PAPER

Effects of short-term feed restriction on milk yield and composition, and hormone and metabolite profiles in mid-lactation Sarda dairy sheep with different body condition score

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Abstract

Ten Sarda dairy ewes (5 with high Body Condition Score: H-BCS, BCS>2.5; BW 48.8±5.4 kg; 5 with low BCS: L-BCS, BCS<2.5; BW 36.2±4.7 kg) were subjected, after 7-day preliminary (Prel) period, to short-term feed restriction (FR, 50% of nutrient requirements) for three days followed by refeeding (Re-Fed, 100% requirements) for three days. Milk yield and composition (protein, fat, lactose, MUN, SCC, fatty acids), and blood parameters (glucose, NEFA, BUN, insulin, GH, IGF-I, leptin) were monitored. Milk yield decreased during FR in both BCS groups: at day 3 it was 38% and 35% of Prel values in H-BCS and L-BCS ewes, respectively, reaching Prel levels at Re-Fed in both groups. Milk fat concentration was influenced by BCS×sampling, increasing in H-BCS ewes during FR, but not varying in L-BCS ewes throughout the trial. During FR, milk protein increased as milk yield decreased. There was no change in milk urea nitrogen concentration during FR, but this decreased in both BCS groups during Re-Fed. FR modified the FA profile of milk fat in both BCS groups, increasing LCFA at the expense of SCFA and MCFA. Some blood parameters (NEFA, GH and IGF-I) were influenced by BCS, whereas almost all parameters were influenced by sampling. There was a rapid return to initial levels in all parameters except milk urea, blood urea and insulin at Re-Fed.

Introduction

In the Mediterranean area, particularly under extensive farming systems based on grazing, dairy ewes can experience temporary nutritional stress due to feed shortage. In such systems, periods of sheep undernutrition can occur during the first third of lactation (December-January), the mating season (May-June), in which ewes are in the last third of lactation, and the end of pregnancy (October-November). During the first period, this is usually caused by adverse weather conditions (e.g. wind and snow storms) which severely reduce grass intake and cause a sharp drop in milk production. During the reproduction periods, undernutrition can occur due to poor quality pasture which can reduce flock fertility (Molle *et al.*, 1996; Rassu *et al.*, 2002). Temporary undernutrition can also be a result of inappropriate management practices (e.g. regrouping of animals) which can lead to competition among sheep, incorrect evaluation of feed intake or inadequate nutritive value of the ration (Cannas, 2004).

Underfed animals initially mobilize their body reserves to compensate the shortage of nutrients, and subsequently adapt their production level to meet the new feed availability. Body fat mobilization during undernutrition varies according to the duration and level of feed restriction, and initial body fatness of the animals (Cowan *et al.*, 1982; Chilliard *et al.*, 2000). Under severe undernutrition, the level of body fat mobilization was higher in ewes that were initially fatter. However, under moderate undernutrition, initial body fatness had only a limited influence on fat mobilization (Cowan *et al.*, 1982).

Some studies have focused on the effects of underfeeding on sheep performance in perinatal (Lawlor and Hopkin, 1981) and early lactation periods (Cowan *et al.*, 1980; Agus and Bocquier, 1995) in which the mobilization of body reserves is supported by the specific metabolic and physiological status of the animal (Blanc *et al.*, 2006). The consequent negative energy balance generally results in a decrease in milk yield and protein concentration, and in an increase in milk fat (Bocquier and Caja, 2001). Feed restriction was found to be associated with variation in circulating concentrations of insulin, leptin, glucose, GH, and IGF-1 (Marett *et al.*, 2011; Gross *et al.*, 2011; Block *et al.*, 2003). In particular, leptin hormone, secreted primarily by adipose tissue, showed to be very sensitive to nutrient restriction in sheep (Delavaud *et al.*, 2000; Chilliard *et al.*, 2001) and was positively related to body fatness or BCS (Delavaud *et al.*, 2000; Thomas *et al.*, 2001; Daniel *et al.*, 2002).

Undernutrition effects are largely documented in early lactation where it is common even with large pasture availability because of the imbalance between feed intake and nutrient requirements. However, ewes in mid or late-lactation may experience a period of moderate or severe undernutrition.

This study investigate the effects of short-term feed restriction on milk production traits, and on metabolic and hormonal responses in mid-lactation dairy ewes with low and high BCS, to test the hypothesis that ewes with different body fat reserves have different strategies to counteract undernutrition.

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Materials and methods

Experimental design

Ten Sarda dairy ewes at mid-lactation (90-120 DIM) were selected according to BCS. Five ewes with high BCS (H-BCS, BCS>2.5; BCS 2.92±0.12; BW 48.8±5.4 kg; mean+SD) and 5 with low BCS (L-BCS, BCS<2.5; BCS 2.28±0.07; BW 36.2±4.7 kg) were chosen from the same flock.

During 15 days prior to the experiment, ewes were acclimated to the single pens where they were also housed throughout the experi-

ment. All pens were inside the same enclosure under natural ambient temperature and photoperiod. The experiment was conducted in accordance with the guidelines of the Council Directive of the EC (European Commission, 1986). Animal health was monitored continuously before and during the experiment.

Ewes were fed a formulated diet to meet their energy requirements using the CNCPS Ovini® software (Cannas *et al.*, 2004). The experimental period lasted 13 days (from 10 to 23 May 2008) and was made up of 3 phases: seven days preliminary (Prel) period during which ewes were fed a total mixed pelleted ration (TMR 2.5 kg/d per head, 100% requirements), three days of feed restriction (FR) at 50% of their previous intake and three days of refeeding (Re-Fed) in which groups were fed TMR as in the Prel period. A feed restriction of 50% of the energy requirement has been considered severe because it falls below required energy maintenance levels. The TMR composition (on a DM basis, 90.84% DM) was: 32.7% NDF, 20.24% ADF, 3% ADL, 17.66% CP and 4.5% EE (rich in palm oil; C16:0 41% of total FA). Ewes were hand-milked twice daily at 7.00 and 18.00 hours. TMR was administered 3 time a day at 8.00, 14.00 and 20.00 hours.

Sampling and measurements

Milk yield was recorded and milk samples were collected daily at each milking. Milk was stored at 4°C until analyses. Undernutrition started at Day 0 (last day of the Prel period) after the afternoon sampling (immediately before the evening milking). Days 1 to 3 represent the FR period. Normal feeding was restored after the afternoon sampling on Day 3. Therefore, Days 4-6 represent the Re-Fed period. Milk samples were analyzed for fat, total protein (N×6.38), lactose and urea (MUN) concentration, somatic cell count (SCC) and fatty acid profile.

Blood samples were collected at the Prel period (0.00 hours), during the FR period (12, 24, 36, 54 and 78 hours) and during the Re-Fed period (126, 174 hours) to determine the plasma concentrations of non-esterified fatty acids (NEFA), urea (BUN), glucose, insulin, growth hormone (GH) and insulin-like growth factor-1 (IGF-I). Except for 172 hours, the same sample times were used to determine leptin. Blood samples were collected before TMR administration (at 8.00, 14.00 and 20.00 hours) in 10 mL vacutainer tubes (Becton Dickinson, Le Pont Claix, France) containing EDTA K3 (to determine NEFA and BUN) or lithium heparin (to determine insulin, GH, IGF-I and leptin), and in 5 mL vacutainer tubes containing lithium heparin and lithium iodo-acetate to determine glucose. Plasma was separated by centrifugation (1500×g, 10 min) within 20 min of sampling and stored at -20°C until analyses.

Milk analyses

Fat, total protein and lactose concentrations of individual milk samples of morning and afternoon milkings were determined according to FIL-IDF (2000) using a Milkoscan-6000 (Foss Electric, Hillerød, Denmark) calibrated for sheep milk. SCC was determined using a Fossomatic 360 (Foss Electric). Milk urea nitrogen was determined by differential pH-metre (Microlab® EFA, Ardea, Italy).

Preparation procedures for milk fat extraction and fatty acid methyl esters (FAME) were as detailed by Nudda *et al.* (2005). The FAME were separated on a capillary column (CB-Fame CP-select; 100 m× 0.32 mm i.d., 0.25 μm film thickness, Varian Inc., Palo Alto, CA, USA). The injector and FID temperatures were 255°C. Programmed temperature was 75°C for 1 min, raised to 165°C at a rate of 8°C/min and held for 35 min, increased to 210°C at a rate of 5.5°C/min, and finally raised to 240°C at a rate of 15°C/min. The split ratio was 1:40 with He at a pressure of 37 psi used as carrier gas. Individual FAME were identified by comparing the relative retention times of FAME peaks from samples using the standards mixture 37 Component FAME Mix (Supelco, Bellefonte, PA, USA). The standards PUFA-2, non-conjugated 18:2 isomer mixture, and individual cis-5,8,11,14,17 C20:5, cis-4,7,10,13,16,19 C22:6 (Supelco, Bellefonte, PA, USA), cis-6,9,12 C18:3 and cis-9,12,15 C18:3 (Matreya Inc., Pleasant Gap, PA, USA) were used to identify polyunsaturated fatty acids. High purity individual conjugated linoleic acid (CLA) cis9, trans11 and trans10, cis12 (Matreya Inc.) were used. Identification of other CLA peaks was made using commercially purchased CLA standard mix (Nu-Check-Prep, Inc., Elysian, MN, USA). Individual trans9 C18:1 and trans11 C18:1 (Supelco) were used to identify trans C18:1 isomers. The relative amount of each FAME was quantified by integrating the area under the peak and dividing the result by the total area of all fatty acids. The internal standard (C19:0) was used to convert peak area percentage to weight percentage (mg/100 mg of total FAME).

Blood analyses

Plasma concentration of metabolites was determined using the colorimetric method. Commercial kits were used to determine plasma glucose and BUN (Adaltis, Bologna, Italy) and circulating NEFA (Randox, Crumlin, UK).

Plasma insulin concentrations were determined in duplicate using the Mercodia® sheep insulin (Uppsala, Sweden) ELISA kit. Sensitivity of the assay was 0.1 μg/L.

Growth hormone was assayed by validated double-antibody radioimmunoassays (RIA) (Baratta *et al.*, 1997). Briefly, GH was radio-iodinated with 125I according to Salacinski *et al.* (1981). Ovine somatotropin (o-GH–LER 1774) was used as labelled ligand (specific activity 32.15). Ovine somatotropin (o-GH–LER 1774) was used as standard. A rabbit anti-bGH antiserum (NIH-GH-B13) was used at final dilutions of 1:7000. Assay sensitivity was 310±26.4 pg/mL. The intra- and inter-assay coefficients of variation were 6.89 and 15.00%, respectively. Leptin concentrations were determined by using a multi-species leptin RIA kit (Linco Research, St. Charles, MO, USA). All samples were analyzed in a single assay with a limit of detection of 1 ng/mL. Plasma concentration of IGF-I was evaluated by RIA according to the method described by Renaville *et al.* (1993).

Statistical analyses

All milk variables, except for fatty acid profile, were analyzed by SAS software (2001; SAS Inst. Inc., Cary, NC, USA) using the following repeated measures linear mixed model:

$$Y=\mu+ B+S+B\times S+E+C+\varepsilon$$

where Y is the dependent variable (milk variables);
μ is the overall mean;
B is the fixed effect of BCS;
S is the fixed effect of sampling (Days 0, 1, 2, 3, 4, 5 and 6);
B×S is the fixed effect of the interaction between BCS and sampling;
C is a covariable represented by the average of the considered traits during the first six days of the Prel period.

The interaction BCS × sampling allows us to evaluate the effect of feed restriction in the two BCS groups. Multiple comparisons were performed using a *t*-test. E is the random effect of the ewe and ε the random residual. SCC was log transformed before statistical analysis. Fatty acids of milk were analyzed using the same model without covariate. Blood variables were analyzed with the model without covariate, with sampling (0, 12, 24, 36, 52, 78, 124 and 172 hours) and BCS as the main factors. All analyses were developed using the MIXED procedure of SAS/STAT software (SAS, 1996). $P\leq0.05$ was considered significant; $P\leq0.10$ showed tendency.

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Results and discussion

Milk yield and composition

Milk yield and composition of H-BCS and L-BCS ewes during the Prel period (mean 7 days) are reported in Table 1. In this initial period, a difference in milk yield, and milk fat and protein concentration was observed between H-BCS and L-BCS groups, whereas no difference was seen in lactose and MUN concentration and SCC (Table 1). During that period L-BCS ewes produced more milk with higher fat concentration but lower protein concentration compared to H-BCS ewes. These data are in contrast to previous observations in early lactating ewes where milk yield was not affected by BCS, whereas milk fat concentration was higher and protein concentration lower for ewes with higher fat reserves (Cowan *et al.*, 1980). In our experiment, the initial differences in milk yield between the two BCS groups supported the use of the covariate analyses to compare the influence of treatments over these productive parameters throughout the trial.

Statistical significance of the factors included in the mixed model analysis for the different dependent variables considered are summarized in Table 2. The effect of sampling was statistically significant for milk yield, milk fat, protein and urea concentration, the proportion of all fatty acid, and blood glucose, NEFA, BUN, insulin, GH and IGF-I. BCS affected several fatty acids (Table 3) and plasma concentration of NEFA, GH and IGF-I. The interaction BCS×sampling was significant only for milk fat concentration (P=0.009) and showed a tendency for leptin (P=0.073), indicating a similar mean response of the two BCS groups throughout the whole experimental period for almost all variables monitored (Figures 1-12).

During the FR period, there was a significant decrease in milk yield but by the end of the Re-Fed period (Day 6) this was completely restored in both BCS groups (Figure 1). The reduction in milk yield during FR found in our experiment is in accordance with other studies on feed restricted cows (McGuire *et al.*, 1995; Samuelsson *et al.*, 1996) and ewes (Agus and Bocquier, 1995). The decrease in milk production at the last day of FR (Day 3) was of 38 and 35% of the milk yield of the Prel period in H-BCS and L-BCS ewes, respectively. The observed reduction in milk yield associated with FR was expected given the design of the experiment: the energy balance, calculated as the difference between energy intake and energy requirement (for milk fat and protein yields and maintenance) as a function of live weight (Cannas, 2004; NRC, 2007) was negative (mean -1.35 Mcal/d) in the FR period for both BCS groups.

Milk fat concentration was not influenced by BCS, but a significant interaction in BCS×sampling was observed (Table 2). In H-BCS ewes, there was an increase in milk fat concentration during the FR period and this returned to Prel levels by the end of the Re-Fed (Figure 2). In contrast, in L-BCS ewes, milk fat concentration remained unchanged throughout the trial. The maintenance of milk fat concentration in L-BCS ewes and its increase in H-BCS, despite the observed changes in milk yield, can be explained by the mobilization of body reserves. In the present study, the likely mobilization of body tissues to provide the mammary gland with fatty acids for milk fat synthesis was confirmed by a corresponding increase in plasma NEFA (Figure 7) during FR. Indeed, plasma NEFA have been shown to reflect mobilization of body reserves in cattle (Chelikani *et al.*, 2004) and sheep (Velez and Donkin, 2005; Chilliard *et al.*, 2007).

There was a significant increase in milk protein (Figure 3) as milk yield decreased due to FR. The pattern of protein concentration showed the well-known concentration effect associated with a reduction in milk yield (Pulina *et al.*, 2006). The initial milk protein concentration was reached at the Re-Fed period. Milk lactose concentration and SCC were not influenced by BCS, sampling or their interaction (Table 2). Milk urea nitrogen concentration was not influenced by BCS and underwent

Table 1. Mean values and standard errors for milk yield and composition of ewes with high (H-BCS, BCS>2.5) and low (L-BCS, BCS<2.5) body condition score (BCS) during the preliminary period (data are mean values of 7 days).

	H-BCS		L-BCS		
	Mean	SE	Mean	SE	P
Yield, g/d	1290	46.4	1494	49.7	**
Fat, %	5.2	0.08	6.3	0.33	**
Protein, %	5.6	0.07	4.8	0.13	**
Lactose, %	5.1	0.03	5.2	0.04	ns
SCC × 1000/mL	91.6	8.60	115.4	12.2	ns
Urea, mg/dL	49.0	0.86	45.6	1.94	ns

**P<0.01; ns, not significant.

Table 2. Statistical significance of effects included in the mixed model analysis for the different dependent variables considered.

	Effect, P		
Variables	BCS	Sampling	BCS×sampling
Milk			
Yield, g/d	0.129	0.000	0.297
Fat, %	0.960	0.002	0.009
Protein, %	0.121	0.039	0.274
Lactose, %	0.142	0.122	0.607
SCC×1000/mL	0.942	0.126	0.733
MUN, mg/dL	0.777	0.000	0.490
Milk fatty acids, % of total FA			
SCFA	0.809	0.005	0.335
MCFA	0.628	0.000	0.553
LCFA	0.484	0.001	0.525
Blood			
Glucose, mg/dL	0.172	0.071	0.421
NEFA, mmol/L	0.050	0.001	0.285
BUN, mg/dL	0.845	<0.001	0.493
Insulin, mcg/L	0.572	<0.001	0.326
GH, ng/mL	0.017	<0.001	0.156
IGF-I, ng/mL	0.070	0.035	0.259
Leptin, ng/mL	0.286	0.360	0.073

SCC, somatic cell count; MUN, Milk urea nitrogen; SCFA, short chain fatty acid; MCFA, medium chain fatty acid; LCFA, long chain fatty acid; NEFA, non-esterified fatty acids.

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no significant variation during the FR period, but it showed a significant linear decrease throughout the Re-Fed period (Figure 4), following the pattern of BUN described below. A reduction in nitrogen losses, in the form of urea in milk, may have occurred in response to a decline in nitrogen intake due to FR, as reported by Silanikove (2000). Mean concentrations of FAs in milk fat in the H-BCS and L-BCS groups and during sampling are reported in Table 3. The milk fatty acid profile was influenced by BCS and sampling. The interaction was not significant for any of the fatty acid analyzed. There was no significant difference in fatty acid groups in the milk (short-, medium- and long-chain fatty acids; SCFA, MCFA and LCFA, respectively) between the BCS groups at any time point (Figure 5). The FR caused a decrease in SCFA and MCFA (Figure 5 A,B) and an increase in LCFA concentration (Figure 5C) compared to the levels of the Prel period. The increase in LCFA was caused mainly by the increase in stearic (C18:0) and oleic (C18:1 c9) acids. Since the adipose tissue of sheep is composed mainly of oleic, palmitic and stearic acid (Nudda *et al.*, 2007), the observed changes in FA in milk likely reflected their mobilization from the adipose tissue, as previously observed by Rossi and Pulina (1991) in Sardinian sheep subjected to undernutrition. During the Re-Fed period, the milk fatty acid profile acquired characteristics similar to those of the starting period, similar to what occurred for milk yield, and fat and protein concentrations.

Metabolic and hormonal profiles

There was no significant difference in indicators of metabolic (blood glucose, NEFA and urea) and hormonal (blood insulin, GH, IGF-I and leptin) status between BCS groups at the Prel period (0.00 hours) (Figures 6-12). Body condition score influenced plasma concentration of NEFA (P=0.050) and GH (P=0.017), and tended to influence IGF-I (P=0.070) concentration. Sampling affected almost all blood measurements, except for plasma leptin (P=0.360) for which the interaction BCS×sampling tended to reach levels of significance (P=0.073) (Table 2).

There was no significant change in blood glucose concentration throughout the trial, except for a temporary decrease at 24.00 hours of FR for both BCS groups (Figure 6). A decrease in glucose due to feed restriction was reported in ewes (Szymanski *et al.*, 2007) and cows (Athanasiou and Phillips, 1978).

Plasma NEFA concentration was higher in L-BCS (0.174±0.077 mmol/L; mean+SD) than in H-BCS (0.125±0.037 mmol/L) ewes. The significant effect of sampling on NEFA showed an increase of this parameter during the FR period in both BCS groups (Figure 7). Such an increase in plasma NEFA concentration indicates the mobilization of body tissues, as previously observed in cattle subjected to short-

Table 3. Fatty acid (% total FAME) of individual milk samples during the preliminary (Day 0), feed restriction (Days 1-3) and refeeding (Days 4-5) periods in ewes with high (H-BCS) and low (L-BCS) body condition score (BCS).

	BCS		Sampling						P	
	High	Low	0	1	2	3	4	5	BCS	Sampling
C4:0	1.27	1.57	1.55	1.32	1.38	1.29	1.50	1.45	**	***
C6:0	1.39	1.54	1.70^{a}	1.35^{ab}	1.38^{ab}	1.27^{b}	1.52^{ab}	1.58^{ab}	*	*
C8:0	1.70	1.72	1.97	1.63	1.64	1.50	1.70	1.82	ns	ns
C10:0	6.10	5.51	6.63	5.54	5.58	5.16	5.64	6.29	ns	ns
C11:0	0.13	0.05	0.15	0.09	0.10	0.06	0.05	0.10	**	ns
C12:0	4.18	3.42	4.36	3.72	3.76	3.49	3.50	3.95	**	ns
C14:0	9.45	8.97	9.49	8.35	8.87	8.80	9.86	9.89	ns	ns
C14:1 c9	0.31	0.19	0.28^{ab}	0.20^{b}	0.20^{b}	0.21^{b}	0.27^{ab}	0.34^{a}	**	**
C15:0	0.88	0.63	0.85	0.74	0.74	0.73	0.69	0.80	*	ns
C15:1	0.19	0.16	0.17	0.18	0.20	0.19	0.16	0.16	*	ns
C16:0	28.32	29.82	28.8^{ab}	27.5^{b}	28.4^{ab}	28.7^{ab}	31.0^{a}	30.0^{ab}	*	*
C16:1 n7	1.55	1.04	1.44^{ab}	1.21^{ab}	1.07^{b}	1.10^{b}	1.34^{ab}	1.61^{a}	**	**
C17:0	0.50	0.49	0.44^{bc}	0.62^{a}	0.55^{ab}	0.56^{a}	0.38^{ab}	0.41^{b}	ns	**
C17:1 c10	0.22	0.16	0.22	0.22	0.20	0.19	0.16	0.15	**	ns
C18:0	6.19	7.50	5.48^{b}	7.96^{a}	7.81^{a}	8.02^{a}	6.34^{ab}	5.47^{b}	**	**
C18:1 t11	0.97	1.80	0.56^{b}	0.79^{ab}	1.60^{ab}	1.97^{a}	1.89^{a}	1.48^{ab}	**	**
C18:1 c9	25.79	24.69	25.41^{ab}	27.11^{a}	26.00^{a}	25.84^{ab}	23.11^{b}	24.11^{b}	ns	*
C18:2c9,c12	5.06	5.08	5.67	5.43	5.09	4.78	4.77	4.66	ns	ns
C18:3 n3	0.35	0.34	0.36	0.39	0.34	0.34	0.34	0.30	ns	ns
CLA c9,t11	1.37	1.61	1.13	1.42	1.53	1.60	1.72	1.52	*	***
EPA	0.01	0.01	0.00	0.02	0.00	0.01	0.00	0.01	ns	ns
DPA	0.08	0.06	0.02^{b}	0.10^{a}	0.09^{a}	0.08^{ab}	0.06^{ab}	0.07^{ab}	ns	*
DHA	0.00	0.01	0.00	0.02	0.00	0.01	0.00	0.00	ns	ns
OBCFA	2.71	2.23	2.46	2.73	2.51	2.61	2.19	2.31	**	ns
SFA	60.32	61.36	61.61^{ab}	59.07^{b}	60.26^{ab}	59.78^{ab}	62.34^{ab}	61.96^{a}	ns	**
MUFA	31.94	30.82	30.44^{b}	32.64^{a}	32.00^{ab}	32.64^{a}	29.93^{b}	30.64^{ab}	ns	**
PUFA n6	7.22	7.36	7.44	7.64	7.26	7.11	7.30	6.97	ns	ns
PUFA n3	0.67	0.61	0.61	0.76	0.63	0.65	0.61	0.56	ns	ns
D9 activity	0.64	0.50	0.67^{a}	0.68^{a}	0.53^{b}	0.49^{b}	0.53^{b}	0.55^{b}	**	*

SFA, saturated fatty acids: sum of C4, C6, C8, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C22:0, C24:0 and odd-branched fatty acids; MUFA, monounsaturated fatty acids: sum of C14:1, ΣC16:1, ΣC17:1, ΣC18:1; PUFA, polyunsaturated fatty acids; sum of total n-6 and total n-3; PUFA n-3: sum of C18:3n-3, C18:4n-3, C20:3n-3, C20:4n-3, C20:5n-3, C22:5n-3, C22:6n-3; PUFA n-6: sum of ΣC18:2n-6 *cis/trans* isomers, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:2n-6, C22:4n-6; OBCFA, Odd and branched fatty acids: sum of C13:0, C13:0 *i*, C13:0 *ai*, C14:0 *i*. C15:0 *i*, C15:0 *ai*, C16:0 *i*, C17:0, C17:0 *i*, C17:0 *ai*, Σ C17:1. (*i* is an *iso*-isomer and *ai* is an *ante-iso*-isomer); D9 activity: C18:1 c9/(C18:1 c9+C18:0). **P≤0.01; *P≤0.05; ***P≤0.10; ns, not significant.

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term fasting (Chelikani *et al.*, 2004). During the Re-Fed period, NEFA concentration returned to initial levels. An increase in plasma concentration of NEFA is usually associated with impaired immune function both in cow (Burvenich *et al.*, 2007) and sheep (Lacetera *et al.*, 2001, 2002), that could increase incidence of mastitis (Janosi *et al.*, 2003) and milk SCC (Nyman *et al.*, 2008). In our experiment, milk SCC was not statistically influenced by sampling (P=0.126).

There was a significant decrease in blood urea nitrogen concentration (Figure 8) towards the end of the FR period and at the Re-Fed period. A peak in BUN occurred in both BCS groups at 24.00 hours, probably due to a sudden increase in glucose demand by the mammary gland which had to be buffered by gluconeogenesis from aminoacids. This explanation is reinforced by the concomitant peak of BUN and drop of blood glucose at 24.00 hours, followed by an increase in glucose and a decrease in BUN (Figures 6 and 8). The return of blood glucose and BUN at 36.00 hours to initial levels was likely a consequence of the observed fall of milk production at Day 2 of FR (Figure 1). A higher efficiency in nitrogen metabolism by recycled urea due to feed restriction could be a cause of the decreasing BUN (Silanikove, 2000). The pattern of BUN was similar to that observed in MUN (Figure 4), in accordance with the strong correlation previously observed between these two variables in dairy sheep (Cannas *et al.*, 1998).

Insulin concentration (Figure 9) remained stable during the FR period and rose in the Re-Fed period to a level markedly higher than those of the Prel and FR periods in both BCS groups. The sudden increase in both BCS groups in plasma insulin concentrations after refeeding had been previously observed in male sheep, regardless of the level of body

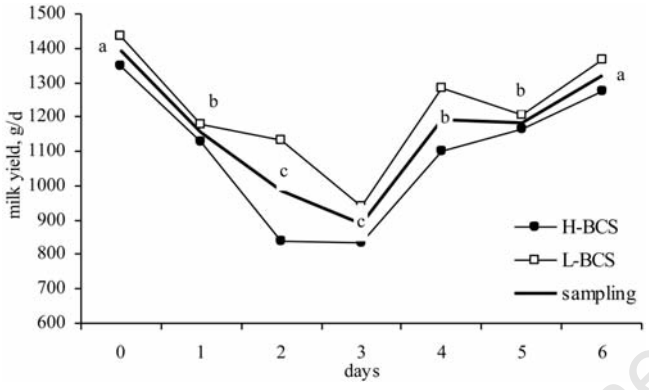

Figure 1. Milk production during the preliminary (Day 0), feed restriction (Days 1-3) and refeeding (Days 4-6) periods in ewes with high (H-BCS) and low BCS (L-BCS) body condition score. Time points throughout the continuous line with different letters are significantly different (P<0.05) during sampling.

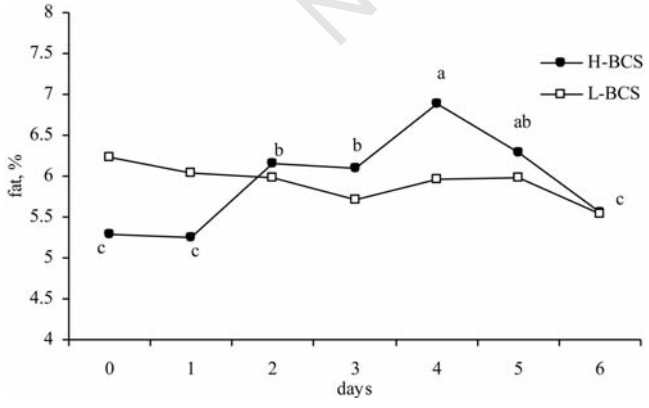

Figure 2. Milk fat concentration during the preliminary (Day 0), feed restriction (Days 1-3) and refeeding (Days 4-6) periods in ewes with high (H-BCS) and low BCS (L-BCS) body condition score. Time points within each BCS group with different letters are significantly different (P<0.05) during sampling.

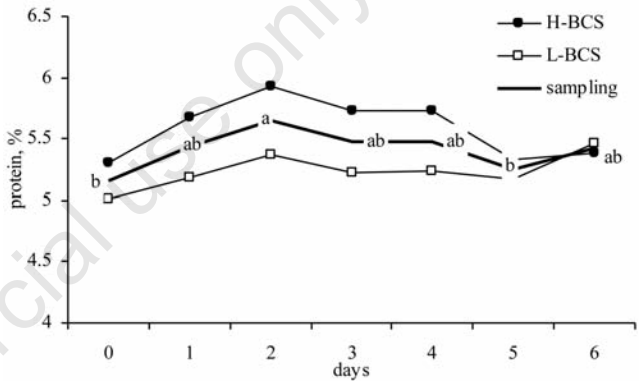

Figure 3. Milk protein concentration during the preliminary (Day 0), feed restriction (Days 1-3) and refeeding (Days 4-6) periods in ewes with high (H-BCS) and low BCS (L-BCS) body condition score. Time points throughout the continuous line with different letters are significantly different (P<0.05) during sampling.

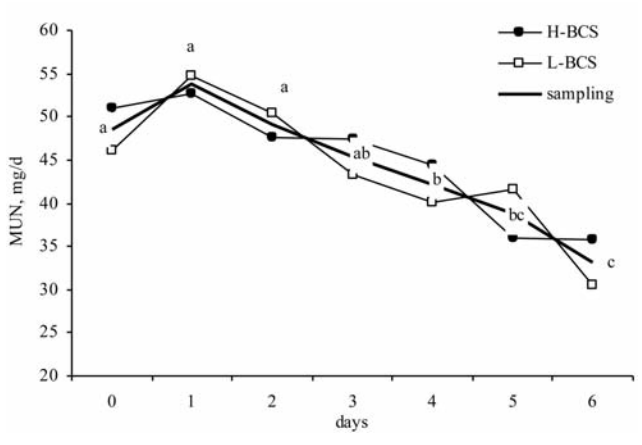

Figure 4. Milk urea nitrogen (MUN) concentration during the preliminary (Day 0), feed restriction (Days 1-3) and refeeding (Days 4-6) periods in ewes with high (H-BCS) and low BCS (L-BCS) body condition score. Time points throughout the continuous line with different letters are significantly different (P<0.05) during sampling.

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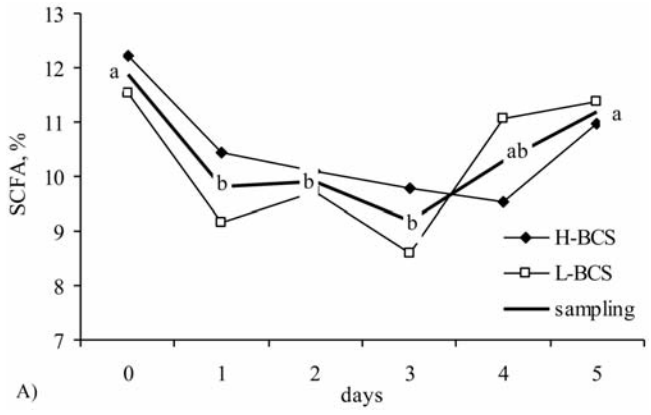

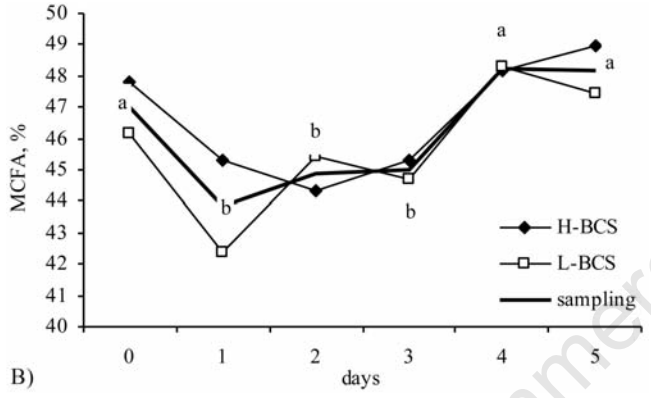

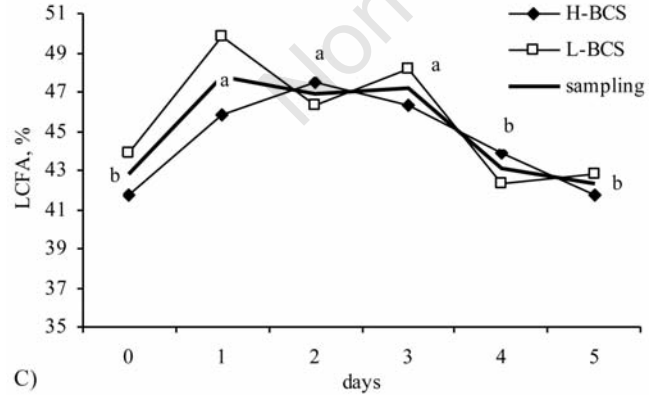

Figure 5. Milk short (SCFA, C4-C10), medium (MCFA, C12-C17) and long (LCFA, (≥C18) chain fatty acids during the preliminary (Day 0), feed restriction (Days 1, 2 and 3) and refeeding (Days 4 and 5) periods in ewes with high (H-BCS) and low BCS (L-BCS) body condition score. Time points throughout the continuous line with different letters are significantly different (P<0.05) during sampling.

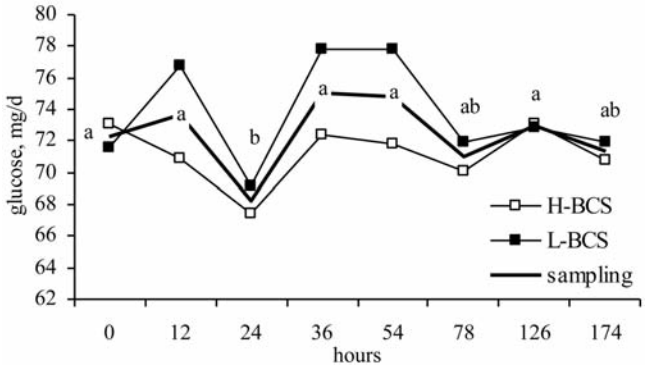

Figure 6. Plasma concentration of glucose during the preliminary (0 h), feed restriction (12-78 h) and refeeding (126-174 h) periods. Time points throughout the continuous line with different letters are significantly different (P<0.05) during sampling.

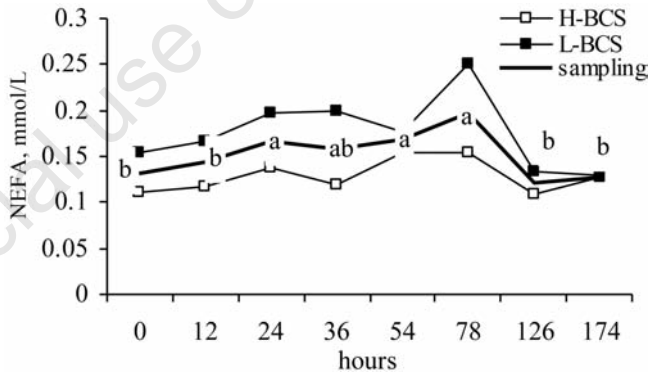

Figure 7. Plasma concentration of non-esterified fatty acids (NEFA) during the preliminary (0 h), feed restriction (12-78 h) and refeeding (126-174 h) periods. Time points throughout the continuous line with different letters are significantly different (P<0.05) during sampling.

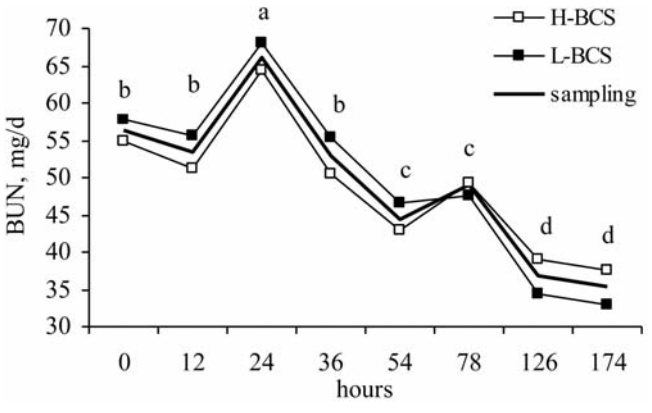

Figure 8. Plasma concentration of urea (BUN) during the preliminary (0 h), feed restriction (12-78 h) and refeeding (126-174 h) periods. Time points throughout the continuous line with different letters are significantly different (P<0.05) during sampling.

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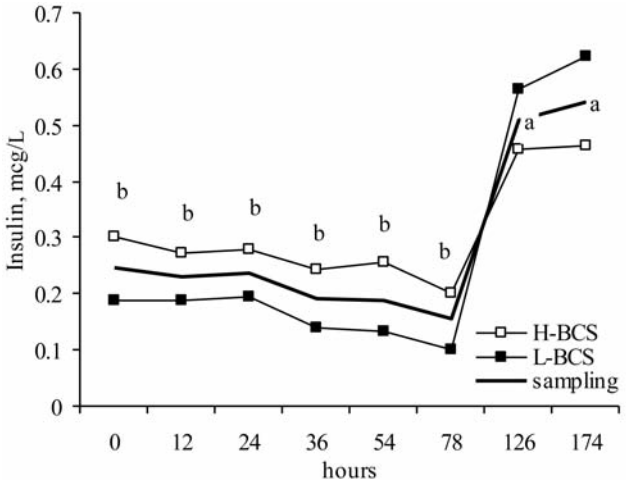

Figure 9. Plasma concentration of insulin during the preliminary (0 h), feed restriction (12-78 h) and refeeding (126-174 h) periods. Time points throughout the continuous line with different letters are significantly different (P<0.05) during sampling.

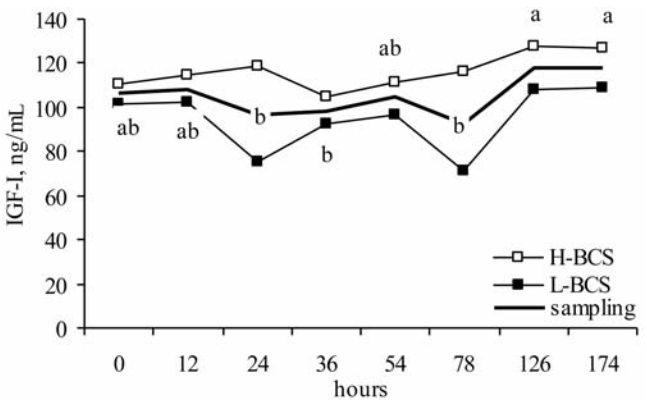

Figure 11. Plasma concentration of insulin-like growth factor-1 (IGF-I) during the preliminary (0 h), feed restriction (12-78 h) and refeeding (126-174 h) periods. Time points throughout the continuous line with different letters are significantly different (P<0.05) during sampling.

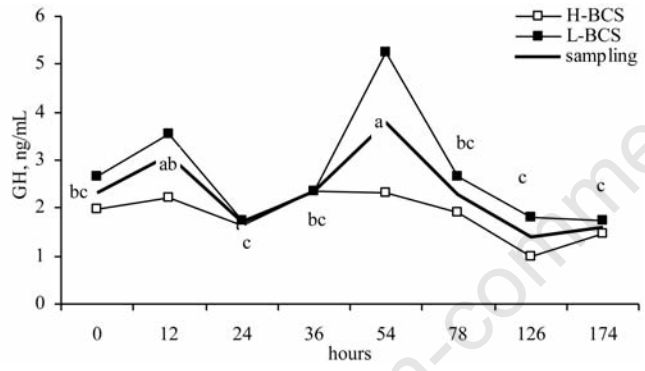

Figure 10. Plasma concentration of growth hormone (GH) during the preliminary (0 h), feed restriction (12-78 h) and refeeding (126-174 h) periods. Time points throughout the continuous line with different letters are significantly different (P<0.05) during sampling.

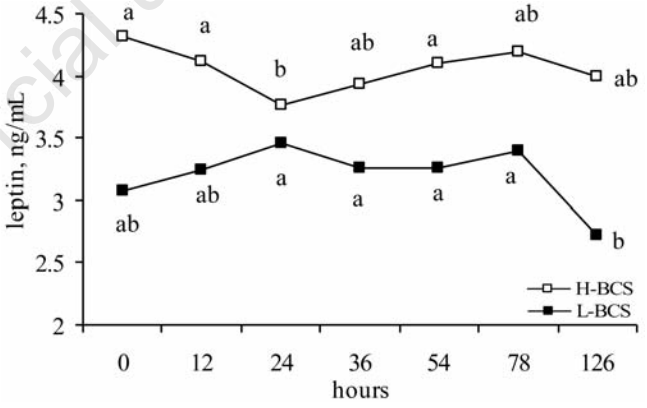

Figure 12. Plasma concentration of leptin during the preliminary (0 h), feed restriction (12-78 h) and refeeding (126-174 h) periods. Time points within each BCS group with different letters are significantly different (P<0.05) during sampling.

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reserves, after an acute increase in the level of nutrition (Zhang *et al.*, 2005). This pattern was a clear metabolic response to a sudden increase in the availability of blood glucose from feeds, which exceeded the level of glucose required by the mammary gland.

Growth hormone concentration (Figure 10) was higher in L-BCS (2.71±1.72; mean±SD) than in H-BCS (1.89±1.00) ewes. Throughout sampling, average GH showed a peak at 52 h. An increase in GH due to food deprivation or undernutrition had been previously observed in different species (Atinmo *et al.*, 1978; Driver and Forbes, 1981; McGuire *et al.*, 1995), including lactating ewes. Even if the interaction between BCS and sampling was not significant, the GH pattern showed a marked peak only in L-BCS ewes. This assessment is reinforced by the IGF-I trend of the L-BCS (Figure 11) that reflected that of GH with a delayed negative peak. The change in IGF-I in such a short time could be related to a variation in local production of IGF-I in skeletal muscles rather than to a general endocrine regulation of IGF-I following an adaptation to undernutrition. Leptin concentration (Figure 12) decreased at 24 h of FR in H-BCS, whereas it decreased at Re-Fed in L-BCS ewes. In general, there is a positive correlation between leptinemia and BCS (Chilliard *et al.*, 2001). Different patterns in plasma leptin in groups with different fat reserves were reported in sheep (Delavaud *et al.*, 2000) and lambs (Altmann *et al.*, 2006). Body fatness strongly regulates leptin and its responses to other factors. Overall, leptin concentration reflects the medium number of adipocytes in the body fat stored in animal tissue (Soukas *et al.*, 2000).

Conclusions

Our results demonstrate that the responses of low BCS ewes to short-term feed restriction were similar to those of high BCS ewes in terms of milk production traits, except for milk fat concentration. This implies that low BCS ewes employ different hormonal and metabolic strategies to high BCS ewes to counteract the temporary feeding shock. In particular, thinner ewes showed higher body fat mobilization which was seen by higher NEFA in the blood and higher GH and lower IGF-I secretion. Furthermore, 3-day feed restriction was sufficient to cause changes in the FA profile of milk fat for both BCS groups, increasing LCFA at the expense of SCFA and MCFA.

Based on this study, Sarda dairy sheep seem to be particularly resilient to environmental constraints, being able to restore fairly quickly the production level and milk composition after a severe but short period of undernutrition, regardless of body condition.

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