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Subcutaneous adipocyte diameter variations during lactation in sheep

E. PIASENTIER
S. BOVOLENTA
S. FILACORDA
P. SUSMEL
DIPARTIMENTO DI SCIENZE
DELLA PRODUZIONE ANIMALE
UNIVERSITA DI UDINE
UDINE
ITALY

SUMMARY - Results are presented for measurements made on 12 lactating Bergamasca ewes and a further 6 dry ewes which grazed together. The lactating ewes had been separated into 2 groups in the first half of pregnancy and fed differently. During this period, from an initial body condition score of 3.0 units, one group (MBC) gained 0.4 points and the other (LBC) lost 0.25 points. Samples of subcutaneous adipose tissue were removed from the rump by human biopsy syringe 3, 11, 21 and 43 days after lambing to determine the average adipocyte diameter. At the same time, the ewes were body scored. The dry ewes (NL) were examined on the first and third measurement day. Both groups of lactating ewes lost body condition, measured both in terms of body score and adipocyte diameter, while the NL ewes maintained their condition. The absolute variation of D was higher in the ewes in group MBC. The adipocyte diameter allowed the identification of a phase of rapid fat mobilization during the first 11 days of lactation. The cell diameter contributed more than BCS to the overall discrimination between clusters of different body condition.

Key words: Subcutaneous adipocyte diameter, body score, lactating ewes.

RESUME - *"Evolution du diamètre des adipocytes sous-cutanés chez des brebis pendant la lactation". La recherche a été effectuée sur 12 brebis de race Bergamasca en lactation ainsi que sur 6 brebis taries, au pâturage. Les brebis en lactation avaient été séparées en 2 groupes alimentés différemment pendant les premiers mois de mise bas. Dans cette phase, en partant de la même note d'état corporel qui était égale à 3,0 unités, les brebis d'un groupe (MBC) ont augmenté de 0,4 points, tandis que les brebis du deuxième groupe (Groupe LBC) ont perdu 0,25 points. Des échantillons de tissu adipeux ont été prélevés au niveau de la croupe, à travers un cathéter pour biopsie humaine, respectivement 3, 11, 21, et 43 jours après la mise bas, afin de déterminer le diamètre moyen des adipocytes (D). Les brebis taries (NL) ont été examinées pendant le premier et le troisième contrôle. L'état corporel, qui a été mesuré soit en tenant compte de la note soit du diamètre des adipocytes, a diminué dans les deux groupes des brebis en lactation, tandis qu'il s'est maintenu constant chez les brebis taries. La variation absolue du diamètre moyen des adipocytes D a été plus grande chez les brebis du groupe MBC. Le diamètre des adipocytes a permis l'identification d'une phase de mobilisation rapide du gras corporel pendant les 11 premiers jours de lactation. Le diamètre des adipocytes a contribué, dans une plus grande mesure que la note d'état corporel, à la séparation de ces mesures en 2 clusters statistiquement différents.*

Mots-clés : *Diamètre des adipocytes sous-cutanés, note d'état corporel, brebis en lactation.*

Introduction

The diameter of subcutaneous adipocytes sampled from the tail base area allows the estimation of the proportion of fat in the whole body of shorn Bergamasca ewes in lactation with a precision of 15.6 g/kg Live Weight (LW) (Susmel *et al.*, 1994).

The method, proposed by Robelin and Agabriel (1986) for use with cattle, appears generally useful for allocating animals into experiment groups with different mean body fat content.

The aim of the present work was to verify the efficacy of the subcutaneous adipocyte diameter to rank ewes in groups on the basis of their body condition *post partum* and to follow the evolution of body reserves during the first weeks of lactation, in comparison with the body condition score technique.

Material and methods

Animals

Results are presented for measurements made on 12 lactating Bergamasca ewes and a further 6 dry animals which grazed the same fescue pasture.

The number of animals originally planned in the experimental design was 21. Three ewes were excluded because it was not possible to perform the sampling of subcutaneous fat from the rump in each measurement period.

During the first 2.5 months of pregnancy the ewes, with an initial mean body condition score of 3.0 (Russel *et al.*, 1969), were divided in two groups and fed at different levels by grazing group 2 after group 1 on the same areas of pasture. Group 1 (Medium Body Condition, MBC) gained a mean of 0.4 units of body condition and group 2 (Low Body Condition, LBC) lost a mean of 0.25 units of body condition in early pregnancy. During the last 10 weeks of pregnancy, the 2 groups were combined and managed together. The dry ewes (group NL) were always in the MBC group. During lactation, half the ewes were milked by machine, while the others were allowed to suckle their lambs.

Weight measurements and body condition

These were performed at 3, 11, 21 and 43 days from lambing on the lactating ewes; those in group NL were measured at the same time as the first and third recordings for the lactating ewes.

LW was recorded on 2 successive days, as was Body Condition Score (BCS), which was measured by a single operator using the Russel *et al.* (1969) scale.

The diameter of the subcutaneous adipocytes (D) was measured on a sample of fat taken from the rump by human biopsy syringe in each of the 3 measurement periods and during slaughter in the fourth period. The procedure adopted for fixation, preparation and measurement has been described by Susmel *et al.* (1994). For each sample, the diameter of at least 100 cells was measured, obtained from a maximum of 4 microscope slides.

Body composition

At the end of the period of lactation considered, the ewes were slaughtered. Their bodies were sampled and analysed as described by Susmel *et al.* (1994). To date, only the determination of body dry matter content has been completed.

Statistical analysis

The data for LW, BCS and D were analysed using the following model:

$$Y_{ijkl} = \mu + A_i + B_j + AB_{ij} + C_{k/ij} + P_l + AP_{il} + BP_{jl} + \varepsilon_{ijkl}$$

testing for the significance of the differences between the levels of body condition (A_i) and the type of lactation, machine milked or suckled (B_j), on the variability of the ewes within the experimental groups ($C_{k/ij}$). The differences between measurement periods (P_l) was tested with the residual error (ε_{ijkl}).

In this paper, the least square means for groups MBC and LBC are presented and discussed.

Cluster analysis (SAS-STAT package, 1988; Fastclus procedure) of the 60 paired observations of BCS and D was used to identify the average parameters of two different groups of measurements which could be attributed to animals in average or poor body condition.

Results

In Table 1, the average weight and body condition of the groups during the whole experimental period are shown. The differences in weight between MBC and LBC ewes were not statistically significant. During the first 6 weeks of lactation, group MBC maintained a higher average body condition than group LBC, expressed either in terms of BCS or D (3.08 *vs* 2.64 and 49 *vs* 30 µm respectively).

At the end of lactation, the ewes in group MBC had a higher content of dry matter in the Empty Body Weight (EBW) than those in group MBC (32.5 *vs* 27.5%) (Table 2).

Table 1. Average live weight and body condition during the first 6 weeks of lactation of Bergamasca ewes in medium (MBC) and low (LBC) body condition at lambing

	MBC	LBC	SE†
Observations (No)	24	24	
Live weight (kg)	67.0	64.9	14.20
Adipocyte diameter (µm)	49[b]	30[a]	16.9
Body condition score	3.08[b]	2.64[a]	0.517

†D.F.: 8
a,b: P<0.05

Table 2. Fleece free empty body weight (EBW) and body dry matter content at 6 weeks of lactation of Bergamasca ewes in medium (MBC) and low (LBC) body condition at lambing

	MBC	LBC	SE†
Animals (No)	6	6	
Empty body weight (kg)	46.4	43.3	5.83
Body dry matter (g/kg EBW)	32.5[b]	27.5[a]	2.03

†D.F.: 8
a,b: P<0.05

Figure 1A shows the evolution of live weight in groups MBC, LBC and NL. A progressive fall in weight during lactation can be observed in both MBC and LBC ewes, from 69.6 to 64.3 kg (P<0.05) and from 67.4 to 63.0 kg respectively (P<0.05). The dry ewes, in the period between the first and third measurement days, did not undergo significant changes in weight, which was, on average, higher than the lactating ewes (72.3 *vs* 67.1 kg).

The variations in body condition in groups MBC, LBC and NL can be seen in Figs 1B and 1C. At each recording date, both D and BCS were higher for the MBC ewes than those in LBC (P<0.05). In both groups, D diminished from one measurement to the next, but the difference only reached significance (P<0.05) between the 1st and 2nd measurements (69 *vs* 46 µm and 41 *vs* 30 µm respectively for groups MBC and LBC) (Fig. 1B). In the period between the 1st and 3rd measurements, D did not change for the dry ewes with an average value (70 µm) similar to that of the MBC group at lambing. As for cell diameter, BCS also demonstrated its greatest variation between the 1st and 2nd measurements (3.22 *vs* 3.09 units and 2.75 *vs* 2.63 units respectively for groups MBC and LBC) (Fig. 1C), although the difference between the measurements did not reach significance. The pattern of BCS changes was similar in MBC and LBC groups. NL ewes had an

average BCS (3.58 units) higher than the initial MBC value and a positive variation of BCS (+0.17 units) in the period between the 1st and 3rd measurements which was at the limit of significance (P=0.06). The total variation of D was higher in the ewes in group MBC than in group LBC (31 *vs* 18 µm, P<0.05), while the total loss of BCS was not significantly affected by the body condition at lambing (0.28 *vs* 0.22).

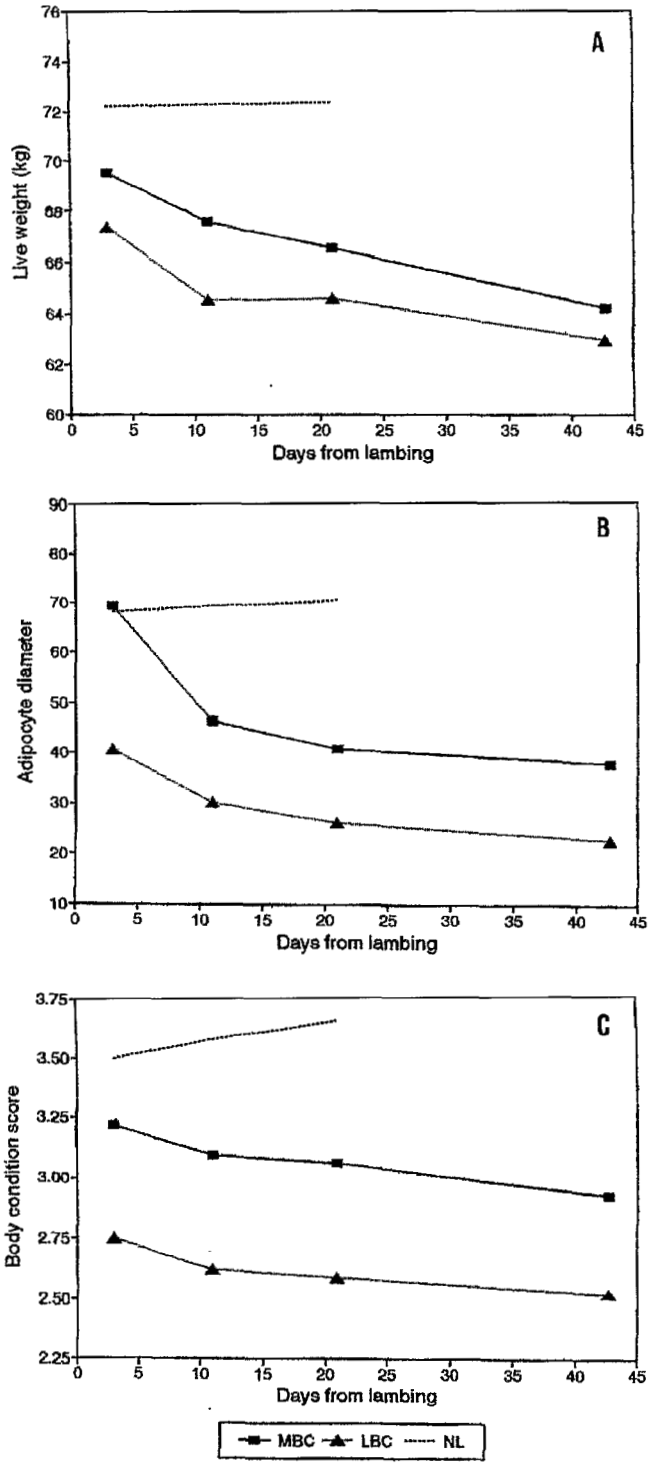

Fig. 1. (A) mean live weight, (B) subcutaneous adipocyte diameter from the rump (D, µm) and (C) body condition score (BCS) of Bergamasca ewes in medium (MBC) and low (LBC) body condition at lambing during the first 6 weeks of lactation and of dry Bergamasca ewes (NL) grazing the same pasture at the same time.

Figure 2 shows the joint sample distribution of BCS and D for the 60 observations made during the experiment; the coefficient of correlation between the variables was 0.82. The observations were partitioned into disjointed groups by cluster analysis. Two clusters were obtained (P=0.001, canonical correlation = 0.89; eigen value = 3.78).

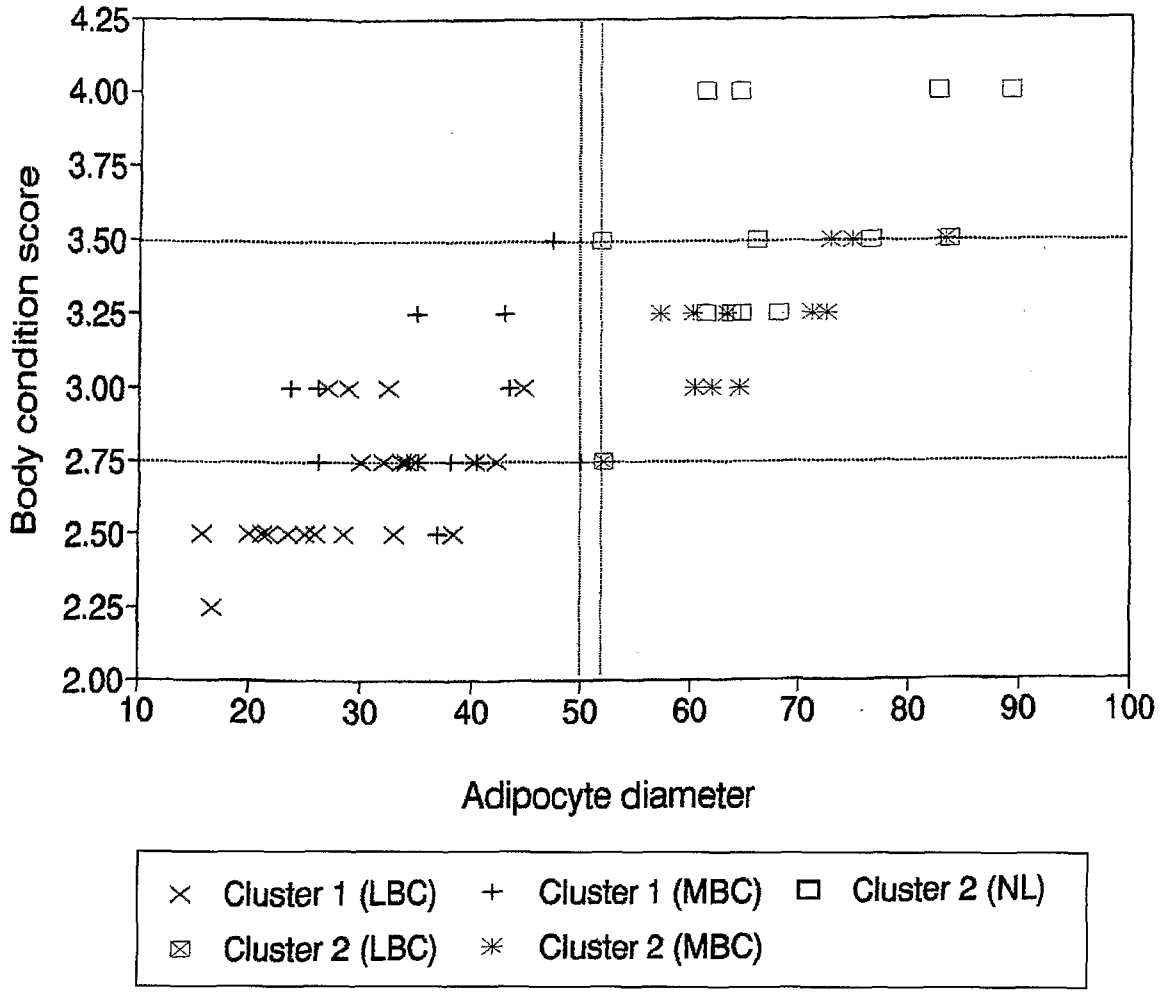

Fig. 2. Joint distribution of subcutaneous adipocyte diameter from the rump (μm) and body condition score measured on Bergamasca ewes in medium (MBC) and low (LBC) body condition at lambing during the first 6 weeks of lactation and on dry Bergamasca ewes (NL), and grouped into clusters of different body condition (clusters 1 and 2).

The observations from the NL ewes, the first measurement of all the MBC ewes and 1 LBC ewe, and 7 recordings from 2 MBC ewes in late lactation were grouped into cluster 2; these latter ewes maintained good body condition even at the end of the experimental period. Descriptive statistics of D and BCS in the clusters are reported in Table 3. The average D was 32 ± 9 μm in cluster 1 and 68 ± 10 μm in cluster 2, with a small overlap around 50 μm into which fell 3 doubtful observations. The mean value of BCS was 2.76 ± 0.26 and 3.40 ± 0.34 for the two clusters which had a large overlap between 2.75 and 3.50, in which more than half cases occurred.

To assess the contribution of the variables to the overall discrimination between groups, the canonical discriminant function standardised coefficients and the total and pooled within groups correlation between the values of the function and the values of variables were considered. These parameters were higher for D (2.124; 1.00 and 1.00 respectively) than for BCS (0.054; 0.82 and 0.55 respectively).

Table 3. Descriptive statistics of the clusters generated for grouping the observations into classes of different body condition

	Cluster 1	Cluster 2
Observations	36	24
Adipocyte diameter (μm):		
mean	32	68
standard deviation	9	10
min	16	52
max	50	89
Body condition score:		
mean	2.76	3.40
standard deviation	0.26	0.34
min	2.25	2.75
max	3.50	4.00

Discussion

The method of obtaining samples of fat *in vivo* gave satisfactory results, better than those reported by Sebastian *et al.* (1989) who performed a biopsy of subcutaneous adipose tissue in the lumbar region of lactating Aragonesa ewes. The sampling difficulties in some animals were probably related to the reduced thickness of the subcutaneous fat in the thin ewes. There was also an individual variability in the ease of performing the operation, which did not allow us to relate the practical failings to a fixed lower limit of fat content or a given body condition score. In any case, there were no signs of suffering in the animals, nor was there any change in behaviour following the samplings. After slaughter, the zone penetrated by the needle did not have any sign of alteration to the structure of the adipose tissue.

Both D and BCS revealed a reduction in body condition in the lactating ewes which did not occur in the dry sheep which were on the same pasture.

Using the prediction equations calculated in a preceding paper (Susmel *et al.*, 1994), it can be estimated that, on the basis of the reduction of D, the MBC ewes lost 4.3 kg of fat (from 8.0 kg at lambing to 3.7 kg), 85% of which during the first 11 days of lactation, while those in group LBC lost 1.6 kg of fat (from 3.7 kg at lambing to 2.1 kg), 66% of which in the initial phase of lactation. Following the reduction of BCS, the estimates of fat mobilisation were 4.5 kg for MBC ewes (from 8.9 kg at lambing to 4.4 kg), 28% of which in the first 11 days, and 4.1 kg for LBC ewes (from 6.9 kg at lambing to 2.7 kg), 35% of which during the initial phase.

Therefore, while measurements of D allowed the estimation of more consistent losses of adipose tissue in MBC ewes than in the LBC group, the variations of BCS did not highlight an effect of body condition at lambing on body fat mobilisation during lactation.

Furthermore, the diameter of the adipocytes showed an early phase of more pronounced reduction than did BCS. At the end of this rapid loss of body condition, after the second week of lactation, the phenomenon slowed. The different initial pattern could have been due, at least in part, to the different sensitivity with which D and BCS detected the reduction in subcutaneous deposits, which are usually lost at a higher rate than total body fat (Vernon, 1986). However, the evolution of D is in agreement with the period of negative energy balance which normally occurs in early lactation when the increase in milk yield is quicker than the increase in appetite.

As in the current experiment, Gibb and Treacher (1982) found that differences in body condition score at lambing, established during pregnancy, had no effects on the pattern of BCS changes by Scottish Halfbred ewes suckling twins, given a plentiful supply of herbage. The BCS showed an initial decline in the first two weeks of lactation and little change thereafter. In contrast, Cowan *et al.* (1980)

found that increased food intake in pregnancy accentuated the amount of fat lost from the empty body during the first 6 weeks of lactation in Finnish Landrace x Dorset Horn ewes fed with a predominantly roughage diet. Furthermore, after 45 days of lactation, as in Bergamasca ewes, sheep fed at a higher level in pregnancy still contained more dry matter, and therefore more fat, in the empty body than sheep fed at a lower level during pregnancy. Similar results were obtained by Geenty and Sykes (1986) with Dorset ewes at pasture.

Considered together, the 2 measurements of body condition, D and BCS, allowed an efficient separation of the observations into 2 clusters which were coherent with what would have been expected from the dietary treatments applied to the ewes during pregnancy and the evolution of the state of fatness during lactation.

Of the 2 variables, D contributed more than the subjective and discrete BCS classification to the overall discrimination between groups of different body condition.

Conclusions

The diameter of subcutaneous fat cells from the rump allowed an efficient separation of animals with different body conditions obtained after different feeding treatments, and an appreciation of the evolution of their body reserves over short periods of time which appeared to be coherent with the expected pattern of mobilisation of adipose deposits during lactation.

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