

Performance of dairy ewes fed diets with a fibrolytic enzyme product included in the concentrate during the suckling period

C. Flores^{1,2}, G. Caja^{1†}, R. Casals¹, E. Albanell¹ and X. Such¹

¹Grup de Recerca en Remugants, Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain; ²Departamento Agrobiología, Universidad Autónoma de Tlaxcala, 90120 Ixtacuixtla, Tlaxcala, México

(Received 30 May 2007; Accepted 14 February 2008)

Seventy-two multiparous ewes from two dairy breeds (Manchega, n = 36 and Lacaune, n = 36) were used in a replicated 2 × 2 factorial design to evaluate the effects of diet supplementation with an exogenous fibrolytic enzyme product on lactation performance and feed intake during the suckling period (weeks 1 to 4) according to breed. Ewes were blocked in groups of nine and fed ad libitum after lambing a diet based on 70% forage and 30% concentrate to which the enzyme was added after pelleting. Experimental concentrates were: control (without enzyme) and enzyme (fibrolytic enzyme complex, included at 0.47% volume to weight of concentrate). Twenty-four dry and open ewes (Manchega, n = 12 and Lacaune, n = 12) were also grouped by breed and used to measure the fill value of the ration used. During the suckling period, milk yield, milk composition, dry matter intake, lamb growth, as well as body weight change and body condition score change were not affected by enzyme supplementation. Breed effect was significant for milk yield, the Manchega ewes yielding less milk with a higher content of milk components than the Lacaune ewes. The opposite was observed for dry matter intake. Enzyme supplementation reduced intake by 9% in the dry ewes, resulting in a greater fill value of the diet. In conclusion, no lactational effects were detected when the fibrolytic enzyme product was added to the concentrate fed to dairy ewes.

Keywords: dairy sheep, fibrolytic enzyme, fill value, intake, suckling

Introduction

More than half of plant dry matter (DM) consists of carbohydrates, of which starch and cellulose are considered to be the most abundant polysaccharides. Natural substances contained in forages and grains may reduce the digestibility of polysaccharides by decreasing the activity of ruminal microbiota or by protecting the cell wall (Minson, 1990). Condensed tannins and methylcellulose prevent microbial attachment or promote microbial detachment, partially or completely inhibiting cellulose digestion. Moreover, the protein matrix of cereal grains is extremely resistant to microbial attachment and penetration (McAllister *et al.*, 1994).

Applied biotechnology and feed industries currently offer exogenous enzymes as feed additives for enhancing the nutritive value of animal diets. There are many studies about adding enzyme preparations to diets for non-ruminant species, but research for ruminants is more limited. Beauchemin *et al.* (2003) reviewed the use of enzymes in ruminants indicating an increase in DM and fibre digestion

in vitro, *in situ* or *in vivo* and an increase in milk yield and weight gain in cattle, although results did not agree in all cases. Much of the variability can be attributed to factors such as type, dose and activity of the enzyme; application method and portion of the diet (forage or concentrate) to which the enzyme was applied and differences in the physiological status of the test animals (Bowman *et al.*, 2002; Beauchemin *et al.*, 2003). Addition of enzymes in the concentrate portion of the diet is especially interesting in practice when enzyme activity is not compromised (Bowman *et al.*, 2002 and 2003).

The use of enzymes in sheep diets has been limited to digestibility trials in wethers (Lee *et al.*, 2000; Pinos-Rodríguez *et al.*, 2002) and to growth trials with fattening lambs (McAllister *et al.*, 2000; Muwalla *et al.*, 2007; Miller *et al.*, 2008). As far as we know, however, only one experiment has been conducted with suckling sheep and goats (Titi and Lubbadah, 2004). The objective of this study was to evaluate the effects of diet supplementation with a commercial xylanase and cellulase fibrolytic enzyme product included in the concentrate on the lactation performances of suckling ewes.

† E-mail: gerardo.caja@uab.es

Material and methods

Two experiments aiming to investigate the effects of adding a fibrolytic enzyme product to the diet of dairy ewes were conducted on the experimental farm of the 'Servei de Granges i Camps Experimentals' of the Universitat Autònoma de Barcelona in Bellaterra (Spain). The experimental and animal care procedures were approved by the Ethical Committee on Human and Animal Experimentation of the Universitat Autònoma de Barcelona (Reference CEEAH 03/429).

Lactation experiment

Animals and treatments. Experiment consisted of a 2 × 2 replicated factorial design in which two dairy sheep breeds in early lactation were used to investigate the effects of two dietary treatments consisting of including or not including a fibrolytic enzyme product in the concentrate. In all, 72 multiparous ewes from two dairy breeds (Manchega and Lacaune) were used from weeks 1 to 4 after lambing. Ewes (Manchega, $n = 36$, 74.6 ± 1.2 kg BW and Lacaune, $n = 36$, 73.1 ± 1.4 kg BW; mean \pm s.d.) were housed in eight balanced groups (nine ewes per group) according to breed, number of lactation, BW and body condition score (BCS) at lambing. Ewes were confined to straw-bedded pens and the balanced groups of each breed randomly assigned to the dietary treatments. Lambs were allowed to suckle from their mothers 24 h a day until week 4 after birth.

Dietary treatments started immediately after lambing and were control (without enzyme) and enzyme (supplemented in the concentrate).

Diets. The fibrolytic enzyme product used was a commercial product (Promote[®]; Agribrands International, St Louis, MO, USA) characterized by high cellulase (130 U/g) and xylanase (120 000 U/g) activities (P. Frumholtz, Agribrands International, personal communication).

Ewes and lambs were moved to the experimental pens after lambing where the ewes received an *ad libitum* total mixed ration based on 70% forage (dehydrated mixture of 50% alfalfa and 50% maize-whole plant) and 30% concentrate pellets to which the fibrolytic enzyme product was or was not added. Lambs were supplemented with creep-feeding using a commercial starter concentrate (DM, 87.9%; CP, 19.3%; ether extract, 3.3%; NDF, 17.0%; DM basis; Fimsa, La Bisbal del Penedés, Tarragona, Spain).

The enzyme product was added to the entire concentrate according to the conclusions of Bowman *et al.* (2002). The liquid enzyme preparation was sprayed (0.47 ml/kg of concentrate) onto the previously manufactured and cooled concentrate pellets in a horizontal mixer. Diet ingredients are shown in Table 1. The total mixed ration was offered twice daily (0900 and 1500 h) at a rate of 115% of the voluntary intake from the previous day. The concentrate was included in the ration at a rate of 0.8 kg per kg of forage offered. Fresh water was permanently available in the pens.

Table 1 Ingredients of the experimental diets

	Forage mixture	Concentrate
Maize-whole plant, dehydrated (g/kg)	500	–
Alfalfa hay, dehydrated (g/kg)	500	–
Alfalfa meal pellets (g/kg)	–	379
Barley meal (g/kg)	–	119
Spanish ground corn (g/kg)	–	117
Soybean-44 meal (g/kg)	–	225
Whole sunflower-seed meal (g/kg)	–	147
Limestone (g/kg)	–	10
Mineral–vitamin mix [†] (g/kg)	–	3

[†]Supplied by Agribrands Europe-España, Barcelona, Spain. The preparation contained per kg of product: 105.0 g Ca, 20.0 g Mn, 17.5 g Fe, 15.0 g Zn, 250 mg I, 100 mg Se, 50 mg Co, 3600 IU of vitamin A, 700 IU of vitamin D₃, 22 000 IU of vitamin E (α -tocopherol).

Fill value evaluation experiment

In all, 24 dry and open ewes from the two dairy breeds (Manchega, $n = 12$, 71.4 ± 1.9 kg BW and Lacaune, $n = 12$, 70.3 ± 1.4 kg BW) were used over a 2-week adaptation period and a 5-week measurement period to estimate the ration fill value (FV). The FV was calculated as defined by the INRA system (Jarrige, 1989) by dividing the voluntary DM intake of the reference pasture hay (75 g DM/kg BW^{0.75}) by the measured DM intake of that forage in wethers. In our case we used dry and open ewes according to Bocquier *et al.* (1987) and Caja *et al.* (1997 and 2002). The experiment consisted of a 2 × 2 replicated factorial design as in the lactation experiment.

The dry and open ewes were distributed into four balanced groups (six ewes per group) according to breed, BW and BCS, and housed in straw-bedded pens next to the dairy ewes. Straw was not replaced during the experiment to prevent consumption. Diets, feeding management, and BW and BCS measurements were similar to those used in the lactation experiment.

Measurements, sampling and analysis

Enzyme activity. Endoglucanase activity of the fibrolytic enzyme product was measured using acetic acid/di-sodium phosphate buffer (pH 6.5) and carboxymethylcellulose as substrate (C-5678; sodium salt, medium viscosity; Sigma, St. Louis, MO, USA). A solution was prepared by adding 20 μ l of enzyme solution (4%) and 250 μ l of substrate solution to a tube containing 250 μ l of buffer. The mixture was incubated at 39°C for 3 h (Pastor *et al.*, 2001), and the reaction was stopped by adding Somogyi reagent and boiling for 10 min. For background corrections, incubations were also carried out with samples in the absence of substrate and enzyme solutions alone plus buffer solution. Concentrations of reducing sugars were determined by the Nelson–Somogyi copper reduction method (Somogyi, 1952) with glucose as the standard. One unit of enzymatic activity was defined as the amount of enzyme that catalyzed the release of 1 nmol of reducing sugar equivalent per min under the assay conditions described. Reducing sugars were

quantified spectrophotometrically (Spectrophotometer UV-120-01; Shimadzu, Kyoto, Japan) at 520 nm, using glucose as the standard (González, 2004).

Animal performance. Individual milk yield during suckling was estimated fortnightly using the oxytocin method (Doney *et al.*, 1979) with machine milking according to Casals *et al.* (1999). Ewes were milked twice at a 4-h interval after intravenous injections of 2 IU oxytocin (Veterin Lobulor; Laboratorios Andreu, Barcelona, Spain). Machine milking was done in a double-12 stall parallel milking parlor (Westfalia Landtechnik, Granollers, Spain) with recording jars. A small amount of concentrate (50 g per ewe) was also offered at each milking in the milking parlor to encourage the ewe to enter. Milking was conducted at a vacuum pressure of 42 kPa, a pulsation rate of 120 pulses/min and a pulsation ratio of 50%, as indicated by Such *et al.* (1999) for Manchega and Lacaune ewes. Between these two milkings, ewes were prevented from suckling their lambs. Milk secretion during 4 h was assumed to be the normal rate of milk secretion and was extrapolated at 24 h to estimate daily milk yield.

Intake of DM was calculated for each ewe group as the difference between the total amount offered and the amount refused daily. Individual lamb BW, and BW and BCS of the ewes were recorded weekly throughout the experiment. The BCS was measured on a scale of 0 to 5 (Russell *et al.*, 1969) to the nearest 0.25.

Samples and analysis. Daily samples of the ration and orts were collected and composited by period for each group and treatment throughout the experiment for analysis of composition. Samples were ground to pass through a 1-mm stainless-steel screen and were analyzed for organic matter and DM (AOAC, 2004). The CP ($N \times 6.25$) was determined using a Kjeltac Auto 1030 Analyzer (Tecator, Hogånäs, Sweden). The method of Van Soest *et al.* (1991) was used to analyze NDF and ADF using the Ankom²⁰⁰ Fiber Analyzer incubator (Ankom Technology, Fainport, NY, USA) adding amylase and sodium sulfite solutions.

Milk samples from the second milking done in the milk yield test-days were taken for milk composition analysis and preserved with potassium dichromate (0.5 ml of a 70 mg/l solution in 100 ml milk). Milk samples were analyzed for total milk solids, fat, total protein ($N \times 6.38$), true protein and casein using a near-infrared spectroscopy analyzer (Technicon InfraAnalyzer-450, Bran + Luebbe, Nordersted, Germany) according to Albanell *et al.* (1999). Calibration was checked using the AOAC (2004) reference methods. Energy-corrected milk (ECM) was calculated from milk composition according to Bocquier *et al.* (1993).

Statistical analysis

Individual data for BW of lambs and for milk yield and composition, BW and BCS of the ewes and group data for

DM intake of the ewes were analyzed using the PROC MIXED procedure with repeated measures of SAS (SAS v. 9.1; SAS Institute, Inc., Cary, NC, USA). The statistical model contained the fixed effects of treatment, parity, prolificacy, breed, week of lactation as the repeated factor, the random effects of the animal inside the group, the first-order interactions of these factors and the residual error. The PROC MIXED was also used in the ration FV experiment to analyze the effects of treatments on DM intake, and BW and BCS change. The covariance structure that yielded the smallest Schwartz Bayesian criterion was considered to be the most suitable analysis (Littell *et al.*, 1998). Differences between means were tested using the PDIFF option of SAS and were considered significant at $P < 0.05$. Trends were discussed at $P < 0.10$.

Results and discussion

Endoglucanase activity of the enzyme mixture indicated 850, 842 and 215 nmol of glucose liberated per min, for pH 4.0, 5.5 and 6.5, respectively. Chemical composition and nutritive value of the forage mixture, concentrate and ration are shown in Table 2. The concentrate fed to the enzyme group contained slightly more CP (4.1 g/kg) than the control group due to differences in the manufacturing process. This difference in CP supply suggested a slightly lower CP intake (<1%), which was not relevant for treatment comparison. No relevant differences between experimental diets were observed for any other nutrient either.

Lactation experiment

Intake of ration during the suckling period was high (Table 3 and Figure 1) for both ewe breeds and feeding treatments, averaging 3.0 kg DM/day. Values observed for DM intake reached 4.3% of BW and were greater than those reported by Molina *et al.* (2001) in Manchega and Lacaune dairy ewes for similar milk yield and exploitation conditions. Despite the differences in estimated milk yield between the two breeds (0.52 l/day; $P < 0.001$), no DM intake differences between them were detected during suckling, as also reported by Molina *et al.* (2001). An initial decrease in DM intake in all ewe groups was observed between weeks 1 and 2, most probably due to the adaptation of the ewes to the lactation diet and to a compensatory intake after parturition.

Enzyme supplementation treatments did not affect DM intake but interaction between treatment and breed was significant ($P < 0.001$) as a result of the different trend of DM intake change according to the week of lactation for each breed (Figure 1).

Supplementation with the fibrolytic enzyme product did not affect actual milk yield (average 2.41 l/day) or ECM (average 2.10 l/day) or milk efficiency (average 0.70 l/kg) during suckling (Table 3), but Lacaune ewes yielded more actual milk (22%; $P < 0.05$) and ECM (28%; $P < 0.001$) than Manchega ewes (Figure 2). Moreover, Lacaune ewes

Table 2 Chemical composition and nutritive value of feeds used in the experimental diets

Item	Forage mixture	Concentrates		Ration	
		Control	Enzyme [†]	Control	Enzyme [†]
Dry matter (g/kg)	936	918	919	931	931
Organic matter (g/kg)	922	901	906	916	917
Crude protein (N×6.25) (g/kg)	117	240	244	154	155
Fat (g/kg)	–	27	26	–	–
Crude fibre (g/kg)	288	176	187	254	257
Neutral detergent fibre (g/kg)	458	275	283	403	405
Acid detergent fibre (g/kg)	273	167	180	241	245
Net Energy [‡] (Mcal NE _i /kg DM)	1.36	1.56	1.51	1.41	1.41
Ca [‡] (g/kg DM)	13.8	11.5	11.3	12.9	12.9
P [‡] (g/kg DM)	3.0	4.3	5.0	3.7	3.7

[†]Fibrolytic enzyme mixture (Promote[®], Agribands International, St Louis, Missouri, USA) applied by spraying onto the concentrate at 0.47 ml/kg of concentrate.

[‡]Estimated from INRA tables (Jarrige, 1989) by using the PreValim 2.7 software.

Table 3 Effects of enzyme supplementation on lactational performance of dairy ewes during the suckling period[†]

Item	Treatment		Ewe breed		s.e.	P value		
	Control	Enzyme	Manchega	Lacaune		Enzyme	Breed	E × B
DM intake								
kg/day	2.99	2.99	2.98	2.99	0.02	0.851	0.843	0.998
% body weight	4.26	4.16	4.14	4.30	0.03	0.436	0.773	0.357
Milk (l/day)								
Actual	2.42	2.40	2.17	2.65	0.07	0.930	0.004	0.529
ECM	2.09	2.10	1.84	2.36	0.07	0.922	0.001	0.579
Efficiency (l/kg DM)	0.70	0.70	0.61	0.79	0.02	0.864	0.008	0.141
Body weight (kg)								
Initial	73.5	74.4	74.6	73.1	0.9	0.864	0.610	0.141
Final	68.7	69.8	70.5	67.7	0.9	0.663	0.007	0.230
Change	−4.8	−4.6	−4.0	−5.4	0.3	0.785	0.008	0.580
BCS								
Initial	2.93	2.94	2.96	2.90	0.04	0.885	0.444	0.831
Final	2.33	2.35	2.40	2.26	0.04	0.486	0.437	0.060
Change	−0.60	−0.59	−0.56	−0.64	0.04	0.802	0.240	0.276

[†]Abbreviations are: ECM = energy corrected milk; BCS = body condition score (scale of 0 to 5); E × B = interaction enzyme × breed.

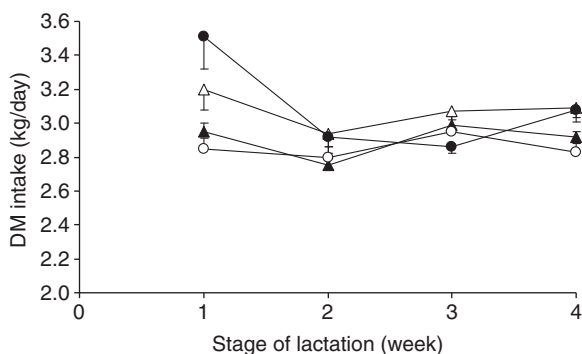


Figure 1 Dry matter intake of dairy ewes during the suckling period according to breed and dietary treatment. Each point represents the mean of 2 observations (Manchega: control, △; and enzyme, ▲; and Lacaune: control, ○; and enzyme, ●).

were 30% ($P < 0.01$) more efficient than Manchega ewes, transforming feed into milk during the suckling period (Table 3) as a consequence of their greater milk yield for a similar DM intake as observed in dairy sheep during milking by Molina *et al.* (2001) and Marie *et al.* (2002).

The enzyme supplementation affected neither final BW nor change in BW, nor BCS during suckling. Ewes of both breeds and both treatments lost BW (−4.7 kg) and BCS (−0.60) during the suckling period (Table 3; Figures 3 and 4), indicating a similar negative energy balance for both dietary treatments. Loss of BW was greater in Lacaune than in Manchega ewes (35%; $P < 0.01$) as a consequence of their greater milk yield with similar DM intake and maintenance requirements. Rode *et al.* (1999) observed an increase in milk yield (10%) in dairy cows after adding the

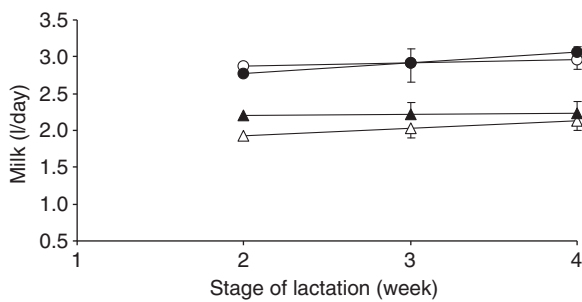


Figure 2 Milk yield of dairy ewes during the suckling period according to breed and dietary treatment. Each point represents the mean of 18 observations (Manchega: control, △; and enzyme, ▲; and Lacaune: control, ○; and enzyme, ●).

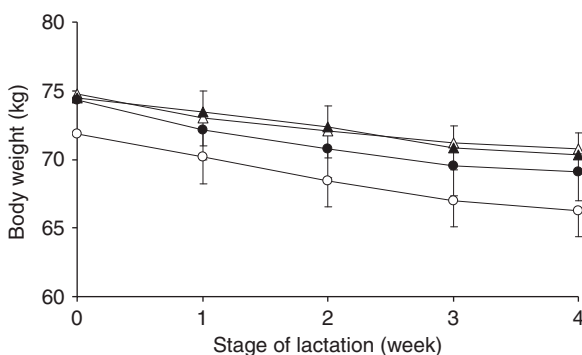


Figure 3 Body weight of dairy ewes during the suckling period according to breed and dietary treatment. Each point represents the mean of 18 observations (Manchega: control, △; and enzyme, ▲; and Lacaune: control, ○; and enzyme, ●).

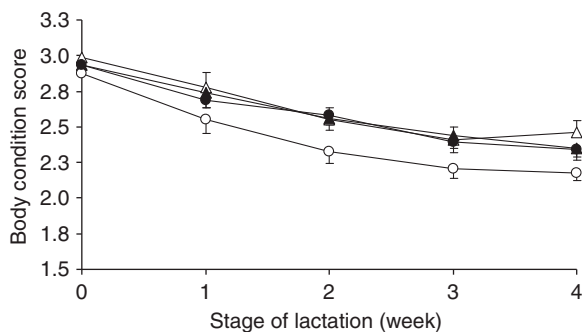


Figure 4 Body condition score of dairy ewes during the suckling period according to breed and dietary treatment. Each point represents the mean of 18 observations (Manchega: control, △; and enzyme, ▲; and Lacaune: control, ○; and enzyme, ●).

same enzyme product as in our experiment to the concentrate, and concluded that this enzyme product has a great potential application in ruminants in negative energy balance. These positive effects of enzyme supplementation were not observed in our case in dairy ewes as well as with other fibrolytic enzyme products in dairy cows at early lactation (Dhiman *et al.*, 2002; Vicini *et al.*, 2003).

Milk composition during suckling was not affected by enzyme supplementation or breed (Table 4). Lamb growth was not affected by the dietary treatments and averaged

276 g/day (Table 5). Breed of ewe significantly affected lamb BW at birth ($P < 0.001$), the Lacaune lambs being lighter than Manchega lambs (-0.6 kg; $P < 0.001$), but the difference was not significant at weaning. Lamb growth rate was similar to the values previously reported by Casals *et al.* (1999) in Manchega sheep. Manchega lambs were apparently more efficient converting milk during suckling ($P < 0.001$) than Lacaune lambs due to the lower milk yield of their mothers as estimated by the oxytocin method. Enzyme \times breed interaction was not significant. The results on lamb growth were congruent with the lack of effect of the enzyme supplementation on milk yield and composition during suckling.

Fill value evaluation experiment

The dry and open ewes used to evaluate the effect of enzyme supplementation on the FV of the ration increased BW and BCS during the ingestion experiment (Table 6) as expected because of the high nutritive value of the ration (Table 2). No significant differences in BW or BCS change were observed for breed or treatment, although Manchega ewes tended ($P < 0.10$) to be heavier than Lacaune at the end of the experiment.

The DM intake of the ration was high for ewes at maintenance requirements and differed ($P < 0.001$) for both breed and enzyme treatment (Table 6). Breed differences disappeared when daily DM intake was expressed as percentage of BW, being 2.6% BW on average. Nevertheless, Lacaune ewes showed a numerically greater DM intake than Manchega ewes (Table 6) when values were expressed per kg of metabolic BW ($P = 0.112$).

Enzyme supplementation reduced daily DM intake by 9.1% (2.03 v. 1.86 kg/day; $P < 0.001$) and intake per metabolic weight by 8.8% (80 v. 73 g/kg $BW^{0.75}$; $P < 0.001$). Differences in intake results between lactation (no change) and FV (increased) experiments may be a consequence of the lower palatability of the enzyme-supplemented ration and to the lower requirements of the dry ewes when compared with the lactating ewes. Since dry ewes were over-fed, they probably refused the unpalatable feed more easily than the lactating ewes. Moreover, enzyme supplementation may have modified the volatile fatty acids pattern in the rumen, as recently reported by Miller *et al.* (2008), being responsible for the altering intake. Beauchemin *et al.* (1995) also reported differences in the ration DM intake according to the enzyme dose in steers fed enzyme-treated forages, DM intake was reduced for medium doses whereas it increased for high and low doses. Moreover, Beauchemin *et al.* (2000) indicated greater improvements in DM intake when the enzyme was added to the concentrate portion of the ration at a low dose rather than at a high dose in dairy cows.

As a consequence of the reduced voluntary intake, sheep FV estimated according to the INRA system (Jarrige, 1989) increased by enzyme supplementation, being 0.94 and 1.03 ($P < 0.001$) for the control and enzyme-supplemented

Table 4 Effects of enzyme supplementation on milk composition of dairy ewes during the suckling period[†]

Item	Treatment		Ewe breed		s.e.	P value		
	Control	Enzyme	Manchega	Lacaune		Enzyme	Breed	E × B
Milk composition (g/l)								
Total solids	170.7	174.1	170.2	174.6	1.5	0.280	0.162	0.659
Fat	59.2	60.9	58.9	61.2	0.8	0.351	0.219	0.716
Total protein	52.1	53.0	52.3	52.8	0.3	0.170	0.458	0.840
True protein	48.4	49.4	48.6	49.1	0.3	0.146	0.415	0.871
Casein	39.6	40.3	39.8	40.1	0.2	0.116	0.431	0.718
Component yield (g/day)								
Total solids	407	403	360	450	12	0.839	0.001	0.363
Fat	149	152	129	172	5	0.818	0.003	0.659
Total Protein	124	125	111	138	4	0.907	0.002	0.570
True protein	115	116	102	128	4	0.859	0.002	0.582
Casein	94	95	84	105	3	0.855	0.002	0.537

[†]Abbreviations are: E × B = interaction enzyme × breed.

Table 5 Effects of enzyme product supplementation on lamb growth during suckling[†]

Item	Treatment		Lamb breed		s.e.	P value		
	Control	Enzyme	Manchega	Lacaune		Enzyme	Breed	E × B
No. of Lambs	49	51	52	48				
Body weight (kg)								
Birth	4.3	4.2	4.5	3.9	0.1	0.633	0.001	0.301
Weaning	11.5	12.2	12.2	11.5	0.3	0.113	0.117	0.393
Daily gain (g)	267	284	276	275	8	0.168	0.838	0.217

[†]Abbreviations are: E × B = interaction enzyme × breed.

Table 6 Effects of enzyme product supplementation on fill units evaluation in dry and open dairy ewes[†]

Item	Treatment		Ewe breed		s.e.	P value		
	Control	Enzyme	Manchega	Lacaune		Enzyme	Breed	E × B
BCS								
Initial	3.02	3.04	3.06	3.00	0.03	0.748	0.340	0.340
Final	3.35	3.44	3.41	3.38	0.04	0.269	0.576	0.269
Change	0.33	0.40	0.35	0.38	0.01	0.257	0.701	0.701
BW (kg)								
Initial	70.6	71.1	71.4	70.3	1.2	0.670	0.840	0.574
Final	74.2	74.6	74.7	74.1	1.2	0.548	0.095	0.735
Change	3.6	3.5	3.3	3.8	0.4	0.955	0.552	0.645
Average BW ^{0.75}	25.4	25.7	25.1	26.0	0.2	0.974	0.131	0.755
DM Intake								
kg/day	2.03	1.86	1.78	2.02	0.02	0.001	0.001	0.158
% of BW	2.74	2.47	2.56	2.64	0.04	0.004	0.411	0.636
kg DM/kg BW ^{0.75}	80	73	75	78	1	0.001	0.112	0.520
FV	0.94	1.03	1.01	0.961	0.01	0.001	0.111	0.520

[†]Abbreviations are: B × E = interaction enzyme × breed; BCS = body condition score (ranging from 0 to 5; FV = fill value estimated in filling units according to INRA methodology (Jarrige, 1989).

ration, respectively. The difference in FV can account for approximately 90 g/kg of ration DM intake, which cannot be attributed to changes in digestibility or rate of passage because their cell wall constituents were unchanged. These sheep FV were used to calculate the voluntary intake of the ewes during the lactation experiment using the

equation proposed by Caja *et al.* (1997 and 2002) for dairy ewes. Predicted DM intake values for the suckling ewes were 3.20 and 2.95 kg/day for control and enzyme treatments, respectively, giving an overestimation of 10.3% and 1.5%, respectively, for the intake of each diet (5.9% on average).

Conclusions

In conclusion, no lactational effects were detected when the Promote[®] fibrolytic enzyme product was added to the concentrate of dairy ewes. Enzyme dose (commercial recommendation) and application method (sprayed onto the concentrate) may have conditioned the response to enzyme supplementation in dairy ewes. No incorporation of the enzyme into the concentrate, for the fibrolytic product and dose used, is recommended in dairy ewes.

Acknowledgments

This study was partially supported by a grant from the MEC (Ministerio de Educación y Ciencia) of Spain (Project CICYT AGL2001-2617) and Agribrands Europe-España, S.A. (Barcelona, Spain). The authors also thank Gastón Vera, Ramón Costa and the team of the Servei de Granges i Camps Experimentals of the Universitat Autònoma de Barcelona for the care of animals, R. Armengol and B. Sánchez for helping in laboratory analyses, and Nic Aldam for the English revision of the manuscript.

References

Albanell E, Cáceres P, Caja G, Molina E and Gargouri A 1999. Determination of fat, protein, and total solids in ovine milk by near-infrared spectroscopy. *Journal of the Association of Official Analytical Chemists International* 82, 753–758.

Association of Official Analytical Chemists 2004. *Official methods of analysis*, vol. 2, 18th edition. AOAC, Arlington, VA, USA.

Beauchemin KA, Rode LM and Sewalt VJH 1995. Fibrolytic enzymes increase fiber digestibility and growth rate of steers fed dry forages. *Canadian Journal of Animal Science* 75, 641–644.

Beauchemin KA, Rode LM, Maekawa M, Morgavi DP and Kampen R 2000. Evaluation of a nonstarch polysaccharidase feed enzyme in dairy cow diets. *Journal of Dairy Science* 83, 543–553.

Beauchemin KA, Colombatto D, Morgavi DP and Yang WZ 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. *Journal of Animal Science* 81(Suppl. 2), E37–E47.

Bocquier F, Thériez M and Brelurut A 1987. Utilisation du foin par la brebis. In *Les fourrages secs: récolte, traitement et utilisation* (ed. C Demarquilly), pp. 423–451. INRA publications, Paris, France.

Bocquier F, Barillet F, Guillouet P and Jacquín M 1993. Prévion de l'énergie du lait de brebis à partir de différents résultats d'analyses: proposition de lait standard pour les brebis laitières. *Annales de Zootechnie* 42, 57–66.

Bowman GR, Beauchemin KA and Sheldford JA 2002. The proportion of the diet to which fibrolytic enzymes are added affects nutrient digestion by lactating dairy cows. *Journal of Dairy Science* 85, 3420–3429.

Bowman GR, Beauchemin KA and Sheldford JA 2003. Fibrolytic enzymes and parity affects on feeding behavior, salivation, and ruminal pH of lactating dairy cows. *Journal of Dairy Science* 86, 565–575.

Caja G, Bocquier F, Pérez-Oguez L and Oregui L 1997. Mesure de la capacité d'ingestion durant la période de traite des brebis laitières des races méditerranéennes. *Rencontres Recherches Ruminants* 4, 84.

Caja G, Marie C, Bocquier F, Ferret A, Gasa J, Pérez-Oguez L, Plaixats J and Oregui L 2002. Capacité d'ingestion des ovins laitiers: Effets des principaux facteurs de variation. *Options Méditerranéennes, Série B, Études et recherches* 42, 9–36.

Casals R, Caja G, Such X, Torre C and Calsamiglia S 1999. Effects of calcium soaps and rumen degradable protein on the milk production and composition of dairy ewes. *Journal of Dairy Research* 66, 177–191.

Dhiman TR, Zaman MS, Gimenez RR, Walters JL and Treacher R 2002. Performance of dairy cows fed forage treated with fibrolytic enzymes prior to feeding. *Animal Feed Science and Technology* 101, 115–125.

Doney JM, Peart JN, Smith WF and Louda F 1979. A consideration of the techniques for estimation of milk yield by suckled sheep and a comparison of estimates obtained by two methods in relation to the effect of breed, level of production and stage of lactation. *Journal of Agricultural Science* 92, 123–132.

González E 2004. Use of fibrolytic enzymes in dairy goats. In vitro evaluation of activity and fermentative characteristics. PhD, Autònoma de Barcelona University.

Jarrige R 1989. *Ruminant nutrition: recommended allowances and feed tables*. Libbey Eurotex, Paris, France.

Lee SS, Hass JK and Cheng KJ 2000. Influence of an anaerobic fungal culture administration on in vivo ruminal fermentation and nutrient digestion. *Animal Feed Science and Technology* 88, 201–217.

Littell RC, Henry PR and Ammerman CB 1998. Statistical analysis of repeated measures data using SAS procedures. *Journal of Animal Science* 76, 1216–1231.

Marie C, Such X, Barillet F, Bocquier F and Caja G 2002. Efficacité alimentaire selon le potentiel laitier des brebis. *Options Méditerranéennes, Série B, Études et recherches* 42, 57–71.

McAllister TA, Bae HD, Jones GA and Cheng KJ 1994. Microbial attachment and feed digestion in the rumen. *Journal of Animal Science* 72, 3004–3018.

McAllister TA, Stanford K, Bae HD, Treacher RJ, Hristov AN, Baah J, Sheldford JA and Cheng KJ 2000. Effect of a surfactant and exogenous enzymes on digestibility of feed and on growth performance and carcass traits of lambs. *Canadian Journal of Animal Science* 80, 35–44.

Miller DR, Elliot R and Norton BW 2008. Effects of an exogenous enzyme, Roxazyme G2 Liquid, on digestion and utilization of barley and sorghum grain-based diets by ewe lambs. *Animal Feed Science and Technology* 140, 90–109.

Minson DJ 1990. *Forage in ruminant nutrition*. Academic Press Inc., San Diego, CA, USA.

Molina E, Ferret A, Caja G, Calsamiglia S, Such X and Gasa J 2001. Comparison of voluntary food intake, apparent digestibility, digesta kinetics and digestive tract content in Manchega and Lacaune dairy sheep in late pregnancy and early and mid lactation. *Animal Science* 72, 209–221.

Muwalla MM, Haddad SG and Hijazeen MA 2007. Effect of fibrolytic enzyme inclusion in high concentrate fattening diets on nutrient digestibility and growth performance of Awassi lambs. *Livestock Science* 111, 255–258.

Pastor FJ, Pujol X, Blanco A, Vidal T, Torres AL and Díaz P 2001. Molecular cloning and characterization of a multidomain endoglucanase from *Paenibacillus* sp BP-23: evaluation of its performance in pulp refining. *Applied Microbiology and Biotechnology* 55, 61–68.

Pinos-Rodríguez JM, González SS, Mendoza GD, Bárcena R, Cobos MA, Hernández H and Ortega ME 2002. Effect of exogenous fibrolytic enzyme on ruminal fermentation and digestibility of alfalfa and rye-grass hay fed to lambs. *Journal of Animal Science* 80, 3016–3020.

Rode LM, Yang WZ and Beauchemin KA 1999. Fibrolytic enzyme supplements for dairy cows in early lactation. *Journal of Dairy Science* 82, 2121–2126.

Russell AJF, Doney JM and Gunn RG 1969. Subjective assessment of body fat in live sheep. *Journal of Agricultural Science* 72, 451–454.

Somogyi M 1952. Notes on sugar determination. *Journal of Biological Chemistry* 195, 19–23.

Such X, Caja G, Fernández N, Molina P and Torres A 1999. The effects of the type of pulsator on the evolution of milk emission kinetics during machine milking in Manchega ewes. In *Milking and milk production of dairy sheep and goat* (ed. F Barillet and NPO Zervas), pp. 227–232. Wageningen Pers., Wageningen, The Netherlands.

Titi H and Lubbadah WF 2004. Effect of feeding cellulase enzyme on productive responses of pregnant and lactating ewes and goats. *Small Ruminant Research* 52, 137–143.

Van Soest PJ, Robertson JB and Lewis BA 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74, 3583–3597.

Vicini JL, Bateman HG, Bhat MK, Clark JH, Erdman RA, Phipps RH, Van Amburgh ME, Hartnell GF, Hintz RL and Hard DL 2003. Effect of feeding supplemental fibrolytic enzymes or soluble sugars with malic acid on milk production. *Journal of Dairy Science* 86, 576–585.