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In vivo prediction of body composition from the dilution space of the deuterium oxide in two lactating Spanish dairy breed ewes

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SUMMARY - Thirteen Churra (5 forty days and 8 seventy days after lambing) and twelve Latxa (6 forty days and 6 a hundred and twenty days after lambing) lactating dairy ewes were used to obtain predicting equations of body fat, protein and energy from the live weight and the dilution space of deuterium oxide (SD_2O). Ewes were injected with 0.6 g D_2O/kg of live weight in the jugular vein and blood samples were collected 5, 7, 29 and 31 hours after infusion. The D_2O content of blood water was determined by infrared spectrometry, and SD_2O was calculated as the ratio between the weight of the injected D_2O and its predicted concentration at zero time. After the last bleeding ewes were sheared and slaughtered and the body water, fat, protein, ash and energy were chemically determined. The Churra ewes presented 2% more water and 1.7% less protein in the fat free empty body 40 days than 70 days after lambing (P<0.01) and fat free empty body water content decreased linearly (R=-0.60) with empty body fat content. There was however a close negative relationship (R^2=0.97) between the fat and water content of the fleece free body weight. Neither the breed nor the stage of lactation significantly affect this relationship. Body water was predicted from SD_2O with a residual standard deviation (RSD) of approximately 1 kg when a pool equation was considered, though for a same SD_2O, the Latxa ewes presented 1.76 kg more water than the Churra ewes. Body fat and energy were predicted from the live weight and measured body water with a RSD of 0.437 kg and 6.85 Mcal, respectively, when a pool equation was considered. The adjusted means of body energy differed significantly between breeds and when this effect was taken into account the RSD diminished to 3.10 Mcal. When SD_2O was substituted for measured body water, the resulting RSD were 0.980 kg and 6.85 Mcal, respectively. The intercept of the prediction equation of body fat differed significantly between breeds and when this effect was taken in account the RSD decreased to 0.703 kg. The accuracy of the body protein prediction from the ewes sheared body weight (RSD=0.569 kg from the pool equation and 0.282 kg for equation taking into account the breed effect) was not improved by the inclusion of SD_2O as a second predictive variable. The stage of lactation did not significantly affect any of the equations studied.

Key words: Deuterium oxide, lactating sheep, body composition.

***RESUME** - "Prédiction de la composition corporelle in vivo à partir de l'espace de diffusion de l'oxyde de deutérium chez des brebis en lactation de deux races laitières espagnoles". Treize brebis traitées de race Churra (5 à quarante et 8 à soixante-dix jours de lactation) et douze brebis traitées de race Latxa (6 à quarante et 6 à cent vingt jours de lactation) ont été employées afin d'établir des équations pour estimer la quantité de lipides, d'azote et d'énergie du corps à partir du poids vif et de l'espace de diffusion du D_2O (ED_2O). Le marqueur a été injecté à raison de 0,6 g/kg de PV par voie jugulaire et des prélèvements de sang ont été effectués 5, 7, 29 et 31 heures après l'injection. L'eau lourde a été dosée dans l'eau du sang par spectrophotométrie infrarouge, et l'ED_2O calculé comme la relation entre la quantité du marqueur injecté et sa concentration théorique au moment de l'injection. Après le dernier prélèvement de sang les brebis ont été tondues, traitées et abattues. On a déterminé le contenu en eau (ECM), lipides, azote et énergie du corps sur* *des échantillons lyophilisés. Les brebis Churras ont montré 2% de plus d'eau et 1,7% en moins d'azote dans le corps vide délipidé (CVD) à 40 jours que celles qui avaient 70 jours de lactation (P<0,01), et la teneur en eau du CVD a montré une évolution négative (R=-0,60) avec le degré d'engraissement des brebis. Néanmois la teneur en lipides du corps vide a été étroitement et négativement corrélée (R^2=0,97) avec leur teneur en eau. Ni la race des brebis ni le stade physiologique n'ont affecté de façon significative cette relation. L'ED_2O a montré une étroite relation avec l'ECM, l'écart type résiduel (ETR) de cette relation étant d'1 kg environ, bien que pour une même ED_2O les brebis Latxa aient en 1,76 kg de plus d'eau que les brebis Churra. La quantité de lipides et d'énergie corporelle peuvent être estimées à partir du poids à l'abattage et de l'ECM avec un écart type résiduel de 0,437 kg et de 6,85 Mcal, respectivement. La relation concernant l'énergie a été affectée significativement par la race des brebis, et quand cet effet a été considéré l'ETR est descendu à 3,10 Mcal. Lorsque l'ED_2O est utilisé à la place de l'ECM, l'écart type résiduel passe à 0,980 kg pour les lipides et à 6,85 pour l'énergie. L'ETR des lipides est descendu à 0,703 kg si l'on tient compte de l'effet race. La précision de l'estimation de la quantité d'azote du corps à partir du poids des brebis (ETR=0,569 kg sans tenir compte de l'effet race et 0,282 kg si l'on en tient compte) n'a pas été amélioré avec l'introduction de l'ED_2O comme deuxième variable indépendante. Le stade de lactation n'a affecté significativement aucune des relations établies.*

***Mots-clés :** Eau lourde, brebis, réserves corporelles.*

Introduction

Among the different methods proposed to estimate *in vivo* the body composition of sheep, the measurement of the dilution space of water tracers has been proved to be one of the most reliable (Robelin, 1973, 1981). Nevertheless, Cowan *et al.* (1979, 1980) criticise the use of the method in lactating sheep because the differences found in the time taken by the marker to equilibrate with body water result in variations in the dilution space when it is calculated from the tracer concentration in blood measured after a estimated time of equilibrium.

The calculation of the tracer dilution space by the extrapolation to zero (time of infusion) of the dilution curve allows some of these problems to be solved. Using this method, prediction equations have been obtained in lambs (Robelin, 1977; Castrillo *et al.*, 1984) and in meat breed ewes at different physiological stages (Tissier *et al.*, 1983; Baucells *et al.*, 1989), which allow us to estimate the fat and energy body content from the *in vivo* body weight and water dilution space, with residual standard errors accounting, respectively, for less than 10% and 7% of those components. Bocquier and Thériez (1984) propose the use of specific equations for early lactation, mid lactation and pregnancy. The use of other multiple-compartment models of water dilution do not provide any advantages over the one-compartment model (Arnold *et al.*, 1985).

There are few reports in the bibliography in which the precision of the deuterium dilution method had been proved in lactating dairy ewes (Echaide, 1989; Ligios *et al.*, 1994), and the objective of this work was to evaluate in two lactating Spanish dairy breeds (Churra and Latxa) the accuracy of the postulates on which the method is based: (i) the existence of a close relationship between the chemical composition of the body and its water content, because of the constant composition of the fat free body, and (ii) the existence of a close relationship between the body water content and the deuterium dilution space. Finally, prediction equations of body fat, protein and energy from the ewes body weight and the dilution space of the deuterium oxide measured *in vivo* have been established.

Material and methods

For this study, thirteen 4-8 years old Churra (CH) ewes (5 forty days after lambing -L1-, 3 days after weaning, and 8 seventy days after lambing -L2-) and twelve 4-6 years old Latxa (LA) ewes (6 forty days after lambing -L1-, 5 days after weaning, and 6 one hundred and twenty days after lambing -L2-), were chosen randomly from experimental dairy flocks. The CH ewes were penned individually just after lambing whereas the LA ewes remained in the flock until 4 days before the deuterium oxide infusion and then were penned individually. All ewes received a 60/40 forage/concentrate diet given a two equal meals at 8 a.m. and 5 p.m. Level of intake from lambing to L2 (for LA) and L2 (for CH) was close to the estimated requirements. Half of the CH ewes from L1 to L2 were fed at a level of intake close to requirements and the other half were restricted on approximately 0.8 Mcal EM/day. All ewes reared one lamb until weaning and thereafter they were milked twice daily, at 9 a.m. and 6 p.m.

Two hours before the morning meal a dose of approximately 6 g/kg live weight of deuterium oxide was infused through a catheter in the jugular vein. A 10 ml blood sample was taken from the jugular vein by needle 5, 7, 29 and 31 hours after infusion, in heparinized glass tubes. Subsequently the ewes were milked, sheared and slaughtered. Feeding and milking patterns were not changed during the two days of sampling. The live weight of the ewes was registered 5 and 7 hours after infusion and just before slaughtering. Blood samples were lyophilized, and the deuterium oxide concentration in the resulting water was measured by infrared spectrometry in a Miran 1FF (Foxboro) at 4.0 µm wavelength, using a CaF_2 cell window with 0.2 mm pathlength.

The empty body weight was calculated by difference between the live weight measured just before slaughtering and the gut content estimated by difference between full and empty stomachs and intestines. The water in the gut content was determined by drying in an oven for 48 hours at 105ºC, and the empty body water was determined by the freeze drying of representative samples of the body, previously minced and homogenised. From the freeze dried samples the ash content was determined at 550ºC for 8 hours, the fat content was measured by extracting samples with diethyl ether after HCl hydrolysis, the N content by the Kjeldhal method, and the energy content was determined in an adiabatic calorimeter.

The dilution space of the deuterium oxide (SD_2O) was calculated as the ratio between the dose of tracer infused and its theoretical concentration in blood water at the time of infusion. This was estimated by extrapolating to zero time the linear regression of the log of the deuterium oxide concentration on the time gone by since the infusion.

The effect of the stage of lactation (L1 *vs* L2) within each breed and the effect of the breed, considering only phase L1 or all of the data, were studied by variance analysis, and the differences due to such factors on the regression equations of body protein (Nx6.25), fat or energy, on the ewes' body weight and their measured or estimated (SD_2O) water content, were studied by covariance analysis. All the statistical analyses were performed following the methods proposed by Steel and Torry (1981), using the BMDP statistical software (1990).

Results and discussion

Body composition and relationship between body fat and water content

Table 1 shows the sheep's mean live weight recorded 5-7 hours after infusion (LW_{5-7}) and at slaughter (LWs), and the fleece free live weight at slaughter (FFLWs) and empty body weight (FFEBW) of milked ewes. The weight of body water, fat, protein and ash, and the energy body content are also included. The chemical composition of empty body (EB) and fat free EB (g/100 g or Mcal/kg) is presented in Table 2.

The live weight of LA ewes was higher than that of the CH ewes and so it was the weights of water, protein and ash. In all cases ewes lost weight between 5-7 hours after marker infusion and slaughter, although the differences were more marked in LA ewes (Table 1). The LA ewes showed a higher protein proportion in the EB than the CH ewes. In the latter, proportions of fat and energy were lower, and that of water higher, at weaning (L1) than at mid lactation (L2).

The fat free EB presents a higher water and a lower protein content at weaning than at mid or late lactation (significant, $P<0.01$ only in CH), and the Churra ewes showed a significantly higher ash content that the Latxa ewes. Cowan *et al.* (1979) and Echaide (1989) also found a slightly higher, but not significant, water content in the fat free EB of ewes 30 and 41 days (74%) after lambing than 111 or 120 days after lambing (73%). Despite the variation in the fat free EB composition found in this work, the mean water, protein and ash content for each slaughter group of ewes never differed by more than 1.5 percentile units from the average (74.1, 20.0 and 5.8%, respectively), and the residual coefficients of variation associated with water and protein content were lower than 1.5 and 3%, respectively. This confirms the very low variation of fat free EB composition in mature animals (Moulton, 1923).

Table 1. Mean body weight (LW_{5-7}, live weight recorded 5-7 hours after infusion; LWs, live weight at slaughter; FFLWs, fleece free live weight of milked ewes at slaughter; FFEBW, fleece free empty body weight of milked ewes at slaughter) and chemical composition of ewes (kg or Mcal)

	Churra		RSD†	Latxa		RSD†	RSD† and significance of breed effect considering	
	L1	L2		L1	L2		Only L1	All data
Number of animals	5	8		6	6			
LW_{5-7}	44.80	49.71	5.48	60.44	55.63	7.60	6.67**	6.76**
LWs	43.55	48.75	5.28	56.65	53.94	7.60	6.89*	6.56**
FFLWs	42.21	47.06	5.18	54.29	50.95	7.65	6.43*	6.55**
FFEBW	35.06	40.20	4.61	45.07	43.90	7.15	6.11*	6.01*
Total body water	25.68	25.54	2.46	33.85	30.26	3.20	2.49***	3.00***
Empty body water	19.78	19.98	1.84	25.81	24.26	2.51	2.11**	2.16***
Fat	8.64	12.78	2.76*	10.70	11.16	4.63	3.64	3.91
Protein	5.05	5.71	0.61	6.78	6.66	0.74	0.57***	0.69***
Ash	1.58	1.72	0.21	1.83	1.87	0.16	0.17*	0.19*
Energy	105.5	150.1	27.7*	151.3	155.5	48.1	38.1	40.5

†RSD: Residual Standard Deviation
*P<0.05; **P<0.01; ***P<0.001

Table 2. Chemical composition of empty body (EB) and fat free EB (g/100g or Mcal/kg)

	Churra		RSD†	Latxa		RSD†	RSD† and significance of breed effect considering	
	L1	L2		L1	L2		Only L1	All data
Composition of EB								
Water	56.8	49.9	3.16**	57.7	55.9	5.48	4.47	5.48
Fat	24.1	31.6	4.47*	23.2	24.5	6.32	4.47	6.32
Protein	14.5	14.2	0.90	15.1	15.3	0.99	0.86	0.91*
Ash	4.6	4.3	0.34	4.1	4.3	0.45	0.29*	0.41
Energy	2.97	3.71	0.40**	3.31	3.47	0.56	0.43	0.54
Composition of fat free EB								
Water	74.9	72.9	0.97**	75.1	74.1	1.19	1.09	1.31
Protein	19.1	20.8	0.66**	19.7	20.3	0.75	0.67	0.94
Ash	6.0	6.2	0.41	5.3	5.8	0.41	0.23*	0.44**

†RSD: Residual Standard Deviation
*P<0.05; **P<0.01; ***P<0.001

Some of the variation in water content of the fat free EB was related to the degree of fattening of ewes. Figure 1 shows the negative correlation existing between the water content of the fat free EB and the fat content of the EB. Fat content of the EB accounted for up to 37% of the variation in the water content of the fat free EB when the values were fitted to a linear regression. Similar results have been found by others (Cowan *et al.*, 1979; Foot *et al.*, 1979; Tissier *et al.*, 1983; Baucells, 1988), but in most of the cases as in ours the degree of fattening is confused with the physiological stage of the animals (Fig. 1).

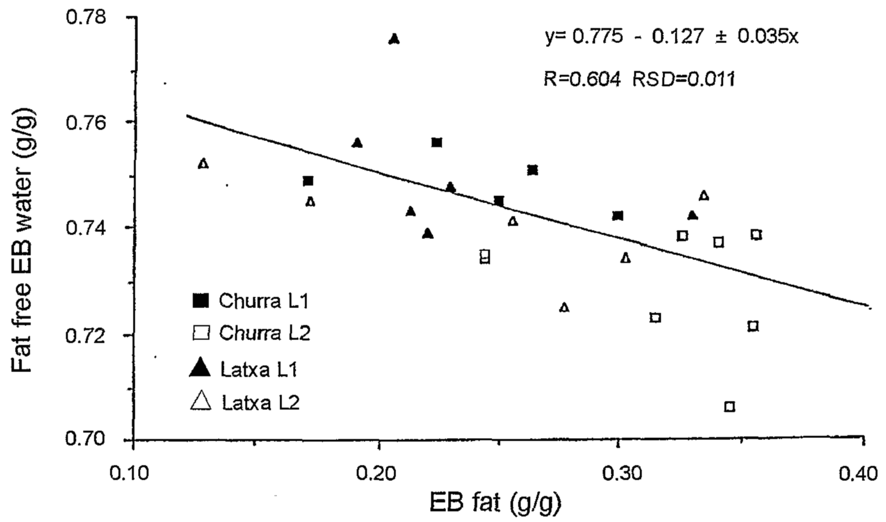

Fig. 1. Relationship between fat free empty body water (fat free EB water) and empty body fat (EB fat) content.

There was a significant, negative correlation between the proportions of fat and water in the body. The linear regression of fat (y) on water (x) body content (both expressed as % LWs) was defined by the equation y= -1.08 ± 0.081x + 82.40 (RSD=2.00, R^2=0.885). The correlation was significantly improved when the variables were referred to the FFLWs (y= -1.09 ± 0.039x + 86.52, RSD=1.03, R^2=0.971), and to the FFEBW (y= -1.17 ± 0.036x + 90.41, RSD=0.955, R^2=0.980), although the improvement obtained by discarding the gut content was small and not significant as previously reported by Foot *et al.* (1979); Cowan *et al.* (1980) and Baucells, (1988). Within the range of empty body water (45-65%) and fat (12-36%) content of the ewes used in this work, the relationship between these two variables was not improved by the introduction of a quadratic term.

In spite of the differences found in the fat free empty body composition (Table 2), the relationships between the proportion of fat and water in the body were not significantly affected by either the breed or the stage of lactation. The equations relating both parameters were very similar to those found by Baucells (1988) using 60 F_1 (Romanov x Rasa Aragonesa) ewes at different physiological stages (Fat, as % EB weight = -1.25 ± 0.026 Water (% EB weight) + 95.19, RSD=1.10, R^2=0.975), by Tissier *et al.* (1983) using 38 meat breed ewes also at different physiological stages (Fat, as % LW= -1.192 Water (% LW) + 93.77, RSD=1.38, R^2=0.963) and by Echaide (1989) in 35 lactating Lacaune (Fat as % EB weight = -1.21 Water (% EB weight) + 93.14, RSD=1.19, R^2=0.956).

Table 3 shows the residual standard deviations (RSD), determination coefficient (R^2) and residual variation coefficients (CV) of the prediction equations of body fat, protein and energy from the FFLWs considered alone, or considering both the FFLWs and the measured total body water (gut + empty body water, W). The stage of lactation did not significantly affect these relationships. On the other hand the means of fat, protein and energy adjusted by covariance for the same FFLWs were 4 kg higher, 0.5 kg lower and 20 Mcal higher (P<0.001), respectively, in Churra ewes than in Latxa ewes. The differences in the adjusted means between breeds disappeared for fat and protein when W was included as a second covariate. In any case the regression coefficients differed significantly between breeds.

Table 3. Determination coefficients (R^2), residual standard deviations (RSD) and residual variation coefficients (CV) associated to the prediction equations of body fat, body protein and body energy (kg or Mcal) from fleece free live weight of milked ewes at slaughter (FFLWs, kg) and measured total body water (W, kg)

Y	X	Pool equation			Breed[†] effect	Considering the breed effect		
		R^2	RSD	CV (%)		R^2	RSD	CV (%)
Fat	FFLWs	0.522	2.704	24.4	***	0.741	2.036	18.4
	FFLWs, W	0.988	0.437	3.9	NS	-	-	-
Protein	FFLWs	0.850	0.569	6.1	***	0.917	0.282	4.7
	FFLWs, W	0.892	0.321	5.3	NS	-	-	-
Energy	FFLWs	0.752	20.835	14.6	*	0.799	19.166	13.4
	FFLWs,W	0.974	6.849	4.8	**	0.995	3.103	2.2

[†]Significance between adjusted means after covariance analysis. There were non significant differences between the regression coefficients in any case
NS: P>0.05; *P<0.05; **P<0.01; ***P<0.001

Considering the pool equations, FFLWs alone accounted for 52, 85 and 75% of the total body fat, protein and energy variation. The percentage of variation explained increased to 74, 92 and 80%, respectively, when the breed effect was considered. The inclusion of W as a second independent variable did not improved the precision of protein prediction, but significantly increased the explained proportion of the body fat and energy variation up to 99 and 97%, respectively (99.5% of the energy when the breed effect was considered). The variation coefficients associated with the equations predicting body fat, protein and energy from FFLWs and W (3.9, 5.3 and 4.8% respectively) are very similar to those obtained by Baucells (1988) (6.5, 5.2 and 4.3%) and by Tissier *et al.* (1983) (6.5, 6.1 and 5.0%), and lower than those obtained by Echaide (1989) (14, 7.5 and 12.4%).

Prediction of body water content from the dilution space of the deuterium oxide

The dilution space of the deuterium oxide overestimated measured body water by, on average, 3.5 kg. This over-estimating (R=0.76) was related to the loss in body weight the ewes underwent between 5-7 hours after infusion and slaughter (Table 1), showing that, in the conditions of the experiment, the SD_2O is more representative of the water content at the time of equilibrium than the water content at slaughter (Castrillo *et al.*, 1984). Nevertheless, the difference in body weight between 5-7 hours after infusion and slaughter accounted only for 1.9 kg and, even if all of these differences were to be considered as representing a loss in body water, the SD_2O continues to overestimate the measured body water by a 5% as an average.

In spite of this, the measured body water (R=0.965) was closely related to the dilution space of the deuterium oxide (Fig. 2). The inclusion of the difference in live weight between 5-7 hours after infusion and slaughter as a second independent variable, significantly increased the proportion of explained measured body water variation from 93 to 95% when a pool equation was considered (Table 4). The stage of lactation did not affect these relationships, as it has been previously reported by Bocquier and Thériez (1984) and Baucells *et al.* (1989), but significantly different adjusted means were obtained for Churra and Latxa ewes. For a same SD20 and a same difference in live weight between 5-7 hours after infusion and slaughter, the Latxa ewes present 1.76 kg more water than the Churra ewes. In any case, the residual standard deviations associated with the pool equation (approximately 1 kg) amounts to 4% of total body water, and this precision is closed to that obtained by others using a similar method (Purroy, 1978; Baucells, 1988; Tissier *et al.*, 1983; Bocquier et Thériez, 1984; Echaide, 1989).

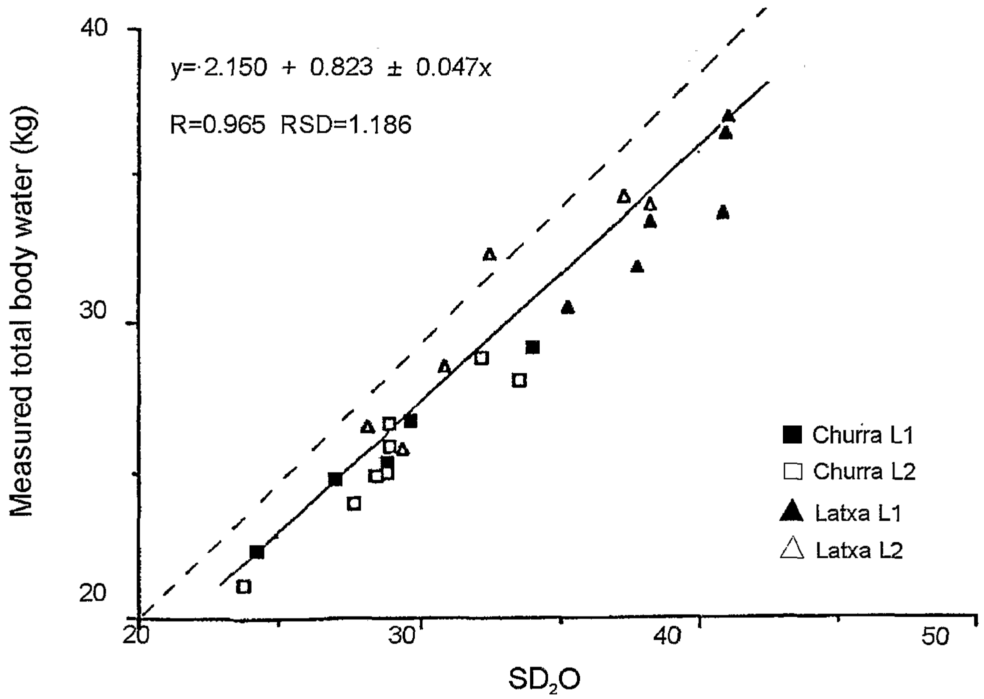

Fig. 2. Regression equation of measured total body water on dilution space of the deuterium oxide (SD_2O).

Table 4. Relationship between measured total body water (W, kg), and the dilution space of deuterium oxide (SD_2O kg) and the difference in ewe live weight between 5-7 hours after infusion and slaughter (LW_{5-7} - LWs, kg)

	Regression coefficient		Intercept	Breed[†] effect	R^2	RSD	CV
	SD_2O	LW_{5-7}-LWs					
Pool equation	0.823 ± 0.047		2.150	*	0.931	1.186	4.13
Churra	0.728 ± 0.058		4.550		0.945	1.080	3.76
Latxa	idem		5.973				
Pool equation	0.976 ± 0.062	- 0.727 ± 0.225	-1.417	***	0.953	0.999	3.48
Churra	0.883 ± 0.051	- 0.846 ± 0.172	0.969		0.975	0.752	2.62
Latxa	idem	idem	2.731				

[†]Significance between adjusted means after covariance analysis. There were non significant differences between the regression coefficients in any case
*P<0.05; ***P<0.001

Prediction of ewes body composition from their body weight and the dilution space of the deuterium oxide

Table 5 shows the prediction equations of fat and energy from the ewes' fleece-free LW5-7 and the SD_2O. Fleece-free live weight 5-7 hours after infusion gave a better fitting than FFLWs when used with SD_2O, as previously found in lambs (Castrillo *et al.,* 1984) and ewes (Baucells, 1988), for the aforementioned reasons. Comparing Tables 3 and 5 it can be seen that when deuterium oxide space was included in the place of measured body water, the RSD of pool equations increased from 0.437 to 0.980 kg for the fat and from 3.10 to 6.85 Mcal for the energy. There were significant differences in adjusted means between the Churra and Latxa ewes with regard to fat content. For a same body weight and a same SD_2O, the Churra ewes have 1.80 kg more fat than the Latxa ewes. When the breed effect was considered the RSD of the prediction equations decreased to 0.703 kg of fat.

Table 5. Prediction equations of body fat (kg) and body energy (Mcal) from fleece free live weight of ewes 5-7 hours after infusion ($FFLW_{5-7}$) and the dilution space of the deuterium oxide (SD_2O)

Dependent variable	Regression coefficient		Intercept	Breed† effect	R^2	RSD	CV
	$FFLW_{5-7}$	SD_20					
Fat							
Pool equation	0.917 ± 0.051	- 1.093 ± 0.080	- 0.232	***	0.940	0.980	8.86
Churra	0.902 ± 0.036	- 0.951 ± 0.065	- 3.176		0.970	0.703	6.35
Latxa	idem	idem	- 4.978				
Energy							
Pool equation	9.171 ± 0.355	- 9.077 ± 0.558	- 29.921	NS	0.974	6.847	4.80

†Significance between adjusted means after covariance analysis. There were non significant differences between the regression coefficients in any case
NS: P>0.05; ***P>0.001

The accuracy of body protein prediction did not improve when the SD_2O was included as a second independent variable together with the fleece free LW5-7, as previously shown by Baucells *et al.* (1989). The relationship between the body protein content (y, kg) and the FFLWs (x, kg) was defined by the pool equation: y= 0.116 ± 0.011x + 0.042 (R^2=0.85, RSD=0.569). As seen in Table 4, the accuracy of the equation improved when the breed effect was considered: y= 0.097 ± 0.093x + A, with A=1.090 kg for CH and 1.697 for LA ewes (R^2=0.92, RSD=0.282).

Conclusions

Neither the breed (Churra *vs* Latxa), nor the stage of lactation (one month after lambing, just after weaning *vs* one-three month after weaning) affected significantly the relationship between the proportions of fat and water (R^2=0.97) in the fleece-free live weight. The relationship between measured and predicted body water was also fairly good (RSD=1 kg, CV=3.5%) although there were significant differences in the adjusted measured body water means of Churra and Latxa ewes. From the residual standard deviations of the pool equations relating body fat and energy to the ewes' live weight and SD_2O (Table 5) it can be estimated that differences of approximately 650 g of fat and 4.52 Mcal could be detected, with a 95% probability, when comparing two groups of ten ewes each.

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