<0.05$) than control goats when expressed relative to $BW^{0.75}$.

Digestibility of DM was significantly higher (4.4%; $P<0.05$) for the enzyme supplemented diet than for the control diet (Table 1), and a similar tendency was observed in OM digestibility (3.6%; $P=0.07$). In contrast, the increase in CP, ADF and NDF digestibilities by enzyme supplementation were non significant ($P>0.05$). Our results in goats agree with other studies in dairy cows (Beauchemin et al., 1999; Rode et al., 1999; Yang et al., 1999) and in fattening cattle (Beauchemin et al., 1995) which reported an improvement (3 to 12%) in total tract DM and OM digestibilities as a result of enzyme supplementation. Nevertheless, the decrease in

Table 1. Effects of enzyme supplementation on intake, digestibility and energy balance of lactating dairy goats

Item	Control	Enzyme	SEM	Effect (P =)
Standard milk yield	1.69	1.69	0.08	0.97
Daily feed intake				
kg DM	2.18	2.13	0.11	0.77
g DM/kg BW $^{0.75}$	128	118	3	0.04
Digestibility, %				
dry matter	68.9	71.9	0.8	0.01
organic matter	70.4	72.9	0.9	0.07
crude protein	59.6	63.0	2.2	0.28
NDF	52.6	55.3	1.5	0.25
ADF	46.4	50.5	2.0	0.19
Body weight, kg	43.63	47.50	2.21	0.26
Change in BW, g/d	7	17	3	0.20
Energy balance, MJ/d				
NE_L intake	13.81	13.84	0.68	0.79
milk energy output[1]	5.30	5.30	0.29	0.95
maintenance requirement[2]	6.19	6.60	0.21	0.25
energy balance	2.35	1.38	0.37	0.18

[1] estimated at 3.14 MJ/L, [2] calculated according to Aguilera et al. (1990)

feed intake relative to $BW^{0.75}$, as indicated above, may also have contributed to the increase of the digestibility values in the goats fed the enzyme supplemented diet.

No differences in ruminal OM digestibilities (apparent and corrected) or in OM entering the small intestine were observed in dairy cows (Beauchemin et al., 1999; Yang et al., 1999), and no differences were observed either in DM and OM total tract digestibilities in wether lambs (Judkins and Stobart, 1987) or in fattening cattle (Krause et al., 1998). Consistent with our results, no significant effects of enzyme supplementation in cell wall digestibility were observed by Burroughs et al. (1960) and Hristov et al. (2000) in fattening cattle and heifers, respectively. Moreover, Judkins and Stobart (1987), Yang et al. (2000) and Pinos-Rodríguez et al. (2002) also reported non significant effects of enzyme supplementation on NDF and ADF digestibilities in lambs.

On the contrary, other studies reported an increase in NDF and ADF digestibilities with a similar fibrolytic enzyme mixture to the one we used (Beauchemin et al., 1999; Rode et al., 1999). Enzyme supplementation also improved NDF and ADF digestibilities in beef steers fed forage diets (Beauchemin et al., 1995; Feng et al., 1996; Krause et al., 1998). Increased digestibility by effect of enzyme translated into increased milk yield (Rode et al. 1999; Yang et al., 1999; Kung et al., 2000) or

into increased growth rate in fattening cattle (Burroughs et al., 1960; Beauchemin et al., 1995).

Yang et al. (2000) tried to support the improvement of digestibility observed in dairy cows with two digestion experiments in wether lambs with no significant effects being found between control and enzyme supplemented diets. They concluded that enzyme supplementation enhanced digestion in dairy cattle because they had a considerably greater intake and ruminal particulate passage, and lower digestibility than sheep or did not achieve the potential digestibility attainable *in vitro*.

As DM intake relative to BW in our experiment in dairy goats (4.8%) was greater than the values estimated from the data of Yang et al. (2000) in dairy cows (3.1%) and in wether lambs (2.4%), this hypothesis seems to be also applicable to explain the increase in DM and OM digestibilities in our case.

Similar to the lactation experiment, there was not difference between treatments for actual or 4% FCM corrected milk yield. No changes in BW were neither found and the energy balance was also similar for both group of goats.

In vitro gas production. The pH in the incubated bottles was maintained in the range of 6.2 to 6.5 for the different treatments, so no effects were expected as a direct consequence of changes in buffer capacity. Cumulative gas production (Table 2) was not affected by enzyme supplementation averaging 14.3±1.8 ml/100 mg (15.7 ml/100 mg OM). There were no effects either of inoculum or of experimental treatments. Total disappearance of DM (517.8±6.5 g/kg)

Table 2. Effect of fibrolytic enzymes on cumulative gas production, and DM and NDF disappearance

Item	Control	Enzyme	SEM	Effect (P =)	
				inoculum	treatment
Gas production					
Ørskov and McDonald (1979)[1]					
a	0	0	-	-	-
b, ml/g DM	142.4	143.9	0.18	0.34	0.44
c, h^{-1}	0.056	0.051	0.001	0.28	0.29
France et al. (1993)[2]					
A, ml/g DM	142.4	143.9	0.18	0.34	0.44
b, h^{-1}	0.093	0.099	0.0037	0.17	0.22
c, $h^{-1/2}$	-0.089	-0.099	0.0408	-	-
L, h	0.91	1.03	0.063	-	-
DM disappearance, %	52.5	51.1	1.1	0.18	0.20
NDF disappearance, %	39.6	35.7	4.1	0.21	0.32

[1] equation: $y = a + b\ (1 - e^{-ct})$ for t time; a initial gas production; (a + b) is potential gas production, and c is the constant rate of gas production per h; [2] equation: $y = A\ [1 - \exp\{ - b(t - L) - c\ (\sqrt{t} - \sqrt{L})\}]$, $t \geq L$ were y denotes cumulative gas production, t is the incubation time, A is the asymptote (total gas), b is a rate constant (h^{-1}), L is lag phase (h) and c is a rate constant ($h^{-1/2}$)

and NDF (376.1±12.5 g/kg) did not vary for inoculums and diets. Thus, the improvement in DM digestibilities observed in the *in vivo* experiment could not be confirmed in the *in vitro* conditions.

Similar lack of effect of the enzyme supplementation on gas production was reported by Yang et al. (2000) in an analogous experiment comparing *in vitro* and *in vivo* conditions. The lack of response in our *in vitro* experiment may also be a consequence of the static fermentation conditions used which agrees with the previously discussed differences between wether lambs and dairy cattle digestibility experiments of Yang et al. (2000).

CONCLUSIONS

Total tract digestibility results could not be supported by the *in vitro* trial in which similar values were observed for DM and NDF degradability and gas production for both diets. Supplementing dairy goat concentrate with a fibrolytic enzyme mixture, under the conditions of this trial, enhanced DM and OM *in vivo* total tract digestibility.

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