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in

Purroy A. (ed.).
Body condition of sheep and goats: Methodological aspects and applications

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 27

1995
pages 59-66

Prediction of body fat in lactating ewes using the diameter of subcutaneous adipocyte cells or body condition score

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SUMMARY - The relationship between cellular dimensions or body condition score and fatness was studied in 35 lactating Bergamasca ewes. Eleven animals (group 1) were slaughtered within 2 weeks of lambing and the remainder (group 2) within 6 or 7 weeks of lambing. The chemical body fat was determined by ether extract analysis of 5 minced fractions of the empty body (23 ewes) or estimated from the weights of empty body, body dry matter and dissected kidney and pelvic fat. The adipocyte diameter (D) was calculated from an average of 200 measurements by light microscopy of 200 µm cryostat sections, obtained from 1 g samples of subcutaneous adipose tissue taken from the rump, fixed in 35% neutral formalin and frozen in liquid nitrogen. The body condition score (BS) was measured 1 hour before slaughter by two operators using a 1 to 5 score range with intervals of 0.25 units. The variation in the degree of fatness between the ewes at different stages of lactation was accompanied by changes in adipocyte dimensions and BS. A significant *intra*-group linear relationship among empty body fat content and both body condition measures was also observed. *Intra*-group and pooled correlation coefficients were higher with D than BS. The best prediction equation of body fat content (BF, g/kg LW) was obtained with D^2 (R^2 = 0.92, RRMS = 15.6 g/kg LW), whose coefficients were not modified by lactation stage. The precision of BF estimated from BS (R^2 = 0.74, RRMS = 28.9 g/kg LW) improved when LW at slaughter was included in the prediction model (R^2 = 0.81, RRMS = 25.1 g/kg LW). The stage of lactation had a significant effect on both simple and multiple regression models for prediction from BS.

Key words: Body fat, subcutaneous adipocyte diameter, body score, sheep.

RESUME - *"Estimation de la teneur en lipides du corps par le diamètre des adipocytes sous-cutanés ou les notes d'état corporel chez des brebis en lactation". La relation entre les dimensions des cellules ou la note d'état corporel et l'état d'engraissement a été étudiée chez 35 brebis en lactation, de race Bergamasca. Onze animaux (groupe 1) on été abattus dans les deux semaines après la mise bas, les autres (groupe 2) entre la sixième et la septième semaine de lactation. Les lipides corporels ont été dosés par l'extrait à l'éther de 5 fractions du corps vide (23 brebis) ou estimés en partant du corps vide, de la matière sèche corporelle et de la graisse pelvienne et rénale disséquées. Le diamètre des adipocytes (D) a été calculé sur la base de la moyenne des 200 mesures au microscope optique des sections de 200 µm, ces dernières ayant été obtenues par des échantillons de 1 g de graisse sous-cutanée. Les échantillons avaient été prélevés sur la croupe, fixés dans la formaline neutre à 35% et puis congelés dans l'azote liquide. La note d'état corporel (NEC) a été mesurée une heure avant l'abattage par deux opérateurs, en utilisant une échelle d'évaluation comprise entre 1 et 5 points et avec des intervalles de 0,25 unités. La variation de l'état d'engraissement entre brebis appartenant aux différents stades de lactation a été accompagnée par des variations des dimensions des adipocytes et de NEC. Une relation linéaire significative intra-groupe a été observée entre la teneur en lipides du corps vide et les deux mesures de l'état corporel. Les coefficients de corrélation intra-groupe et ceux "pooled" ont été supérieurs pour D par rapport à NEC. Les meilleures équations de prévision du contenu des lipides corporels (MG, g/kg de poids vif, PV), dont le coefficient de régression n'a pas été influencé par le stade de lactation, ont été obtenues avec D^2 (R^2 = 0,92, écart-type résiduel, RRMS = 15,6 g/kg PV). La précision de l'évaluation de MG à partir de NEC (R^2 = 0,74, RRMS = 28,9 g/kg PV) s'est améliorée lorsque le poids à l'abattage a été inclus dans le modèle de prévision (R^2 = 0,81 RRMS = 25,1 g/kg PV). Le stade de lactation a eu un effet significatif sur les modèles de régression simple et multiple obtenus à partir de NEC.*

Mots-clés : *Lipides corporels, diamètre des adipocytes sous-cutanés, note de l'état corporel, ovins.*

Introduction

The methods and techniques for the *in vivo* evaluation of body fat reserves are numerous and differ in their complexity, cost and accuracy. At the extremes are the technique of body condition scoring (Russel *et al.*, 1969) and the measurement of the diffusion space of water-isotopes (Tissier *et al.*, 1983). Robelin and Agabriel (1986) have proposed an intermediate method for cattle, which estimates the quantity of body fat from the diameter of subcutaneous adipocytes. Susmel *et al.* (1991) evaluated the efficiency of this method with growing lambs.

The aim of the current work was to verify the possibility of using the average diameter of subcutaneous adipocytes as a criterium for the estimation of the quantity of adipose tissue in lactating Bergamasca ewes. The efficiency of this prediction technique was compared with body condition score.

Material and methods

Animals and slaughter measurements

The measurements were performed on 35 animals from 3 experiments, designed to study the performance of lactating Bergamasca ewes maintained at pasture with different protein supplements, stocking rates and body conditions. Eleven animals (group 1) were slaughtered within two weeks of lambing and the remainder (group 2) within 6 or 7 weeks of lambing. The animals were taken from the pasture about 1 hour before slaughter, shorn, milked (after an injection of 6 IU of oxytocin), weighed (LW) and scored for body condition (BS). The BS of the ewes was determined by two operators using the method described by Russel *et al.* (1969). During slaughter a sample of about 500 g blood was taken and the total weight of blood (B) was calculated from the difference in weight *pre* and *post mortem*. The following fractions were separated and weighed: the skin and the distal part of the limbs (SF); the head, tail and diaphragm (HTD); the organs (O) including all the viscera, emptied of their contents, the udder and the kidney and pelvic dissected fat-weighed alone (KPFW) before inclusion in this fraction; the net carcass (C). After storage for 24 h in a refrigerator, SF, HTD, O and C were weighed again and then frozen separately. The empty body weight (EBW) was calculated by summing the warm weights of SF, HTD, O, C and B.

Determination of body composition

The four body components were ground 3 times through an industrial mincer with a plate with 8 mm holes. The blood sample, two samples of C and one sample of SF, HTD and O, all weighing about 500 g, were freeze dried. The freeze dried samples were reminced prior to analysis for residual moisture (at 105°C) and ether extract (Soxhlet method preceded by acid hydrolysis), henceforth referred to as chemical fat. The dry matter (DM) content of each body component was calculated from the losses of water during refrigeration, freeze and oven drying.

For 23 animals, for which the analyses have been completed, DM and total body chemical fat weight (BFW) after shearing were calculated from the analysis of the different body fractions. For the other 12 ewes, BFW has been estimated from the following prediction equation, calculated from the measurements made on the first 23 ewes (all measurements in kg):

$$BFW = -3.39 + 0.814 \cdot DM - 0.123 \cdot EBW + 2.96 \cdot KPFW \pm 1.1;\ R^2 = 0.96$$

Sampling of adipose tissue and measurement of the adipocytes

Immediately after slaughter, samples (about 1 g) of subcutaneous fat were taken from the base of the tail, 2 cm to the right of the body central line, in correspondence with the articulation between the second and third coccyges vertebrae. The tissue sample was fixed in 35% neutral formalin for 7 minutes (Sjöström *et al.*, 1971), frozen in liquid nitrogen and stored at -20°C. Each sample was sectioned (200 µm) with a Leitz 1720 cryostat, with care being taken to collect only those sections

occupying the inside of the sample. The sections were collected and arranged on a slide, covered with a film of water and a slide-cover. The measurements of the adipocytes, 100 per prepared slide, were made under a Leitz orthoplan optical microscope. Fifty measurements of cell diameter were taken at random along the line of the micrometer scale and then a further fifty measurements were taken at right angles to this direction to compensate for any polarisation of the adipocytes. Diameters ≥15.4 µm were considered, which corresponded to 4 intervals (4 x 3.84 µm) on the micrometer scale. Two hundred measurements were made for each sample, on 2 different sections, which were then used to calculate the average diameter (D) of the subcutaneous adipocytes for each experimental animal.

Statistical analysis

The closeness of the linear relationship between body composition and body condition (BS or D) was analysed within-group. For each measurement of the body composition, the Ho hypothesis was verified to ensure that the within-group correlation coefficients (r) were estimates of the same ρ, tabulating the pooled r if Ho was not rejected.

The relationships between the proportion of total chemical fat in the body weight (BF, in g/kg LW) and the *in vivo* predictors were studied by analysis of covariance, initially considering two factors of variation: experimental treatment prior to slaughter and group. The first factor did not affect the relationships between the continuous variable examined, and so only the significant differences due to the "group" factor are presented and discussed. D or BS and their derived variables (natural logarithm, square and cubic power), together with LW and days from lambing (d) were considered as independent variables. The estimated prediction models of BF were compared by the multiple correlation coefficient squared (R^2) and the residual root mean square (RRMS). Only the parameters of the more precise models are reported.

Results

The animals in weeks 6 or 7 of lactation weighed less than those in group 1 (EBW: 54.8 *vs* 48.3 kg), had a lower body DM content (396 *vs* 321 g/kg EBW) and a lower total chemical fat (167 *vs* 87 g/kg EBW). Both D and BS were lower in the thinner group of ewes. An average between group variation in body fat of 80 g/kg EBW corresponded to a variation in BS and D of 0.4 points and 29 µm respectively (Table 1).

Table 1. Body weight and body composition and condition

	Group 1	Group 2	Root MSE	CV (%)
Live weight (kg)	70.0	66.8	8.6	12.7
Empty body weight (kg)	54.8^b	48.3^a	7.6	15.2
Dissected kidney and pelvic fat (kg)	0.66^b	0.30^a	0.28	68.9
Dry matter (g/kg EBW)	396^b	321^a	44	12.7
Total body chemical fat (g/kg EBW)	167^b	87^a	56	49.7
Body condition score	3.3^b	2.9^a	0.5	17.7
Adipocyte diameter (µm)	72^b	43^a	20	38.5

a,b: P<0.05

The measurements of body condition were positively correlated with DM and chemical fat content, independently of the stage of lactation (Table 2). The hypothesis that *intra*-group rs were estimates of the same ρ was not rejected for both D and BS. The pooled rs were higher with D than BS.

Table 2. Correlation coefficients between body condition variables and body composition (g/kg empty body weight)

	Adipocyte diameter (µm)			Body condition score		
	Group 1	Group 2	Pooled	Group 1	Group 2	Pooled
Body dry matter	0.84	0.87	0.86[b]	0.66	0.69	0.68[a]
Total body chemical fat	0.94	0.95	0.95[b]	0.78	0.76	0.77[a]

a,b: P<0.05, on the same row, between pooled correlation coefficients

The relationship between the BF and D (Fig. 1) was not linear; in fact, the best fit was obtained by considering D squared, and the relationship with BF was not significantly affected by the stage of lactation. The parameters which express the precision of the regression equation between BF and D^2 are reported in Table 3, while the coefficients of the model are in Table 4. The proportion of the variability of BF explained by the variability of D^2 was high (R^2 = 0.92) and did not increase further with the inclusion of LW (P>0.05) or the number of days from lambing (P>0.10) in the regression equation.

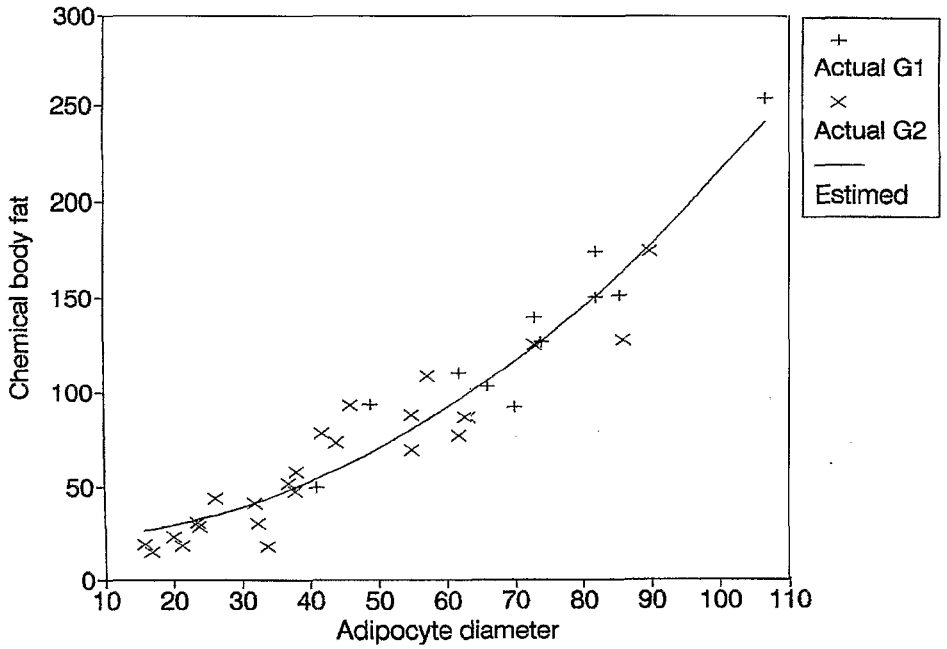

Fig. 1. Relationship between chemical body fat (g/kg LW) and the diameter (µm) of subcutaneous adipocytes from the rump in Bergamasca ewes at 1st (G1) and 6th (G2) week of lactation.

The fraction of total variation of BF accounted for the variation of BS was important (R^2 = 0.74, RRMS = 28.9 g/kg). The stage of lactation had a highly significant effect on the fitted equation, whose constant coefficient was -89.1 g/kg LW in group 1 and -103.8 g/kg LW in the ewes later in lactation. In both groups the relationship between variables was the same: for each BS unit, the variation of BF was 68 g/kg LW.

The LW, which alone accounted for 64% the total variation of BF, provided a significant improvement in precision when added to BS in the BF prediction model (R^2 = 0.81, RRMS = 25.1 g/kg LW) (Table 3). In effect, a positive and significant (P<0.01) linear relationship, not affected by lactation stage, linked LW with BS: LW = 43.6 + 8.1 BS. However, the degree of agreement between

the two variables was low (r = 0.52) and the estimated standard deviation of LW was high (RRMS = 7.6 kg). Therefore, if BS was constant, a significant fraction of the variation of BF may be accounted by LW, as expressed by the partial R^2 between LW and BF (0.27). The effect of the stage of lactation on the prediction model was significant (P<0.001), demonstrating that the difference in BF between the two groups was not completely explained in terms of the variations of BS and LW. When these two variables were used as co-variates, the ewes at the beginning of lactation were always fatter than those at the end of lactation (114 *vs* 72 g/kg LW). When the number of days after lambing (d) were included with BS and LW in a single multiple regression equation for all the ewes, there was a significant linearisation of the stage of lactation effect expressed in days, which allowed the production of a prediction equation which could be applied over the entire lactation period (R^2 = 0.77, RRMS = 27.6 g/kg LW). If BS and LW were constant, d would explain a significant fraction of the variation of BF (partial R^2 = 0.24). The square root of this value was the part of the simple correlation between d and BF (*r* = -0.57), which was not simply a reflection of their relationships with LW and BS. This fraction was high, due to the low association between d and BS plus LW. The partial regression coefficients from the regression equations are reported in Table 4.

Table 3. Precision of estimation (R^2, RRMS) of total body fat in live weight (g/kg) based on live weight (kg), days from lambing (no) and body condition expressed as subcutaneous square adipocyte diameter from rump region (μm^2) or body condition score

Independent variables	Square adipocyte diameter		Body condition score	
	R^2	RRMS	R^2	RRMS
Live weight			0.64	34.9
Body condition	0.92	15.6	0.74	28.9
Body condition, live weight	0.93	15.3	0.81	25.1
Body condition, live weight, days from lambing			0.77	27.6

Table 4. The more precise prediction equations of total chemical fat (g/kg LW) from body condition: adipocyte diameter squared from rump region (D^2, μm^2) or body condition score (BS), live weight (LW, kg) and days from lambing (d, no)

Independent variables		Coefficients of independent variables			
		Constant	D^2 or BS	LW	d
D^2	Total	21.6	0.0194	--	--
BS, LW	Group 1	-176.3	51.9	1.99	--
	Group 2	-217.7	51.9	1.99	--
BS, LW, d	Total	-168.8	52.4	1.93	-1.01

Discussion

The positive relationship between variations of body fat content and cell size of subcutaneous adipose tissue from the rump, observed both between and within groups of Bergamasca lactating ewes, showed that the reduction in the mass of adipose tissue during lactation also led to a reduction in the volume of the subcutaneous adipocytes. A consistent reduction in the volume of lumbar subcutaneous adipocytes (-283 picolitre, pl) during lactation has also been observed by Sebastian *et al.* (1989) in Aragonesa ewes. Vernon *et al.* (1987) found that mean cell volume of subcutaneous

adipose tissue from the flank of Finn x Dorset-Horn ewes varied in a range from 440 to 1,260 pl in non-lactating sheep and from 220 to 890 pl in lactating animals.

With both cattle (Robelin and Agabriel, 1986) and Bergamasca lambs (Susmel *et al.*, 1991) a linear relationship has been demonstrated between D and dissected body fat, as a proportion of EBW, while in this study the best relationship between BF and D for lactating ewes was obtained with a curvilinear equation. In addition to the effect of the gut content, this difference could have been due to the physiological states of animals, which could have affected the rate of growth and/or mobilisation of body fat deposits. For example, Hood and Thornton (1979) observed that the decrease in the mass of ovine adipose tissue, which accompanied nutritional restriction, was only due to decreased adipocyte size with no change in number of adpocytes per carcass, while both hypertrophy and hyperplasia characterised the cellular growth of adipose tissue until the sheep were about 11 months old.

The precision of the estimate of the level of fat was relatively good: RRMS was 18.4% of the mean of dependent variable (85 g/kg LW). In cattle, Robelin and Agabriel (1986) recorded RRMS values of 28.9 and 30.3 g/kg EBW respectively for growing male and adult female animals, corresponding to 22.6% and 21.5% of the mean of independent variable. In lambs of the same breed, Susmel *et al.* (1991) obtained a residual coefficient of variation of 14.2%. The comparison of these levels of precision is not corrected due to the variability of conditions under which these estimates were made. However, there is a good agreement among authors and types of animal, which clearly indicates the limits of this method for the prediction for BF. In fact, the standard error of the prediction for an individual new value of BF with D equal to 72 or 43 µm was 16 g/kg LW (95% confidence limit: 122 ± 32 g/kg LW and 58 ± 32 g/kg LW respectively). In contrast, the standard error of the difference between the means of 2 experiment units of 6 ewes was 9 g/kg LW.

The residual standard deviation of the prediction equations for body lipids from body weight and water dilution space for various kinds of animals, as reviewed by Robelin (1984), amounted to a mean value of 10% of body lipids. The lower precision of the adipocyte size method could be due to the measurement of a prediction variable for body composition which is performed on a small sample of the whole body component, itself not homogeneous in structure or metabolism (Vernon, 1986). For example, in human female adipose tissue, abdominal adipocyte cells appear to store fat for everyday lipid mobilisation while the adipocytes in the gluteal-femoral region could function as a fat reservoir for lactation (Björntorp and Sjöström, 1985). Thus, adipocytes from the abdominal region are more inclined to change their size than those in the femoral-gluteal region.

Body condition score, which mainly assesses subcutaneous fat cover in the lumbar region with some indications of muscle thickness and inter muscular fat, allows the production of less precise prediction equations for body fat content in lactating ewes than D. This result was expected given the subjective nature of the method and the discrete classification adopted for a continuous variable. The R^2, although rather low, fell within the range of values reported in the literature (Russel *et al.*, 1969; Remond *et al.*, 1988; Teixeira *et al.*, 1989; Morand-Fehr *et al.*, 1991; Sanson *et al.*, 1993).

The inclusion of LW and stage of lactation as additional variables to BS in a multiple regression model increased the precision of the estimate but limited the field of its application to the three-dimensional space defined by the specific combinations of the independent variables observed. Similar results have also been reported by other authors. Houghton *et al.* (1990), for beef cattle *post partum*, obtained improvements in the prediction of body composition including LW in multiple regression with BS. Morand-Fehr *et al.* (1991), for lactating goats, found that the best fitting equations of BF were those incorporating BS measurements with LW and a physiological state index. It can be hypothesised that the variation in the relative proportion of the body adipose deposits and the body components during lactation diminished the between-period accuracy of the estimates of body fat reserves by BS. Other authors (Russel *et al.*, 1969; Teixeira *et al.*, 1989; Sanson *et al.*, 1993), who have demonstrated different relationships, have worked with dry sheep in which BS and LW were better correlated, so that the introduction of weight to explain the variation of body fat did not increase the precision beyond that obtainable using BS alone.

Conclusions

Adipose cell size is a rapid and cheap method for estimating the proportion of body fat in ewes, and it may be used under experimental conditions to predict mean body fat mobilisation or deposition in a relatively small group of animals. In fact, to demonstrate a 24 g/kg LW difference in body fat content at the 5% significance level requires 6 animals per treatment. On the other hand, it may be used to predict fat mobilisation on a single animal only when a large variation in body fat content can be expected. Using a biopsy sample, it would also appear to be possible to repeat, with a certain frequency, the observations on the same animal within one experiment, thus improving the precision of the method and monitoring the evolution of the body adipose reserves.

BS remains the most rapidly applicable technique and is the only method practicable on-farm. The precision level limits its usefulness under experimental circumstances to the creation of groups of animals with relatively large differences in their average proportion of body fat.

Acknowledgements

Research performed with MURST 40% funding, U.O. Piero Susmel.

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