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Effects of crude protein level in the concentrate and time allotment on pasture on milk yield, urinary nitrogen, and purine derivative excretion in lactating Latxa ewes

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Abstract. This study assessed the influence of reducing the crude protein (CP) content (from 190 to 130 g/kg CP/kg) in the supplementary concentrate and time allotment on pasture (TAP) on the milk yield, bodyweight, and the urinary nitrogen (N) and purine derivatives (PD) in lactating Latxa ewes. Animals were reared in a production system that restricted the amount of time that ewes spent on pasture. In mid-April, at the start of the 42 days experiment, 40 dairy Latxa ewes were assigned to one of four groups on the basis of their initial milk yield, days in lactation (DIL), bodyweight (BW), and condition score; thereafter, each group grazed in its own fenced paddock. The experiment was based on a 2 × 2 factorial design that included two CP levels and two pasture grazing regimes: 4 h continuous grazing (CG) in the morning or 2 h grazing in the morning and 2 h in the afternoon (DG). Individual milk yield was recorded three times a week, and time spent grazing and BW was recorded weekly. In the middle (day 15–17; P1) and at the end (day 36–38; P2) of the experiment, urinary spot samples were collected using a catheter. At the end of the experiment, ewes were confined to metabolic cages and urine was collected. CP level of the concentrate was not correlated with time spent grazing; however, the ewes that were permitted access to pasture twice per day spent more time grazing (223 min/day vs 207 min/day, $P < 0.01$) and were more efficient with their time (56 min/h grazing vs 52 min/h, $P < 0.05$) than the ewes that were permitted access to pasture once per day. Concentrate CP levels were not correlated with milk yield or composition, although ewes that received the high protein (HP) lost more

weight than did those that received the low protein (LP) concentrate. Concentrate CP level and TAP were not correlated with creatinine (CR) excretion rate (mean = 315 mmol/kg LW^{0.75} s.e. 0.0161). Urea-N was the largest component of urinary-N (68.7 s.e. 2.33%; $P > 0.05$), and urea-N waste was higher in HP ewes (202.7 mmol/day) than it was in LP ewes (159.5 mmol/day) (s.d. 27.83; $P < 0.01$). Ewes subjected to the DG regime had significantly ($P < 0.01$) higher urinary PD excretion (23.6 vs 21.4 mmol/day s.d. 4.01; $P < 0.01$), and tended ($P < 0.1$) to excrete less urea-N (175.1 vs 188.3 mmol, s.d. 27.83) than did the CG ewes. A reduction in the CP in the supplementary concentrate led to a reduction in N waste without having a detrimental effect on performance or milk production.

Additional keywords: dairy ewes, grazing time, milk production, PD excretion.

Introduction

In the Basque Country, Spain, dairy sheep production is based on a semi-intensive system that includes a single spring lambing, early weaning (28–30 days), and ewes that are milked for 150–180 days. Approximately 55% of the milk is used to produce high quality cheese (NASDAP 2012). Although the system entails high pasture use, grasses are scarce in the region; therefore, shepherds attempt to balance herbage, forage, and concentrate with the nutritional requirements of lactating ewes.

Well managed pastures have high concentrations of rumen degradable protein (RDP) and, in grazing lactating ewes, the ratio of RDP to fermentable metabolisable energy (FME) can exceed rumen microbial requirements, which results in the inefficient use of nitrogen (N). Dietary supplements that optimise the RDP : FME ratio can improve performance and N efficiency. For instance, a managed grazing system (grazing 3–7 h outdoors) and a dietary supplement that contained 19–20% crude protein (CP), mostly from legumes (e.g. soybean), appeared to optimise the performance of Latxa ewes (Oregui *et al.* 1997).

The objective of our study was to investigate the response of lactating Latxa ewes reared in a rationed grazing system to a reduction in the CP of their concentrate and differences in time allotment on pasture (TAP).

Materials and methods

Location and types of pastures

The experiment was conducted at the Neiker-Technalia Research Station, Arkaute, Spain (42°51'0" North, 2°37'0" West), at an elevation of 520 m.a.s.l. The swards were permanent pastures in which perennial rye grass (*Lolium perenne*) cocksfoot, (*Dactylis glomerata*), and white clover (*Trifolium repens*) were the predominant species. One section of the grassland was fenced to create four 1.5-ha paddocks. To minimise the differences in the availability of forage among the paddocks, the fenced area was chosen based on the homogeneity of that section of the grassland.

Experimental design

Based on a 2 × 2 factorial design, the 42 days experiment began in mid-April. Forty multiparous Latxa dairy ewes were assigned to one of four groups of 10 animals based on individual milk yield, days in lactation (DIL), and bodyweight (BW). Average initial mean milk yield, BW, and DIL were 1530 ± 83.8 mL, $59.5 \text{ kg} \pm 0.87$, and $45 \text{ days} \pm 0.79$ respectively. Data were collected from six ewes in each group. The four groups were randomly assigned to one of the following experimental treatments: (1) high protein (190 g CP/kg) concentrate (Table 1) and 4 h continuous grazing in the morning (HPCG); (2) low protein (130 g CP/kg) concentrate and 4 h continuous grazing in the morning (LPCG); (3) high protein concentrate and 4 h discontinuous grazing (2 h in the morning + 2 h in the afternoon) (HPDG); and (4) low protein concentrate and 4 h discontinuous grazing (2 h in the morning + 2 h in the afternoon) (LPDG). Each ewe received its daily supplement after the morning (225 g DM) and after the afternoon milking (225 g DM). When not on pasture, ewes were housed individually in a stall free barn and had unlimited access to drinking water. In the afternoon, each ewe was offered 250 g DM of alfalfa hay. Grazing and feeding protocols (concentrate and roughage supply) were based on the typical dairy sheep production system in the region. The amount of CP in the concentrates was based on the levels used in the local system (high, 190 g CP/kg DM; Oregui *et al.* 1997), and the low protein concentrate (130 g CP/kg) was formulated to meet ewe requirements based on known milk production, grass intake and forage composition.

Measurements and sampling

The height of pasture grass was estimated based on 50 random measurements taken in each paddock three times per week using a sward stick. Grass height was maintained at 6–8 cm by adjusting

the number of grazing ewes. Grass samples were hand-plucked (2–3 cm from the ground) from each plot every 2 weeks. Concentrate, lucerne hay, and residues were sampled weekly. Individual grazing time was recorded once a week when ewes had access to pasture. To measure grazing activity, the number of ewes engaged in grazing was recorded at 5 min intervals. Ewes were weighed weekly, immediately after the morning milking, which minimised the effects of fluctuations in their weight caused by changes in the contents of the mammary glands and gut. Milk yields were recorded three times per week, and fat and protein concentrations were measured weekly at the time of the morning and afternoon milkings. Individual urinary spot samples (≤ 50 mL) were collected using Foley urethral catheters (Folatex, 18 mm $\varnothing$; 10 mL balloon) twice a day after each milking in the 3rd week (P1: day –17), which corresponded to 60–62 DIL, and at the end (P2: day 36–38) of the experiment, which corresponded to 81–83 DIL. After 42 days (87 DIL) of the experiment, to measure total urine excretion, the ewes were housed individually for 1 week (day 87–94 DIL) in metabolic cages. During that week, to simulate grazing conditions, ewes were offered hand-picked pasture grass either once (between 1000 h and 1400 h) or twice per day (between 1000 h and 1200 h, and between 1500 and 1700 h). Grass refusal was maintained at ~10% of the total amount offered. Daily urine samples (5% of total urine production) were collected from each ewe. To maintain pH<3, sulphuric acid (100 mL; 0.1 M) was added to the samples, which were pooled and stored (20°C).

Chemical analyses

The concentrations of fat and protein in the milk were measured using an infrared analyser (Foss System 4000, Foss Electric, Hillerød, Denmark). To measure the dry matter content of pasture grass, alfalfa hay and concentrates samples were dried at 60°C for 48 h and ground to pass through a 1-mm screen. Ash content was measured after the samples were burned in a muffle furnace at 550°C overnight. CP was measured using the Kjeldahl Procedure (AOAC 1990). Concentrations of neutral detergent fibre and acid detergent fibre were quantified using the Goering and Van Soest (1970) method. Urea concentrations were measured using the diacetyl-monoxine reaction (Technicon Instruments Co. Inc. 1972). The PB and CR concentrations in the urine samples were measured using high performance liquid chromatography (Balcells *et al.* 1992).

Calculations and statistical analyses

Statistical analyses were performed using the PROC MIXED procedure for repeated-measures (SAS 2000), with ewe as a random effect. In the model, a compound symmetry structure was used that was based on the following equation:

$$y_{ijk} = \mu + \text{TAP}_i + \text{CP}_j + (\text{TAP} \times \text{CP})_{ij} + \epsilon_{ijk}$$

where Y is the dependent variable, μ is the mean value, (TAP) [fixed factor] is the time allotment on pasture, CP_j [fixed factor] is the protein level, and ϵ_{ijk} is the error term. For CR, PD/CR ratio, total urea, and PD excretion, the model included two additional factors, GP_k [fixed factor], the grazing period, and ST_l [fixed factor], the sampling time, and their interaction. Sampling time (ST) was a within animal factor, and treated as a repeated measure.

Results and discussion

All of the multiparous Latxa ewes remained healthy throughout the experiment, which began immediately after the weaning period. Flooding in the region prevented grazing on pastures until mid-April; consequently, the experiment began when the ewes were ≈ 45 DIL. Although the grass paddocks were in the same meadow, which was chosen because of the homogeneity of the plant community and the availability of forage, the results might have been influenced by a paddock effect, which have been incorporated into the treatment effect. The paddocks did not differ significantly in the chemical composition of the vegetation (Table 1). In the statistical model, interaction effects were not significant ($P > 0.05$); therefore, only the main effects are presented in the tables.

Animal behaviour

Ewes in DG group spent more time grazing (223 vs 207 min/day, $P < 0.01$) and grazed more efficiently (56 min/h grazing vs 52 min/h, $P < 0.05$) than did CG ewes (Fig. 1). Ewes in CG and DG groups spent most of the first 3 h grazing; however, in the fourth hour, DG ewes grazed more efficiently than did CG ewes ($P < 0.01$). CP concentration in the supplementary concentrate and individual time spent grazing were not correlated. Possibly, DG ewes might have learned faster the restriction on pasture availability than did the CG ewes and modified their grazing behaviour, accordingly. Perojo *et al.* (2005) reported that Latxa ewes responded to a reduction in access to pasture (from 7 h to 4 h by increasing time spend grazing (min/h)).

Milk yield and liveweight

Grazing regime did not have a significant effect on the final BW of ewes; however, those fed HP concentrate lost more weight than did those fed LP concentrate ($P < 0.05$; Table 2). Ewes can use excess protein in the diet as a source of energy, but doing so increases the costs of the synthesis and excretion of urine, which is unfavourable to the animal's energy balance. Furthermore, lactating cows (Ørskov *et al.* 1987) and ewes (Castrillo *et al.* 1995) can increase body fat mobilisation after consuming undegradable dietary protein. Those factors might have contributed to the reduction in the performance of the ewes in the HP group, although milk composition and yield were unaffected by differences in CP intake.

Average milk yield decreased significantly ($P < 0.01$; from 1.19 mL/day to 0.93 mL/day) between the third and the sixth week of the experiment because of the physiological changes that occur in the mammary glands during lactation (Ruiz *et al.* 2000). In our experiment, the treatments did not have a significant effect on milk yield, although Perojo and Oregui (2001) reported a negative correlation between a reduction in TAP and milk yield in Latxa ewes.

Urinary excretion of creatinine

Creatinine is a product of the random degradation of phosphocreatine, and the amount excreted in urine is influenced by lean body mass (Van Niekerk *et al.* 1963) and the concentration of creatine in muscle (Waterlow 1969), and is independent of the animal's diet. Therefore, CR excretion can be used as index of urinary volume and metabolite excretion (Faichney *et al.* 1995). In our experiment, CR excretion was measured when the ewes were housed in metabolic cages and did not have access to pasture. We assumed that CR excretion measured in the cages ($\text{mg/kg LW}^{0.75}$) was equivalent to the CR excretion when ewes were permitted to graze on pastures.

Neither TAP nor CP level in the supplementary concentrate were significantly correlated with the CR excretion rate in lactating ewes. Creatinine excretion rates (mean = 6.61 mmol/day s.e. 0.371 or 315 mmol/kg $\text{LW}^{0.75}$ s.e. 0.016) were similar to those reported in lactating Rasa Aragonesa ewes (Martín-Orúe *et al.* 1996; Dapoza *et al.* 1999).

Urinary excretion of urea and purine derivatives

Urea-N was the largest proportion of urinary-N (68.7% s.e. 2.33; $P > 0.05$). Urea-N excretion reflects the difference between N intake and N requirements; thus, an excess intake of N increases urea output. Urea excretion rates as estimated by the urinary urea/ CR ratio and total CR excretion rates was higher in ewes fed a HP diet than it was in those fed a LP diet (202 and 159 mmol/day respectively s.d. 27.83; $P < 0.01$).

The higher protein intake by ewes in the HP groups and the absence of effects on milk yield and animal performance (Table 2) suggest that N overfeeding led to an increase in urea-N excretion, only. Furthermore, in the rumen, excess degradable protein is fermented into ammonia, and converted into and excreted as urinary urea. As an index of microbial rumen yields, urinary PD excretion can help to identify the source of urinary urea.

The PD/CR (mol/mol) ratio did not differ significantly between the ewes fed the HP diet (3.61) and those fed the LP diet (3.29) (s.d. 0.502, $P > 0.01$), which suggests that rumen microbial yield were similar in the two treatments and, therefore, the rumen microbial requirements of the ewes in the LP groups appear to have been met. If the supplementary concentrate fed to the ewes in the LP groups met their rumen microbial requirements, and given that soybean meal was the source of the additional protein in the HP diet (Table 1), the ewes fed the HP diet received excess degradable protein, which contributed significantly to the high urea-N excretion rates in this group.

In Latxa ewes, the PD/CR ratio was higher in P1 (60–62 DIL) than it was in P2 (81–83 DIL) (3.87 vs 3.03 ± 0.502 , for P1 and P2 respectively; $P < 0.01$), which reflected a reduction in DOM intake. The reduction in milk yield (1.19 and 0.93 l/day for P1 and P2 respectively; Table 3) during lactation period implies a reduction in the ewe's nutritional requirements and, probably, a reduction in DOM intake. In addition, DOM intake and urinary PD are positively correlated (Balcells *et al.* 1993; Surra *et al.* 1997).

In our experiment, TAP influenced the PD/CR ratio, and the DG animals had higher urinary PD/CR ratios (3.89 vs 3.01 mol/ mol ± 0.502) than did the CG ewes.

Furthermore, CG ewes (175.1 mmol urea/day) wasted less N more efficiently than did the DG ewes. It appears that the former had a better balance between nutrient supply and microbial requirements than did the latter. Theoretically, carbohydrate and N availability should have been more synchronised in the DG ewes than it was in the CG ewes because the former grazed at times that were close to the times at which the supplementary concentrate was provided (during milking at 0730 h and 1730 h); therefore, microbial yield and PD excretion should have been higher in the DG ewes than in the CG ewes.

The urinary PD/CR ratios of Latxa ewes were significantly lower in the morning than they were in the afternoon (2.98 vs 3.92; s.d. 0.502; $P < 0.01$). Creatinine excretion reflects the size of the phosphocreatine/CR pool, which should be constant throughout the day (Van Niekerk *et al.* 1963); however, changes in rumen microbial outflow can influence urinary PD/CR excretion rates. Some studies have found that sampling time had no effect on the PD/CR ratio (Chen and Gomes 1995; Dewhurst *et al.* 1996; George *et al.* 2006), whereas other studies found a significant correlation between PD/CR and time of day (Susmel *et al.* 1995; Shingfield and Offer 1998). In our study, the changes in PD/CR ratio throughout the day suggest that frequent sampling can improve the accuracy of PD/CR measurements.

Conclusions

A reduction in the protein in the supplementary concentrate fed to lactating Latxa ewes contributed to a reduction in the ration cost and N waste without a measurable effect on ewe performance or milk yield; however, the experiment might not have lasted long enough to detect the effects of a reduction in the protein content of the concentrate on lactation persistence, body condition and interbreeding interval.

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Table 1. Ingredients (% DM) and chemical composition (% DM) of the **roughages and concentrates offered to multiparous lactating Latxa ewes**

	Concentrate		Alfalfa hay	Grass
	Low protein (LP)	High protein (HP)		
<i>Ingredients (% DM)</i>				
Soybean meal	5.0	25.0	–	–
Molasses	1.3	1.3	–	–
Barley grain	68.4	53.2	–	–
Corn grain	23.8	19.0	–	–
Ca CO ₃	0.7	0.7	–	–
Ca PO ₄	0.3	0.3	–	–
Micro-minerals ^A	0.5	0.5	–	–
<i>Chemical composition (% DM)</i>				
DM (% fresh matter)	87.7	88.2	88.7	19.1
Crude protein	13.7	19.5	15.2	13.5
OM	95.5	95.3	91.7	90.8
NDF	14.5	14.0	50.0	48.6
ADF	5.4	6.2	44.9	32.3
Energy (ME ^B (MJ/kg))	11.4	11.4	–	–

^A Mineral composition of the concentrates: 10 mg/kg zinc oxide, 60 mg/kg sodium selenate, 60 g/kg cobalt carbonate, 9 mg/kg magnesium oxide, 7.4 mg/kg copper carbonate, 2.5 mg/kg copper sulphate.

^B Estimated metabolisable energy (ME) based on FEDNA tables (De Blas *et al.* 2003).

Fig. 1. Grazing behaviour in lactating Latxa ewes at different time allotment on pasture.

Table 2. Final liveweight, milk yield, milk composition, protein level (high or low) in their concentrate, and the time allotment on pasture by Latxa ewes

*, $P < 0.05$. NS, not significant ($P > 0.05$); s.e.m., standard error of the mean

	Liveweight (kg)	Milk yield (mL/day)	Milk composition	
			Fat (g/kg)	Protein (g/kg)
<i>Pattern of time allotment in pasture (TAP)</i>				
4 h	57.6	1182	66.7	53.7
2+2 h	57.5	1152	68.9	51.9
<i>Protein concentration (CP)^A</i>				
HP	56.6	1194	69.5	53.1
LP	58.4	1140	66.1	52.6
s.e.m.	0.6	53.0	1.5	1.2
<i>Statistical significance</i>				
TAP	NS	NS	NS	NS
CP	*	NS	NS	NS

^A130 or 190 g CP/kg of the concentrate.

Table 3. Liveweight, milk yield, urinaryPD/CR ratio, daily excretion of urea and PD, protein level (high or low) in their concentrate and the time allotment on pasture by Latxa ewes.

Estimates of total daily PD excretion were based on the urinary creatinine excretions of Latxa ewes held individually in metabolic cages for 42–50 days. Overall performance and milk yield of Latxa ewes between the 3rd (59–65 DIL) and 6th (80–87 DIL) week of the experiment. Urine samples were collected at 15–17 days (P1: 60–62 DIL) and 36–38 days (P2: 81–83 DIL). *, $P < 0.05$. **, $P < 0.01$. ***, $P < 0.001$. NS, not significant ($P > 0.05$); s.d., standard deviation

Item	LW (kg)	Milk yield (mL/day)	PD/C ^A (mmol/ mmol)	Urea (mmol/ day)	PD ^B (mmol/ day)
<i>Grazing period</i>					
P (1)	58.3	1187	3.9	182.5	25.2
P (2)	57.4	929	3.0	177.5	19.8
<i>Time allotment on pasture (TAP)</i>					
4 h	57.8	1059	3.0	188.3	21.4
2+2 h	57.9	1057	3.9	175.1	23.6
<i>Protein concentration (CP)^C</i>					
HP	57.5	1068	3.6	202.7	22.2
LP	58.2	1047	3.3	159.5	22.8
<i>Sampling time (ST)</i>					
Morning	—	—	3.0	176.4	19.6
Afternoon	—	—	3.9	183.3	25.4
s.d.	2.0	50.9	0.5	27.8	4.0
<i>Statistical significance</i>					
Grazing period	NS	***	***	NS	***
TAP	NS	NS	***	NS	*
CP	NS	NS	NS	**	NS
ST	—	—	***	NS	***

^APurine derivative/creatinine ratio.

^BPurine derivative.

^C130 or 190 g CP/kg in their concentrate.