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SHORT COMMUNICATION

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Production performance and plasma metabolites of dairy ewes in early lactation as affected by chitosan

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Abstract

The objective of this study was to evaluate the effects of chitosan (CHI) supplementation on production performance and blood parameters in dairy ewes. Twenty-four multiparous Latxa dairy ewes at d 16 of lactation were divided into two groups of 12 ewes each. Ewes were fed one of two experimental concentrates (0.840 kg dry matter/d), control or supplemented with 1.2% CHI, on a dry matter basis. Ewes also had free access to tall fescue hay, water, and mineral salts. The experimental period lasted for 25 d, of which the first 14 d were for treatment adaptation and the last 11 d for measurements and samplings. Supplementation with CHI decreased total (p=0.043) and fescue (p=0.035) dry matter intake (DMI), but did not affect concentrate DMI. Supplementation with CHI, moreover, increased plasma glucose (p=0.013) and BUN concentrations (p=0.035), but did not affect those of non-esterified fatty acids. Dietary supplementation with CHI, however, did not affect milk yield, 6.5% FCM, milk composition, or BW, but it improved dietary apparent efficiency by increasing the milk yield-to-DMI (p=0.055) and 6.5% FCM-to-DMI (p=0.045) ratios. In conclusion, dietary supplementation of chitosan maintained ewe performance while reducing feed intake and improving dietary apparent efficiency.

Additional key words: animal nutrition; Latxa ewe; milk production.

Abbreviations used: BCS (body condition score); BUN (blood urea nitrogen); BW (body weight); CHI (chitosan); DM (dry matter); DMI (dry matter intake); FCM (fat corrected milk); NDF (neutral detergent fibre**);** NEFA (non-esterified fatty acid).

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Chitosan (an N-acetyl-d-glucosamine polymer, CHI) is a natural, nontoxic, biodegradable biopolymer (Muzzarelli, 1993) derived from deacetylation of chitin, a major component of the shells of crustaceans and insects. The antimicrobial activity of chitosan has emerged as one of its most interesting properties (Matsuhashi & Kume, 1997), which has led to evaluation of its use in ruminant nutrition. Benefits observed seem to be caused by changes in ruminal fermentation, in particular by increased propionate proportion (Goiri *et al.,* 2010) and decreased methane production (Goiri *et al.,* 2009), which in turn lead to energetically more efficient fermentation patterns. Such shifts in the fermentation pattern could result in increased plasma glucose concentrations (Sauer *et al.,* 1989) and as a consequence in improved productive performance or improved efficiency (McGuffey *et al.,* 2001). The application of CHI as an animal feed additive to modify animal productive performance, however, is little studied to date. The objective of this study was, therefore, to evaluate the effects of CHI supplementation on lactational performance and plasma metabolites in dairy ewes.

The experiment was carried out in accordance with the European Council Directive 86/609/ECC (EC, 1986) for the protection of animals used for experimental and other scientific purposes.

Twenty-four blackfaced multiparous Latxa dairy ewes at early lactation from the Neiker-Tecnalia experiment station (42º51'N, 2º37'W) were blocked according to milk yield (1976 ± 276 mL), days in milk (16 ± 9 d postpartum), and BW (67.5 ± 8.5 kg). Ewes were group housed. Tall fescue (*Festuca arundinacea*) hay was group fed *ad libitum* in a feed bunk (0.5 m/

ewe), water, and mineralized salt block. The quantity of offered fescue hay was based on morning bunk readings, and the amount of feed offered was adjusted daily to allow 10% refusals.

In each block ewes were randomly assigned to one of two experimental concentrate supplements that contained either 0 (control) or 1.2% CHI (Chitoclear; Deacetylation degree: > 95%; viscosity: < 500 mPa·s; Trades S.A., Barcelona) on a dry matter (DM) basis. Ingredients of both experimental concentrates are shown in Table 1. The experimental concentrates were offered in individual feeders in the milking parlour as two equal meals (420 g DM) during the morning and evening milkings.

The experimental period lasted for 25 d, of which the first 14 d were for treatment adaptation to allow the ruminal microflora to adjust to the supplemental chitosan, and the last 11 d for measurements and samplings.

Quantities of concentrate offered and refused were recorded 4 d/wk on an individual basis throughout the experiment. Individual fescue hay dry matter intake (DMI) was determined using the n-alkanes technique. Paper pellets containing the alkane C32 were prepared following the technique described by Mayes *et al.* (1986), with slight modifications. Twenty sheets of Albert 135 filter paper, 45 cm wide and 55 cm long, were placed in a stainless steel tray and dried at 105°C for 2 h in a forced-air oven. Meanwhile, 30 g of C32 were measured into a 1000-mL Pyrex screw-top flask and dissolved in 900 mL heptane by heating to 80°C and maintaining this temperature for 15 min. The rest of the process was conducted as described by Mayes *et al.* (1986). Pellets were made by introducing 1.5 g of shredded paper into an adapted reloading press. Average concentration of C32 in the dosed pellets was 85.2 ± 6.0 g/kg DM. Beginning on d 16 until the end of the experiment, a once-daily dose of paper pellet containing 128 mg of C32 alkane was given to each ewe with a dosing gun during the morning milking. Faecal grab samples were collected twice daily from each in the last 3 d of the experiment. The faecal samples from each ewe were kept at -20ºC until the end of the collection and bulked to a single sample per ewe for alkane analysis.

Weekly experimental concentrate and fescue hay samples were collected and composited by group. Half of the sample was dried at 60°C for 48 h for DM determination, and the other half was kept at -20ºC for chemical composition and alkane analysis.

Ewes were milked daily at 07:30 and 18:00 h, and milk yield was recorded individually four times weekly with an electronic meter (MM25 SG, DeLaval, Madrid). On d 16, 18, 23, and 25, a portion of milk from each ewe was stored with potassium bichromate (0.3 g/L) at 4ºC and analysed (ILL, Lekumberri, Spain) by near-infrared spectroscopy (Foss System 4000, Foss Electric, Hillerød, Denmark) for fat, crude protein, and lactose.

Blood samples (10 mL) were collected 2 h after morning milk feeding on d 19, 23, and 25 via jugular venipuncture into evacuated tubes containing potassium-EDTA. Once collected, blood samples were centrifuged (1,500 × g for 15 min at 4°C), and plasma was recovered and frozen at -20°C for glucose, non-esterified fatty acids (NEFA), and blood urea nitrogen (BUN) determinations.

Fescue hay, concentrates, and faeces were freeze-dried (Christ Alpha 1-4 LD Plus, Fisher Bioblock Scientific, Madrid) and ground to pass a 1-mm screen. Dry matter (method 934.01) and N (method 984.13) contents were determined following AOAC (1999).

Table 1. Ingredients and chemical composition of experimental concentrates and fescue hay.

Item (% of dry matter)	Concentrate[1]		Fescue hay
	Control	CHI	
Cold pressed rapeseed meal	55.8	55.3	
Chitosan		1.2	
Barley	18.3	18.3	
Corn	21.4	21.4	
Molasses	4.0	4.0	
Vitamin-mineral premix[2]	1.0	1.0	
Organic matter	94.0	92.2	91.2
Crude protein	15.2	15.4	15.4
Neutral detergent fibre	24.2	23.5	18.0
Acid detergent fibre	11.1	10.1	27.3
Fat	5.0	5.1	2.6

[1] It refers to concentrates containing 0 (Control) or 1.2% of chitosan (CHI) on dry matter basis. [2]Vitamin and mineral premix contained per kg of DM: 2500 IU of vitamin A, 400 mg of vitamin D, 2.5 IU of vitamin E, 4.9 mg of Zn, 4.05 mg of Mn and 0.1 mg of Se (Calseaphos, Saint Malo, France).

Nitrogen content was determined using the macro-Kjeldahl procedure on a Kjeltec Auto 1030 (Foss, Hillerød, Denmark). Neutral detergent fiber (NDF) was determined by the method of Van Soest *et al.* (1991) using an alpha amylase, but without sodium sulphite in the neutral detergent solution, and was expressed free of ash. Acid detergent fiber, expressed exclusive of residual ash, was determined by the method of Robertson & Van Soest (1981). Fat content was determined without hydrolysis by the automated soxhlet method (Selecta S.A., Barcelona) using hexane for 6 h as solvent. Ewes were weighed and body condition score (BCS) was determined on day 1 and 25.

The extraction of n-alkanes was carried out following the technique described by Mayes *et al.* (1986), with the modifications suggested by Vulich *et al.* (1995) and Olivan & Osoro (1999).

Plasma samples were analysed for glucose (glucose oxidase/peroxidase method; Karkalas, 1985), NEFA (NEFA-C kit; Wako Chemical, Richmond, VA, USA; Johnson & Peters, 1993), and BUN (liquid urea nitrogen reagent set, Pointe Scientific Inc., Canton, MI, USA).

Day milk production was adjusted to 6.5% fat-corrected milk (FCM) based on the following equation developed by Pulina & Nudda (2002):

$$FCM = M(0.37 + 0.097F)$$

where M is milk yield (kg) and F is fat concentration (%).

Concentrate DMI was determined as the difference between quantities offered and refused. Fescue hay DMI was calculated from the alkanes C33 and C32 as follows (Mayes *et al.,* 1986):

$$I_h = \frac{\frac{F_i}{F_j}(D_j + I_c C_j) - I_c C_i}{H_i - \frac{F_i}{F_j} H_j}$$

where F_i and F_j are the respective concentrations of C33- and C32-chain alkanes in the faeces, D_j is the quantity of C32 administered daily (mg), I_c the concentrate DMI (kg/d), C_j and C_i the respective concentrations of C32- and C33-chain alkanes in the concentrate, and H_i and H_j the concentration of C33- and C32-chain alkanes in the hay (all concentrations of n-alkanes in mg/kg of DM).

Day milk yield, FCM, milk fat and protein contents, milk fat and protein yield, and blood NEFA, glucose, and BUN concentrations (n=24) were analysed using the MIXED procedure (SAS, 2002) for repeated measures, with random (sheep) and repeated (day) statements. The statistical analysis of results was subjected to three covariance structures: compound symmetric, autoregressive order one, and unstructured covariance. The covariance structure that yielded the smallest Schwarz's Bayesian criterion was considered the most desirable analysis, and least squares means for treatments are reported. Total, hay, and concentrate DMI data were analysed using the MIXED procedure (SAS, 2002). All traits were analysed according to the following statistical model:

$$Y_{ij} = \mu + A_i + \varepsilon_{ij}$$

where Y_{ij} is the value of each individual observation, μ the average, A_i the effect of the ith additive (i=chitosan or control), and ε_{ij} the residual error.

Effects of CHI on DMI, milk yield, milk composition, body weight, plasma metabolites, and apparent efficiency are shown in Table 2. Mean CHI intake was 135 mg/kg of BW, a dose that has been shown to shift ruminal fermentation toward energetically more efficient routes (Goiri *et al.,* 2010). In the present study, supplementation of CHI caused a 9.3% reduction of fescue hay DMI (p=0.035) that resulted in a 6.4% reduction of total DMI (p=0.043).

Reasons for decreased feed DMI when CHI was fed cannot be elucidated from this study, and more research is warranted to ascertain this effect, but the reduction of DMI could be associated with the magnitude of changes in ruminal fermentation patterns and digestibility. Chitosan has been shown to reduce NDF apparent digestibility and to increase ruminal proportions of propionate (Goiri *et al.,* 2010), a metabolite involved in stimulating oxidative metabolism in the liver (Allen, 2000).

In the present study lambs were removed immediately after parturition, before suckling, for artificial rearing, but we did not observe a decline in milk yield as anticipated. In dairy ewes after lambing, milk production —and thus the energy requirements of ewes— grows more rapidly than the intake of energy from the diet, and, therefore, a negative energy balance is inevitable in the first months of lactation (Cannas, 2002). In the present study, therefore, as a consequence of the reduced DMI, decreased productive performance and increased NEFA concentrations would have been expected in CHI-supplemented ewes. Dietary supplementation with CHI, however, did not affect milk yield, milk composition, BW, BCS or NEFA concentrations. The reason underlying this may relate to glucose synthesis, as factor that influences the energy status of an animal. Based on the 6.5% increase in plasma glucose concentrations (p=0.013) found in the ewes receiving CHI, it could be postu-

Table 2. Mean effects of diet supplementation with chitosan (CHI) on DMI, milk yield, milk composition, BW, plasma metabolites and apparent efficiency (n=12).

Item[1]	Treatment		SEM[2]	*p*[3]
	Control	CHI		
DMI, kg/d				
Total	2.64	2.47	0.070	0.043
Fescue hay	1.83	1.66	0.064	0.035
Concentrate	0.81	0.80	0.014	0.826
Milk yield, g/d				
Milk	1940	2016	125.1	0.544
6.5% FCM	1865	1976	115.6	0.342
Fat	118.1	126.9	8.16	0.283
Protein	84.12	88.5	4.34	0.315
Lactose	99.2	102.0	6.05	0.653
Milk composition, %				
Fat	6.07	6.34	0.292	0.368
Protein	4.35	4.40	0.099	0.612
Lactose	5.10	5.07	0.052	0.483
Body weight, kg				
Initial	70.7	70.5	3.51	0.946
Final	70.3	69.1	3.43	0.744
Body condition score				
Initial	2.77	2.75	0.284	0.871
Final	2.52	2.58	0.354	0.743
Plasma metabolites				
Glucose, mg/dL	55.7	59.3	1.37	0.013
NEFA, mEq/L	0.26	0.26	0.019	0.971
BUN, mg/dL	20.9	22.8	0.59	0.035
Apparent efficiency, g/kg				
Milk yield/DMI	721	812	41.7	0.055
6.5% FCM/DMI	703	796	32.2	0.045

[1] DMI: dry matter intake, FCM: adjusted to 6.5% fat corrected milk, NEFA: non-esterified fatty acid, BUN: blood urea nitrogen. [2] SEM: standard error of the mean. [3] Probability of significant effects.

lated that the administration of CHI may have increased the hepatic synthesis of glucose, thereby improving the energy balance and alleviating the detrimental effect on productive performance of a reduced DMI.

Several factors could be implicated in explaining the observed greater plasma glucose concentrations. Supplementation with CHI resulted in an 9.1% increase in BUN (p=0.035), which could reveal an increase in the use of aminoacids for the hepatic synthesis of glucose. In the present study, ruminal escape of amino acid N to the abomasum was not measured, but Goiri *et al.* (2010) reported CHI to decrease the branched-chain proportion and the NH_3-N concentration in the rumen of sheep, which is usually associated with a reduction in aminoacid degradation (Chalupa *et al.,* 1980). Finishing pigs consuming CHI, however, decreased populations of lactobacilli in the distal gastrointestinal tract resulting in an increase of concentrations of luminal NH_3-N and pH, indicating a greater bacterial proteolysis (O'Shea *et al.,* 2011). Therefore, the effect of CHI on BUN concentration in dairy ewes could depend on the net effect on proteolysis in the rumen and the distal gastrointestinal tract.

The increase in plasma glucose concentrations in the current study is in agreement with the results reported by Araújo *et al.* (2015) who also observed an increase in plasma glucose concentrations in Nellore steers. These results are consistent with the observed increase in the molar proportion of propionate and the propionate-to-acetate ratio in the rumen of sheep (Goiri *et al.,* 2010) and Nellore steers fed chitosan (Araújo *et al.,* 2015). It is well established that other growth promoters, such as ionophores, increase ruminal propionate production and thereby increase the supply of this glucogenic substrate to the hepatic tissues, stimulating the production of glucose via gluconeogenesis (Sauer *et al.,* 1989).

The combined decrease of DMI and maintenance of milk yield or of FCM support the observed increase in dietary apparent efficiency by increasing the milk yield-to-DMI ratio by 12.6% (p=0.055) and the FCM-to-DMI ratio by 13.2% (p=0.045). It would seem, therefore, that increased conversion of dietary DM to product at the same level of production with chitosan supplementation has the potential to have a positive impact on farm productivity.

In conclusion, chitosan increased plasma glucose concentrations translated into potential improved efficiency through lower DMI while milk production was maintained.

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