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Original article

Comparison of three methods for the in vivo estimation of body composition in dairy ewes

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Abstract — Three methods for estimating in vivo body fat in sheep, by the assessment of body water using the deuterium oxide (D_2O) dilution technique, adipose cell size (ACS) measurement and body condition scoring (BCS), were studied in mature ewes and compared to results obtained after slaughter and chemical analyses. Twenty Lacaune dairy ewes were slaughtered at three stages of lactation (30 days [n = 8], 60 days [n = 8] and 110 days [n = 4]), with a mean body weight (BW) of 72.7 ± 7.6 kg, 16.0 ± 4.7 kg of body lipids (LIP) and 42.7 ± 3.8 kg of total body water. Body fatness ranged from 10.3 to 28.8 % of empty body weight. Although the ewes were relatively fat, the mean adipose cell volume of subcutaneous pericaudal or sternal adipose tissue (209 or 287 pl, respectively) was in the lower range of previously reported values, while cells from omental adipose tissue had a larger mean volume (804 pl) than has been previously reported. Due to differences in the frame of ewes, prediction of body lipids through D_2O, ACS and BCS was improved by including body weight in the model. The best equations using ACS were obtained with subcutaneous fat tissues (sternal tissue: residual coefficient of variation (rCV) = 13.9 % and pericaudal: rCV = 17.8 %), followed by internal tissue (omental: rCV = 19.5 %). The rCVs of the prediction of body lipids with these in vivo methods were in general agreement with published works on cattle or sheep i.e. D_2O: 8.8 %, pericaudal ACS: 13.9 % and BCS: 16.7 %. The interest and limitations of the methods studied are discussed in terms of different experimental objectives and constraints. (© Elsevier / Inra)

sheep / body composition / adipose cell size / deuterium oxide / body condition score

Résumé — Estimation in vivo de la composition corporelle de brebis laitières par trois méthodes. Trois méthodes pour estimer in vivo la composition corporelle des ovins ont été comparées : la technique de l'espace de diffusion de l'eau lourde (D_2O), la mesure du diamètre moyen des adipocytes

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(ACS) et la notation de l'état corporel (BCS). Vingt brebis adultes de race Lacaune ont été abattues à trois stades de lactation (30 j (n = 8), 60 j (n = 8) et 110 j (n = 4)) et analysées chimiquement. Le poids vif moyen (BW, *tableau I*) était de 72,7 ± 7,6 kg, les lipides corporels (LIP) de 16,0 ± 4,7 kg et la quantité totale d'eau corporelle de 42,7 ± 3,8 kg. L'état d'engraissement (lipides en % du poids vif vide) de ces brebis a varié de 10,3 à 28,8. Bien que les brebis aient été assez grasses, le volume moyen (209 ou 287 pl) des adipocytes du tissu sous-cutané péricaudal ou sternal (respectivement) est faible par rapport aux valeurs rapportées dans la bibliographie, alors que les adipocytes du tissu omental sont plus volumineux (804 pl ; *tableau I*). En raison des différences de format entre brebis, la prédiction des lipides corporels est améliorée en incluant le poids vif aux modèles. Les meilleures équations sont obtenues avec les cellularités des tissus sous-cutanés (sternal ; rCV = 13.9 et péricaudal ; rCV = 17.8), suivies par celles obtenues avec le tissu adipeux interne (omental ; rCV = 19.5). Les coefficients de variation résiduelle des relations de prévision des lipides corporels sont conformes à ceux déjà publiés sur des ovins ou des bovins : D_2O : 8,8 %, ACS du tissu adipeux sous-cutané péricaudal : 13,9 % et BCS : 16,7 (*tableau II*). L'intérêt et les limites des méthodes étudiées sont discutés en fonction de contraintes et des objectifs expérimentaux. (© Elsevier / Inra)

brebis / composition corporelle / taille des adipocytes / eau lourde / note d'état corporel

1. INTRODUCTION

The energetic efficiency of sheep during periods of alternate undernutrition and recovery can only be assessed if the accompanying changes in body composition are known. Changes in body composition can be studied according to variations in body chemical content (lipids, protein, water and minerals) or energetic content. It may, however, also be of interest to know the quantitative variations of tissues (adipose tissues, muscles) and the characteristics of these tissues (cellularity or metabolic activity).

The extent to which lactating dairy animals can use their body reserves [9] cannot be determined without effective means of estimating body composition serially in the same animal during lactation [15, 22, 38]. Methods for estimation of body composition in live animal are numerous but, to our knowledge, no evaluation of the relative interest of these methods is available for dairy ewes. The purpose of this work was to carry out a direct comparison of three methods (body condition scoring [BCS], adipose cell size measurement [ACS] and deuterium oxide [D_2O] dilution technique), applied to adult Lacaune dairy ewes before slaughtering and chemical analyses. Discussion is focused on the interest and limitations of these methods and on variations in the cellularity of three adipose tissue sites (omental, sternal and pericaudal).

2. MATERIALS AND METHODS

Twenty mature Lacaune dairy ewes were randomly chosen from the large flock of La Fage (Inra, Aveyron, France) and allocated in three groups according to their lactation stage: 30 days (n = 8), 60 days (n = 8) and 110 days (n = 4), and variability in body condition score. Ewes were fed ad libitum in two meals (0730 and 1500 hours) and had free access to water. They were milked twice daily (0830 and 1730 hours).

2.1. Body condition scoring

Subjective estimates of body condition (0–5 scale) were assessed by palpation of the lumbar region [25]. The BCS of the 20 ewes was assessed daily on the 4 days before slaughter and scores reflected agreement between two trained scorers. The intra-ewe and inter-day coefficient of variation was 4 %. The score obtained on the day before slaughter (S-BCS) and the mean of the four scores (M-BCS) were used to develop equations to predict body composition.

2.2. Deuterium oxide method

Deuterium oxide (D_2O, 99.8; Euriso-top/CEA, France) was infused at 0900 hours (t = 0) through a catheter into a jugular vein and rinsed with physiological saline. Syringes used to inject D_2O (0.5 $g \cdot kg^{-1}$ body weight [BW]) were precisely weighed (mg) before and after infusion. After infusion, ewes were weighed (body weight at injection [BWI]) and six blood samples were obtained by venipuncture at 1400, 1600 and 1700 hours on the 1st and 2nd day, i.e. ewes were sampled at +5, +7, +8, +29, +31 and +32 h after D_2O injection. Blood samples were stored at −20 °C pending analysis. Blood water was extracted by freeze-drying and condensed at liquid nitrogen temperature [35]. D_20 concentration was determined by infrared spectrophotometry at 4 µm, on a MIRAN-1 FF apparatus (Foxboro, USA) calibrated with standard solutions (from 0 to 1 250 ppm D_2O w/w). Precision of D_2O determination was 9 ppm. The deuterated water space (DWS, kg) was determined as the amount of D_2O injected divided by the concentration of D_2O (Co) at zero time [2, 36]. The Co value was extrapolated from a one pool model [12] based on the regression of log D_2O concentration (Ct) and sampling time (t).

2.3. Adipose cell size measurement

Samples of three adipose tissues: omental (OM), subcutaneous pericaudal (SP) and subcutaneous sternal (ST) were studied [21]. Adipose tissues were collected immediately after slaughter and kept in physiological saline at 39 °C for less than 1 h. The adipose tissues were fixed by osmium tetroxide during a 10-day incubation period and then digested by urea (8 M) for 1 month. The fixed and dissociated adipocytes were spread on a filter (0.47 µm) and photographed under microscope (× 80). The diameters of approximately 100 cells per tissue sample were measured in a range of 25.0 to 200 µm by 12.5 µm increments. The mean cell volume (M-VOL, pl) in each sample (OM, SP and ST) was calculated by multiplying the volume of each class by its frequency. This mean volume was then transformed into mean diameter (M-DIAM, µm). Repeatability of mean diameter measurement between two operators was high (r^2 = 0.97) and residual standard deviations were low (rSD = 3.8 µm; n = 30). Mean diameter was not altered (r^2 = 0.924; rSD = 5.2 µm; n = 31) when a larger sample population (200 adipocytes/tissue) was used.

2.4. Slaughter procedure

Ewes were shorn and weighed just before slaughter (BWS), which occurred between 1800 and 1900 hours (i.e. +33 and +34 h after D_2O injection). Before slaughter they were normally fed (1500 hours) but were not milked (mammary gland not emptied). Blood was weighed and aliquoted for further chemical analysis. Parts of the digestive tract were weighed before and after being emptied and the dry matter of gut content was determined (103 °C/48 h). The non-carcass components were sealed in plastic bags as soon as possible, to minimise water losses by evaporation. The cold carcass was weighed and backfat thickness (BFT, mm) in the lumbar region was measured before storage of the carcass in plastic bags. The content of each bag was weighed on removal from cold storage (−20 °C) and any losses since the initial weighing were assumed to be water. The content of the bags was minced using an industrial grinder and mixed. A 3-kg aliquot was obtained for analyses. Triplicate samples were freeze-dried (dry matter content) before being minced through a fine mincer. Samples were analysed for ether-extracted fat, ash (furnace), protein (N × 6.25; Kjeldhal method) and energy (calorimeter). Validation of analyses was assessed by comparing measured energy to calculated energy content from fat and protein content [36].

2.5. Statistical analysis

The statistical analysis was carried out using the GLM procedure of SAS [27]. Simple and multiple regression analyses were used to evaluate relationships between different parameters. The lactation stage was also tested as a three-level fixed factor.

3. RESULTS

3.1. Body composition determined by analysis after slaughter

The sum of analysed body components (*table I*) was close to body weight before slaughter, the average uncontrolled loss of material was calculated to be lower than 3.2 %. The range of variation of body lipid content in body weight at slaughter was from 10.3 to 28.8 %, while the water content

Table I. Anatomical measurements, chemical composition, body condition score (BCS) adipocyte size and milk yield in ewes slaughtered at different lactation stages (30, 60 or 110 days).

Stage (days)	30		60		110		All	Stage effect	
Number of ewes	8		8		4		20		
Anatomical measurements (kg)									
Infusion BW	74.3	(4.8)	71.6	(8.5)	77.7	(3.9)	73.9	(6.5)	$P > 0.32$
Slaughter BW	73.6	(4.8)	69.4	(9.7)	77.9	(4.8)	72.8	(7.6)	$P > 0.17$
Gut content	12.0	(1.2)	9.9	(2.3)	11.0	(1.1)	11.0	(1.9)	$P < 0.07$
Empty BW	58.1	(4.5)	56.7	(7.9)	63.7	(4.5)	58.6	(6.4)	$P > 0.20$
Mammary weight	1.7[a]	(0.3)	1.1[b]	(0.3)	1.2[b]	(0.2)	1.4	(0.4)	$P < 0.01$
Uterus weight	0.2	(0.1)	0.3	(0.1)	0.2	(0.1)	0.2	(0.1)	$P > 0.39$
Chemical composition									
Body water (kg)	44.1	(2.6)	41.5	(4.8)	42.5	(3.7)	42.7	(3.9)	$P > 0.54$
Lipids (kg)	15.1[a]	(2.7)	14.5[a]	(5.9)	20.8[b]	(1.1)	16.0	(4.7)	$P < 0.06$
Proteins (kg)	8.3	(0.4)	7.9	(0.8)	8.4	(0.4)	8.2	(0.6)	$P > 0.35$
Minerals (kg)	2.3	(0.2)	2.3	(0.2)	2.5	(0.2)	2.4	(0.2)	$P > 0.29$
Energy (Mcal)	202[a]	(28)	197[a]	(58)	258[b]	(11)	211	(46)	$P < 0.06$
Body condition score									
S-BCS (once, day before slaughter)	2.8[a]	(0.1)	3.1[ab]	(0.3)	3.3[b]	(0.3)	3.0	(0.3)	$P < 0.04$
M-BCS (mean)	2.8[a]	(0.1)	3.0[b]	(0.3)	3.4[b]	(0.2)	3.0	(0.3)	$P < 0.01$
Back-Fat thickness (mm)	9.5[a]	(1.9)	5.6[b]	(3.8)	11.0[a]	(3.2)	8.2	(3.7)	$P < 0.02$
Adipocyte size									
Pericaudal									
Diameter (µm)	73	(12)	65	(12)	81	(15)	71	(13)	$P > 0.91$
Volume (pl)	214	(107)	160	(85)	296	(152)	209	(115)	$P > 0.85$
Sternal									
Diameter (µm)	81	(12)	75	(14)	87	(6)	80	(12)	$P > 0. 52$
Volume (pl)	299	(136)	243	(116)	350	(75)	287	(120)	$P > 0.55$
Omental									
Diameter (µm)	118.8	(13)	103	(19)	122	(16)	113	(18)	$P > 0.37$
Volume (pl)	906	(281)	620	(332)	970	(382)	804	(343)	$P > 0.29$
Milk Yield (L/d)	2.00[a]	(0.82)	1.55[ab]	(0.33)	0.88[b]	(0.39)	1.60	(0.7)	$P < 0.02$

Tabular values represent means and standard deviations.
[ab] Within a row, means with different superscripts differ ($P < 0.05$). BW: Body weight.

ranged from 48.5 to 63.5 %. Most of the measurements were not significantly different among groups of ewes (*table I*), except for fat mass (110-day ewes being fatter; $P < 0.06$), and for mammary gland development. The greater mammary weight at 30 days ($P < 0.001$) is likely due to greater amounts of residual milk in the gland, because the ewes were not milked prior to slaughter. As expected, because none of the ewes was pregnant, uterus weight were not different (*table I*).

3.2. Relationship between water and lipid contents

The relationship between the proportion of lipid (LIP) and water (EBW) in empty body mass (EBM), was precise ($r^2 = 0.979$; *figure 1*), and was not affected ($P > 0.91$) by stage of lactation:

$$\text{LIP \% EBM} = -1.209\ (\pm 0.04) \times \text{EBW \% EBM} + 92.69\ (\pm 2.26);$$
$$\text{rCV} = 3.18\ \%\ r^2 = 0.979$$
$$\text{rSD} = 0.87\ n = 20$$

Terms ± 0.04 and ± 2.26 are standard deviations associated with the regression coefficients listed earlier (i.e. –1.209 and 92.69, respectively)

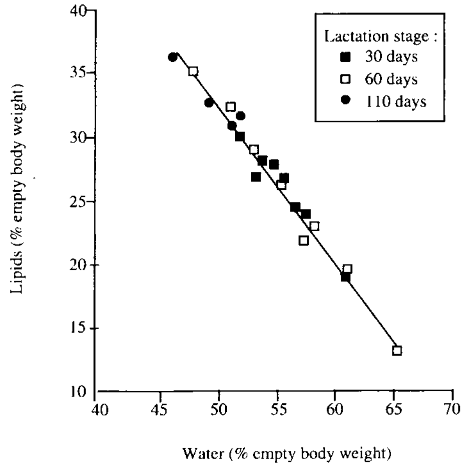

Figure 1. Relationship between proportions of water and lipids in empty body weight of lactating ewes.

3.3. Prediction of body water

Among the six blood samples ($t = 5$ to $t = 32$ h post-injection), all combinations of sampling times were tested in order to minimise the coefficient of variation attached to Co (CVCo). The use of all the collected samples (+5, +7, +8, +29, +31 and +32 h) led to a high mean CVCo (18.7 %). The best fit was observed (CVCo = 7.5 %) with the four samples taken at +5, +7, +29 and +31 h after infusion. These samples correspond to prefeeding times (+5 and +29 h) and two proximate postfeeding samples (+7 and +31 h), the afternoon meals being at +6 and +30 h after injection. In these conditions, the relationship between total body water (TBWater, kg) and DWS (kg) was precise (rCV = 2.3 %) and the rSD of the prediction was ±0.99 kg. However, the slope of this relationship (0.788 ± 0.054) was significantly different from 1.0, with a constant term (8.43 ± 2.41 kg) that was significantly different from zero and without any effect of lactation stage.

3.4. Prediction of body components from body weight and deuterated water space

Body weight at injection did not give a precise estimate of the ewes' body lipid content ($r^2 = 0.527$; rSD = 3.32 kg and rCV = 20.7 %). The simultaneous inclusion of DWS and BWI in equations for predicting body lipids and body energy explained more than 91 % of the variance (*table II*). For the prediction of body lipids, the coefficients of regression of BWI and DWS were of same magnitude but of opposite signs, and the rSD of the prediction was of 1.4 kg (i.e. rCV = 8.8 %). As shown earlier, the same equation established with empty body components was more precise (rSD = 0.42 kg; rCV = 2.6 %). Body protein estimation was less accurate ($r^2 = 0.749$). Prediction of both body lipids and body protein was not affected by lactation stage.

3.5. Adipose cell size at the different anatomical locations

For a mean fatness of 21.7 % BWS, adipocyte mean diameters (*figure 2*) did not differ between the two subcutaneous tissues: SP = 71.3 ± 3.1 μm and ST = 80.2 ± 12.1 μm, but were larger in omental fat tissue: OM = 112.9 ± 17.5 μm. The first class of diameter (above 25 μm and under 37.5 μm) was rather different between tissues: higher

Table II. Prediction of body components measured after slaughter with independent variates measured in vivo, associated with body weight at injection (BWI, kg).

Methods	Variates			Statistics		
	X_1	X_2	Constant	rSD	rCV (%)	r^2
Dilution technique	DWS (kg)	BWI (kg)				
Water (kg) =	+0.788 (0.054)	– –	+ 8.4 (2.4)	0.99	2.3	0.923
Lipids (kg) =	–0.865 (0.095)	+0.863 (0.062)	–9.1 (3.9)	1.40	8.8	0.919
Proteins (kg) =	+0.024 (0.021)	+0.070 (0.014)	+ 1.9 (0.9)	0.32	3.9	0.749
Energy (Mcal) =	–7.73 (0.82)	+8.54 (0.54)	–74.4 (34.1)	12.2	5.8	0.937
Adipocyte diameter	Diam. (μm)	BWI (kg)				
Sternal Lipids (kg) =	+0.212 (0.044)	+0.397 (0.082)	–30.4 (6.0)	2.2	13.9	0.798
Pericaudal Lipids (kg) =	+0.150 (0.055)	+0.388 (0.111)	–23.4 (7.4)	2.8	17.8	0.670
Omental Lipids (kg) =	+0.087 (0.048)	+0.402 (0.127)	–23.5 (8.1)	3.1	19.5	0.604
Body condition score	S-BCS (kg)	BWI (kg)				
Lipids (kg) =	+1.178 (0.280)	– –	–19.9 (8.6)	3.42	21.4	0.495
Lipids (kg) =	+0.793 (0.246)	+0.369 (0.105)	–35.4 (8.0)	2.68	16.7	0.707

DWS: deuterated water space; rSD: residual standard deviation; rCV: residual coefficients of variance.

in subcutaneous caudal (16.6 %), lower in omental tissue (5.7 %) and intermediate in sternal tissue (11.5 %). Correlations between the proportion of body lipids in empty body weight and adipose cell mean diameter of the three fat tissues (*figure* 3), were not very high: OM: $r = +0.57$; SP: $r = +0.66$ and ST: $r = +0.79$. Multivariate analyses gave better relationships using, simultaneously, the mean diameter and the empty body weight: OM: $r = +0.87$; SP: $r = +0.89$ and ST: $r = +0.93$. For the purpose of in vivo prediction of body lipids (*table II*), we used the mean diameter of each of the three adipose tissues together with body weight at injection. The best fit, smallest rCV, was still obtained with sternal tissue (rCV = 13.9 %), followed by pericaudal tissue (rCV = 17.8 %) and by omental tissue (rCV = 19.5 %).

3.6. Body condition score

The prediction of body lipids by BCS was improved when BWI was included in

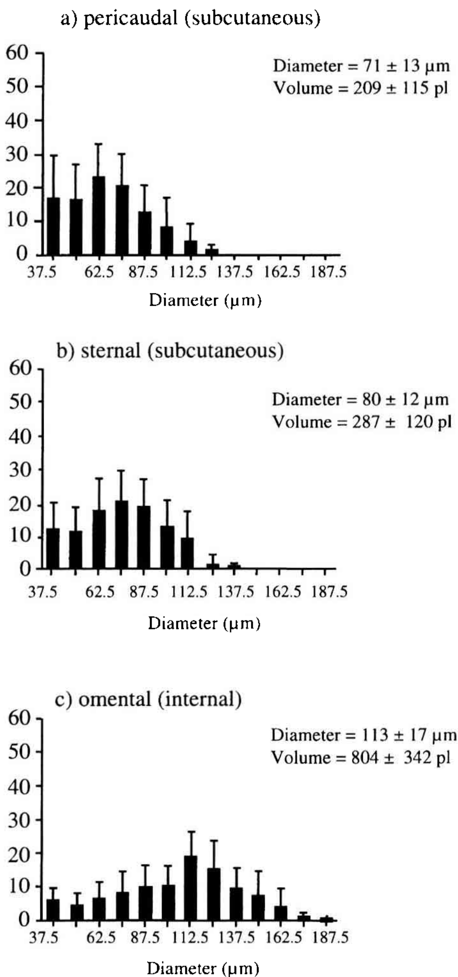

Figure 2. Distribution of adipose cells according to their diameter, measured by steps of 12.5 µm, in three adipose tissues (a, b, c) of lactating Lacaune dairy ewes (means and standard deviations).

the equation (*table II*). A single assessment of S-BCS allows a prediction of body lipids (*table II*) with a rCV of 16.7 %, while the mean value of four assessments leads to a small reduction (less than 2 percentage units) in the rCV (r^2 = 0.76 and rCV = 15.0 %). BCS was positively correlated to adipose cell diameter: the best correlation was obtained with sternal tissue (r = +0.48; P < 0.03), followed by pericaudal tissue (r = +0.46; P < 0.04) and omental tissue (r = +0.43; P < 0.06). Correlations were lower with mean adipose cell volume than diameter, although the three fat tissues were ranked in the same order.

3.7. Back-fat thickness

Back-fat thickness, which was only measurable in slaughtered animals, ranged from 1.0 to 14 mm with a mean value of 8.2 mm (*table I*). Correlations with adipose cell diameters were r = +0.75 with sternal, r = +0.66 with omental and r = +0.54 with pericaudal tissue. Correlation between back-fat thickness and BCS was not significant (P > 0.22). The prediction of total body lipid with back-fat thickness was possible with a rCV = 17.3 % and rSD = 2.77 kg (r^2 = 0.687).

4. DISCUSSION

The body fatness of these lactating Lacaune ewes, estimated by BCS, was within the range of commonly observed values. The minimum lipid content (10.3 % BWS) was not really low compared to those reported in underfed suckling ewes (2.2 % [10]; 5.7 % [15]), and the maximum value of lipid content (28.8 %) was in the upper range of values reported for non-lactating sheep (33 % [10]). The non-significant difference in body protein between early and late milking period (30 to 110 days) is not surprising since ewes were not underfed and body protein mass has been shown to be less variable than body lipids, even in suckling ewes [14].

4.1. Dilution technique

The literature on labelled water technique (tritiated water and D_2O) is well documented in cattle [12, 21], sheep [2, 4, 9, 18, 34] and goats [1, 13]. The first basic principle used for estimation of in vivo body composition

is that fat-free empty body mass can be considered to be of a constant composition and independent of large variations in empty body lipid content. The constancy of the water proportion in the fat-free mass is, however, relative. Results obtained in adult sheep of different breeds in different experiments can be considered to be relatively close: 72 % [17]; 73 % [10]; 74.1 % [4], 74.8 % [36] and 74.9 % [18]. However, within a same experiment, Foot et al. [14] noted that, in ewes of the same breed and in the same range of body fatness, the proportion of water in fat-free empty body mass was slightly greater in lactating ewes (76 %) than in dry ewes (74 %). This phenomenon has also been observed in cattle [7]: lactating and/or lean cows having a higher proportion of water than dry and/or fat cows (72.4, 73.3, 73.5 and 74.3 in dry-fat, dry-lean, lactating-fat and lactating-lean, respectively). In the present experiment with lactating ewes of the same breed the water content of fat-free empty body was fairly constant 74.4 % ± 1.1. Consequently, if the composition of fat-free mass is constant, estimation of one of its components (water) allows the calculation of total fat-free mass. It is then possible to calculate fat mass from the difference to whole body mass. The accuracy of this procedure depends on the ability to estimate body water.

The second principle is concerned with the practical use of the marker for the estimation of body water. The main assumption is that the elimination rate of the marker is constant. This is not easy to control because water fluxes are not constant but rather depend on water inputs (drinking and eating) and outputs (urine, faeces, milk, transpiration and respiration). The main problem, therefore, is to find a reliable time for sampling to obtain a good estimation of the initial concentration of the marker. The simplest way would be to use a single measurement of the marker concentration at the equilibrium between the diffusion phase and the elimination phase. Unfortunately this sampling time is not constant and depends on water turnover. Cowan et al. [11] indeed showed that a single measurement of the marker at a supposed equilibration time is not suitable for the prediction of body water in lactating ewes. The choice of extrapolation to zero time from several concentrations measured within 2 days after infusion has been shown to be reliable [20]. However, according to known variations in water fluxes due to meals (food and water) and milkings (twice a day), the present study confirms the previous findings [36] that sample collections must be carried out before and after afternoon meals and before evening milkings (+5, +7, +29 and +31 h) in order to reduce the error in predicting Co. In our study, elimination of the samples collected just before the evening milking reduced this error from 18.7 to 7.5 %. We can hereby confirm that a carefully designed protocol of blood sampling can limit errors due to water variations.

Lipid prediction from empty body weight and empty body water is precise (rSD = 0.42 kg; rCV = 2.6 %), while in vivo prediction from BWS and DWS is less accurate (*table II*; rSD = 1.4 kg; rCV = 8.8 %). One well known source of error in predicting empty body lipid content is that associated with predicting gut water. Gut water content is highly variable in ad libitum-fed animals. Gut water changes introduce an error in lipid prediction that is difficult to avoid with in vivo measurements. However, in calculations with the present data, the removal of gut water from deuterated water space and from body weight did not improve the prediction of body lipids (rSD = 1.44 kg; rCV = 9.0 %). This is probably because errors in the estimation of body water through D_2O are already considerable (rSD = 0.99 kg). Hence, the use of in vivo methods for the estimation of gut content would probably not improve the in vivo prediction of body composition. Furthermore, attempts to estimate in vivo gut water from two-pool models were not satisfactory [12]. The lactation stage may introduce an error due to mammary development or water

turnover changes [11] but, in our conditions, this effect was not statistically significant, as previously observed in goats [13] and cows [5]. However, controversial opinions can be drawn from works on dairy cow [5, 12] concerning the lactational stage effect. The precision (i.e. rCV) of in vivo lipid prediction in dairy ewes (8.9 %) was similar to that found in other dairy ewes (8.9 % [4] and 13.4 % [18]) in dairy cows (8.7 % [5]) and in dairy goats (13.3 % [1] and 13.2 % [13]).

4.2. Adipose tissue cellularity and body composition

Among sheep fat tissues, the cellularity of subcutaneous adipose tissue is well documented. The mean volume of adipocytes has been reported in ewes at different physiological stages. For example, subcutaneous adipocyte volumes have been reported for dry mature ewes (185 pl: [28]; 467 pl: [3]; 1 140 pl: [33]), in pregnant ewes (478 pl: [37]), at lambing (444 pl: [28]) or post-weaning (369 pl: [37]; 161 pl: [28]). These variations of adipose cell size are probably not mainly related to physiological stages or to breed characteristics, but mostly dependent on animals' nutritional status or history [30]. Although the Lacaune dairy ewes were relatively fat, mean adipose cell volume of subcutaneous pericaudal or sternal adipose tissue (209 and 287 pl, respectively), was in the lower range of previously reported values.

For internal fat tissues, the observed mean volume of adipocytes of these Lacaune ewes (804 pl; *table I*) was much greater than that observed in Rasa aragonesa ewes, in which it varied from 102 pl at weaning to 293 pl 10 weeks later [29]. The differences observed between internal (omental) and subcutaneous (sternal or pericaudal) tissues are in general agreement with the literature; omental adipose tissue contained larger adipose cells than subcutaneous sites in growing lambs [17], in mature wethers and rams [32] and in mature ewes [33]. The same tendency was observed in mature dry or lactating Holstein cows [6] and in steers [21].

In early works on the cellularity of adipose tissues in ruminants, it was generally assumed that changes in weight of extramuscular fat depots result primarily from hypertrophy (enlargement) rather than hyperplasia (increase of the number of adipose cells). This assumption was made based on the absence of small-sized adipocytes in 12–16-month-old fat lambs [16] or very fat adult wethers [34]. This is in agreement with a unimodal distribution of adipocytes in the subcutaneous adipose tissue of adult lean ewes [28]. In normal adult animals, however, bimodal distributions of adipocyte diameter has been observed both in fat cattle [21] and in mature sheep (rams, wethers and ewes [32]). Bimodal distributions have been also observed in some of the dairy ewes in the present work, and were probably responsible for the variability observed in the mean adipose cell diameter for a given lipid content of empty body weight *(figure 3)*.

Cellular hypertrophy is, however, the major factor contributing to overall fat deposition in adult ruminant [3]. This is why the mean cell size of subcutaneous fat adipose tissue has been successfully used to predict either total body lipids [24] or total adipose tissue [23] in cattle. In this species, although relationships were established in a wide range of fatness, residual errors (rCV) of equations for the prediction of body lipids using fat cell diameter and body weight were comprised between 13 [8] and 17 % [23]. An rCV of 24 % was logically obtained when empty body lipid was predicted from adipose cell [23]. In sheep, the only published relationship has been established between subcutaneous cell volume and body condition score (r = +0.71; [28]), but not with chemical composition. Nevertheless, for the dairy ewes in the present study, the residual errors of prediction of body lipids from body weight and mean adipocyte diameters and in three different adipose tissues (i.e. sternal rCV = 13.9 %; pericaudal

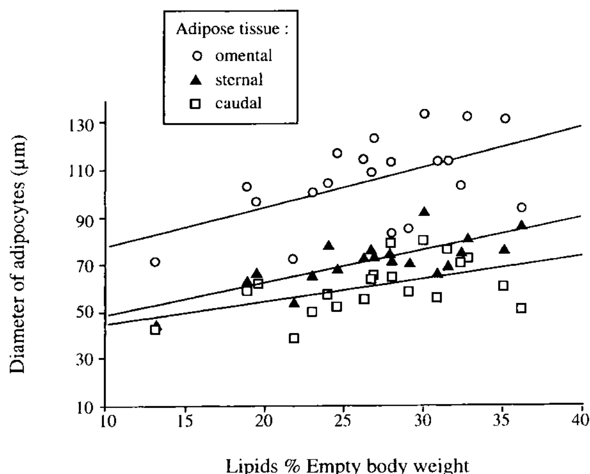

Figure 3. Relationship between omental, sternal and pericaudal adipose tissues and body lipid content of lactating ewes.

rCV = 17.8 % and omental rCV = 19.5 %; *table II*) were within the range of values observed in cattle.

4.3. Body condition score

Direct evaluation of body condition scoring methods are scarce. Rather high correlations between BCS and fatness expressed either as lipid percentage (Lipid % EBW: r^2 = 0.88 [24] or r^2 = 0.95 [26]) or as log of fat dissected tissue (r^2= 0.90 [31]) have been reported. The coefficients of variation that can be calculated from these reports are low (12 % [25]; 14 % [31]) or unavailable [26], compared to the value of 21 % obtained in the present work (*table II*). In the present experiment, ewes were chosen regardless of their frame size. Therefore, fatness, i.e. lipids as a percentage of BW, was poorly correlated (r = +0.31) to BW. Inclusion of BW together with BCS in the model of prediction of body lipids in dairy ewes reduced the rCV from 21 to 17 %. Furthermore, prediction is even slightly better (rCV = 15 %) when using the mean of four BCS measurements. The usefulness of BW in the prediction equation may be due to the fact that the BCS better reflects the proportion of fat than the absolute amount of fat. It has been shown that in ewes of approximately the same frame, BCS is sufficient for the prediction of the total amount of body lipids [25, 26] or fat tissues [31], and that BW does not significantly increase the variation accounted for with the single best predictor, i.e. BCS [25, 26, 31]. In dairy cattle [19], it was possible to predict body lipids directly from the BCS (r = +0.83; rSD = 2.7 lipids BW; rCV = 19 %), probably because the cows were of similar frame size. In the present work, with Lacaune dairy ewes differing both in frame and in fatness, the prediction of the amount of body lipid must rely on a model using both BCS and BW.

5. CONCLUSION

Whatever the method used to estimate body composition, slaughter and chemical analysis are necessary to validate the reliability of prediction equations. The interest of in vivo prediction of body reserves (i.e. mainly fat) rely on the possibility of repeated measurements on the same animal. It is difficult to compare the results presented here with the literature since no direct comparisons of these have been carried out in sheep. Considering the rCV of each method as a criterion of precision, the order ranking we obtained: dilution technique (8.8 %), adi-

pose cell size (13.9 %) and body condition score (16.7 %) is in good agreement with published results obtained in other ruminants. Our results are very similar indeed to those obtained by Chilliard et al. [8] in 20 dairy cows which had the following rCVs: D_2O: 9 %, ACS: 13 % and BCS: 21 %.

The interest and limitations of each method are, however, different in terms of cost and objectives. The water isotope (D_2O) dilution technique is the best of the three methods evaluated here. However, its cost and time-consuming operations clearly restrict its use to experimental purposes. Furthermore, it seems, at least in cattle [12], that equations obtained in one place may not provide accurate estimates of body composition when applied elsewhere. Measurement of adipose cell size by biopsies on sheep for the sole purpose of estimating body composition is not frequent. This method is costly, but of real interest when associated with measurements of biochemical characteristics on the same samples of adipose tissue. Furthermore, problems may be encountered for subcutaneous adipose tissue biopsy in very emaciated ewes. Body condition scoring, which is a subjective method, might be useful with trained operators. It is an easy means of adjusting feeding programmes to ewes' requirements in large flocks.

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