In vivo prediction of goat kids body composition from the deuterium oxide dilution space determined by isotope-ratio mass spectrometry[1]

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ABSTRACT: Deuterium oxide dilution space (D_2OS) determination is one of the most accurate methods for in vivo estimation of ruminant body composition. However, the time-consuming vacuum sublimation of blood preceding infrared spectroscopy analysis, which is traditionally used to determine deuterium oxide (D_2O) concentration, limits its current use. The use of isotope-ratio mass spectrometry (IRMS) to determine the deuterium enrichment and thus quantify D_2O in plasma could counteract this limitation by reducing the sample preparation for plasma deproteinisation through centrifugal filters. The aim of this study was to validate the D_2OS technique using IRMS in growing goat kids to establish in vivo prediction equations of body composition. Seventeen weaned male Alpine goat kids (8.6 wk old) received a hay-based diet supplemented with 2 types of concentrates providing medium ($n = 9$) or high ($n = 8$) energy levels. Kids were slaughtered at 14.0 ($n = 1$, medium-energy diet), 17.2 ($n = 4$, medium-energy diet, and $n = 4$, high-energy diet), or 21.2 wk of age ($n = 4$, medium-energy diet, and $n = 4$, high-energy diet). Two days before slaughter, D_2OS was determined after an intravenous injection of 0.2 g D_2O/kg body mass (BM) and the resulting study of D_2O dilution kinetics from 4 plasma samples (+5, +7, +29, and +31 h after injection). The deuterium enrichment was analyzed by IRMS. After slaughter, the gut contents were discarded, the empty body (EB) was minced, and EB water, lipid, protein, ash, and energy contents were measured by chemical analyses. Prediction equations for body components measured postmortem were computed from in vivo BM and D_2OS. The lack of postmortem variation of fat-free EB composition was confirmed (mean of 75.3% [SD 0.6] of water), and the proportion of lipids in the EB tended ($P = 0.06$) to be greater for the high-energy diet (13.1%) than for the medium-energy diet (11.1%). There was a close negative relationship (residual CV [rCV] = 3.9%, $R^2 = 0.957$) between EB water and lipid content, whereas D_2OS was closely related to total body water (rCV = 2.9%, $R^2 = 0.944$) but D_2OS overestimated it by 5.8%. Adding D_2OS to BM improved the in vivo predictions of EB lipid and energy content (rCV = 13.1% and rCV = 7.9%, respectively) but not those of protein or ash. Accuracy of the obtained prediction equations was similar to those reported in studies determining D_2OS by infrared spectroscopy. Therefore, the use of IRMS to quantify D_2OS provides a highly accurate measure of the in vivo body composition in goat kids.

Key words: body composition, deuterium oxide, goat kid, isotope-ratio mass spectrometry

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INTRODUCTION

Body composition and its variations are of major interest in several animal research fields, including growth and developmental physiology, nutrition, and genetics. Therefore, numerous methods for in vivo prediction of body composition have been developed (Speakman, 2001). Among them, the labeled water (often deuterium oxide [**D_2O**]) dilution technique (Hevesy and Hofer, 1934) remains one of the most precise. Its principle use consists of determining the amount of body water by studying the dilution kinetics of D_2O injected within the body. Knowing both body mass (**BM**) and body water allows the prediction of the other body components (i.e., lipids, protein, and ash), as fat-free body composition is fairly constant (Robelin, 1973; Speakman et al., 2001). This method was widely calibrated and used in ruminant physiology from 1960 to 2000 (Speakman et al., 2001). Since then, its use has been more parsimonious, mainly because of the cost and the time-consuming determination of D_2O concentration in blood, because it requires a water sublimation step preceding infrared spectroscopy analysis (Tissier et al., 1978; Byers, 1979).

Isotope-ratio mass spectrometry (**IRMS**) is a more sensitive and selective technique for deuterium enrichment analysis and D_2O concentration determination than infrared spectroscopy (Wong et al., 1987). Therefore, IRMS could allow reducing the cost and time needed for blood sample preparation, because only deproteinisation of plasma through centrifugal filters is required before analysis (Ripoche et al., 2006). This method was applied with success to accurately predict hen body composition (Mata et al., 2006) as well as the water intake in goats and ewes (Al-Ramamneh et al., 2010). To our knowledge, no study has yet reported the prediction of ruminant body composition by D_2O dilution technique using IRMS.

The objective of this study was to validate the D_2O dilution technique using IRMS in growing goat kids to establish in vivo prediction equations of body composition. Two groups of goat kids consuming either medium- or high-energy diets, and of contrasting body composition, were slaughtered between 14 and 21 wk of age for this study.

MATERIALS AND METHODS

Animals and Diets

All experimental procedures were conducted in accordance with the French guidelines for experimental animal use, within the protocol agreement number 00270.01 approved by the French Ministry of Research and Higher Education. Seventeen Alpine weaned and noncastrated male goat kids (*Capra hircus*; mean of 8.6 wk old [SD 0.4]; mean of 18.2 kg BM [SD 2.5]) were selected from the experimental farm of Bouzule (Laneuvelotte, France), located in Lorraine region in the northeastern part of France. They were allocated to 2 groups according to BM and age. One group received a diet of meadow hay (first cut permanent grassland mainly composed of 31% *Agrostis vulgaris*, 21% *Dactylis glomerata*, and 15% *Lolium perenne*) for ad libitum intake supplemented with 20 g DM·kg $BM^{-1}\cdot d^{-1}$ of a commercial concentrate mixture for growing goat (Fluvial Junior; Sanders, Einville au Jard, France; medium-energy diet; $n = 9$). The second group received the medium-energy diet with an additional 13 g DM·kg $BM^{-1}\cdot d^{-1}$ of corn grain (high-energy diet; $n = 8$) to provide an additional daily energy intake of +26 kcal of NE·kg $BM^{-1}\cdot d^{-1}$ compared with the medium-energy diet.

One kid from the medium-energy diet was slaughtered at 14 wk of age (i.e., after 5.5 wk on the medium-energy diet). At 17.2 ± 0.4 wk old, the remaining kids were allocated within each diet to 2 subgroups of 4 kids according to their BM. Kids from 1 subgroup of each diet were then slaughtered (8.6 wk on experimental diets and 4 kids in each diet; total $n = 8$) and the remaining kids were reared until 21.2 ± 0.4 wk of age (12.6 wk on experimental diets) and then slaughtered (4 in each diet; total $n = 8$). Over the entire 13-wk study, kids were allowed free access to water and mineral blocks and were housed in a free stall barn on barley straw with individual feed bunks, allowing controlled access and individual feeding of the concentrate supplement. Hay was distributed at 0800 h and concentrates were prepared daily and offered at equals amounts at 0700 and 1500 h.

The amounts of concentrate distributed were registered daily. No concentrate refusals were recorded over the entire study. Representative samples of hay, commercial concentrate mixture, and corn grain were sampled every 7 d over the entire study, before being pooled. Samples were ground and sieved through a 0.6-mm screen and analyzed according to standard methods (AOAC, 1997) by In Vivo Labs (Château-Thierry, France) for DM (103°C for 4 h), ash (method 942-05), CP (method 984-13), and ether extract (only for concentrates, after acid hydrolysis; method 920-39). For NDF and ADF, a sequential procedure with prior amylolytic and proteolytic treatments was used (Van Soest et al., 1991). The content of energy and protein truly digestible in the small intestine were calculated for all of the feeds using their ash, CP, ADF, and ether extract contents (PrevAlim 3.23; INRA, 2006). Chemical composition of the feed and values for energy and protein truly digestible in the small intestine are presented in Table 1.

Table 1. Chemical composition, energy, and protein contents of hay and concentrates

Item	Percent of DM							Energy, Mcal of NE/kg of DM[2]
	OM	CP	NDF	ADF	Fat	PDIN[1,2]	PDIE[2,3]	
Meadow (grass) hay	93.7	12.1	76.3	44	ND[4]	7.5	6.4	0.75
Concentrate mixture[5]	91.7	20.0	33.3	14.1	4.2	13.4	11.0	1.60
Corn grain	98.5	9.5	9.6	2.5	5.0	4.6	10.0	2.01

[1]PDIN = true protein truly digestible in the small intestine when protein supply is limited (INRA, 2007).

[2]Determined from chemical analyses using the PrevAlim software (INRA, 2006).

[3]PDIE = true protein truly digestible in the small intestine when energy supply is limited (INRA, 2007).

[4]ND = not determined.

[5]Fluvial Junior (Sanders, Einville au Jard, France) composed of 25% wheat bran, 12% corn, 10% rootlets, 10% corn germ meal, 10% sunflower meal, 8% beet pulp, 6% alfalfa, 5% wheat, and 5% rapeseed meal (wt/wt).

Deuterium Oxide Dilution Technique

During all the D_2O dilution technique procedures, kids had free access to feed and water. Two days before slaughter, each kid was infused at 0900 h with D_2O (0.2 g/kg BM, 99.96%; Euriso-top, Saint-Aubin, France) in the jugular vein through a catheter (polyurethane, 16 gauge × 45 mm; BD Medical, Le Pont-de-Claix, France), which was flushed with physiological saline (15 mL of NaCl solution 0.9%; Lavoisier, Paris, France). Amounts of D_2O administered were precisely determined by weighing the syringes (polypropylene, 10 mL; BD Medical) used to inject D_2O to the nearest 0.1 mg before and after injection. Within 30 min after infusion, kids were weighed to the nearest 0.5 kg (to determine BM at injection). Six blood samples (9 mL each) were collected into tubes containing lithium heparin (polyethylene terephthalate, 9 mL, 19 IU/mL; BD Medical) via a jugular catheter immediately before D_2O injection (to determine background baseline concentration of D_2O [**Cbg**]) and by venipuncture at 1400 and 1600 h the first and the second day and at 0900 h the third day immediately before slaughter. The 5 last samples corresponded to +5, +7, +29, +31, and +48 h after D_2O injection. Plasma was separated within 1 h by centrifugation at 1,400 × *g* at 20°C for 10 min and then transferred to glass tubes and stored at –20°C pending analysis.

Plasma samples were thawed at room temperature and 0.5 mL was subjected to deproteinisation by filtration through regenerated cellulose-filtered centrifuge tubes of 10 kDa (Amicon Ultra 0.5; Millipore, Carrigtwohill, Ireland) at 14,000 × *g* at 4°C for 2 h (Ripoche et al., 2006). Filtrates were then transferred to glass tubes sealed with butyl/polytetrafluoroethylene caps and stored at 4°C before analysis within within one week for $^2H/^1H$ enrichment by IRMS at the Unité Mixte de Recherche (**UMR**) 1137 Ecologie et Ecophysiologie Forestières (INRA, Champenoux, France). Isotope ratios of ^2H to ^1H were measured using a continuous flow EuroPyrOH (EuroVector, Milano, Italy) coupled, via a gas box interface, to an Isoprime IRMS (GVI, Manchester, UK). The liquids were sampled by a 1-µL syringe (SGE Analytical Science, Melbourne, Australia) rinsed with acetone and water. The liquid auto sampler HT300A (EuroVector) injected 0.4 µL into a quartz tube filled with Cr powder and heated to 1,050°C. The Cr reduces to H_2 ($^1H_2$, mass 2) and HD ($^1H\ ^2H$, mass 3) gases. The helium gas was used to carry H_2 or HD from the EuroPyrOH to the IRMS. Two international standards (Vienna Standard Mean Ocean Water [**VSMOW**] and Greenland Ice Sheet Precipitation) from the International Atomic Energy Agency (Vienna, Austria) and 4 homemade standards (distilled seawater, enriched water by evaporation, ultra pure water and filtered sleet) were used to analyze samples. The results were expressed as delta values relative to VSMOW.

Slaughter Procedure and Determination of Body Composition by Chemical Analysis

Goat kids were fed (0700 h) and weighed immediately before slaughter (slaughter BM) by electronarcosis followed by exsanguination, which occurred between 0900 and 1100 h (i.e., 48 to 50 h after D_2O injection). Blood, liver, and perirenal adipose tissue were collected and weighed. Parts of the digestive tract were weighed before and after being emptied (gut content weight determination) and the gut content DM was determined (desiccation at 103°C for 48 h). The hair was shorn from the skin and the horns were removed, and both were weighed before the rest of the body was cut into 4 to 5 pieces. All the collected parts of the empty body (**EB**; i.e., whole body including blood minus gut contents, hair, and horns) were stored at –20°C in hermetic plastic bags. The content of each bag was weighed before and after removal from cold storage and any losses observed since the initial weighing were assumed to be water. For each kid, on removal from cold storage, the frozen content of the bags (including blood and emptied digestive tract but excluding liver and perirenal adipose tissue) was minced, mixed, and homogenized at the UMR 1348 Physiologie, Environnement et Génétique

pour l'Animal et les Systèmes d'Elevage (INRA, Saint Gilles, France) using an industrial flaker (Rotary Meat Flaker, model RF15; Hobart Cesson Sevigne, Rennes, France) to render meat blocks into a size suitable for grinding, and then an industrial mixer–grinder (Mixer-grinder, model 4346; Hobart Cesson Sevigne) was used to grind and homogenize all samples. A homogenized 1-kg aliquot was obtained and stored at –20°C. Liver, perirenal adipose tissue, and aliquots of the minced remaining EB were lyophilized (DM content determination) before being minced through a fine mincer. Samples were analyzed again for DM (desiccation at 103°C for 48 h), lipid (Folch et al., 1957), protein (N × 6.25; Kjeldhal method; AOAC, 1997; method 981-10), and ash (550°C furnace for 6 h) content. Energy content was calculated from lipid and protein content using calorific values of 5.6 and 9.4 kcal/g for protein and lipids, respectively (Tissier et al., 1983).

Calculations and Statistical Analysis

Parameters of D_2O dilution kinetics were computed for each kid by extrapolating the regression of the D_2O concentrations (C*t*) over time by the regression equation

$$C_t = C_0 \exp^{-k \times t},$$

in which $\mathbf{C_0}$ (intercept) is the theoretical D_2O concentration at injection ($t = 0$) if dilution was instantaneous, k (slope) is the water turnover, and t is the time elapsed since D_2O administration (Robelin, 1973; Speakman et al., 2001). Deuterium oxide C0 and Cbg were then used to calculate the D_2O dilution space (**D_2OS**) in kilograms by using the equation from Schoeller et al. (1986):

$$D_2OS = (Q_{D2O} \times APE_{dose} \times MW_{H20})/ [MW_{dose} \times 100 \times (C_0 - C_{bg}) \times R_{std}],$$

in which Q_{D2O} is the dose of D_2O administrated in grams, APEdose is the deuterium atomic enrichment of the dose in percentage (i.e., 99.96%), MWH20 is the molecular weight of the body water (H_2O: 18.02 g/mol), MWdose is the molecular weight of the dose (i.e., D_2O: 20.02 g/mol), C0 and Cbg are expressed as delta ^2H vs. VSMOW, and Rstd is the ratio of deuterium to hydrogen (i.e., ^2H to ^1H) in VSMOW, that is, 1.5576×10^{-4}.

From postmortem measurements and analysis, EB weight was computed by subtracting the weight of gut contents from BM at slaughter, whereas the weight of each EB component (liver, perirenal adipose tissue, and the rest of the EB [excluding hair and horns]) was computed by summing the amounts of water, lipids, protein, and ash determined. The energy content of these component parts was also determined. Moreover, total body water was computed by summing EB water and water in gut contents.

Body composition data determined after slaughter were analyzed by ANOVA using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC), with a model that included age (17 or 21 wk old) and diet (medium or high energy) as fixed effects and animal as a random effect. The interaction between age and diet was not significant ($P > 0.10$) and was removed from the model. A first-order autoregressive covariance structure was used. Simple and multiple regression analyses were performed using the GLM procedure of SAS to evaluate relationships among different parameters and to develop prediction equations. Age and diet effects on regression coefficients were also tested as fixed factors. Significance was declared at $P \leq 0.05$, and trends were considered at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Body Composition Determined after Slaughter

The sum of the analyzed body components (Table 2) was very close to the slaughter BM. The average uncontrolled loss of material was calculated at less than 1.0%. Among the 17 male goat kids slaughtered between 14 and 21 wk old, slaughter BM varied from 22.0 to 35.5 kg (mean of 28.8 kg [SD 4.0]), gut contents weighed between 5.1 and 9.3 kg (mean of 7.1 kg [SD 1.1]), and EB weight was between 16.1 and 27.3 kg (mean of 21.7 kg [SD 3.3]). Empty body water ranged from 11.0 to 17.5 kg (mean of 14.4 kg [SD 1.9]) and represented 61.5 to 70.2% (mean of 67.1% [SD 2.1]) of EB weight. Conversely, when reported on fat-free EB (EB weight – EB lipids), only small variations in the proportion of EB water were observed across the 17 kids (i.e., range from 74.1 and 76.6% and mean of 75.3% [SD 0.6]). Protein was, on average, the most abundant component of the EB DM, varying from 2.7 to 4.6 kg (mean of 3.7 kg [SD 0.5]) followed by lipids (1.5–4.7 kg and mean of 2.6 kg [SD 0.8]) and ash (0.6–1.1 kg and mean of 0.8 kg [SD 0.1]). Therefore, protein represented between 16.8 and 17.9% (mean of 17.4% [SD 0.4]) of EB weight, lipids represented between 8.2 and 18.3% (mean of 12.0% [SD 2.2]), and ash represented between 3.4 and 4.3% (mean of 3.8% [SD 0.2]). Moreover, EB energy ranged from 30.4 to 67.8 Mcal (mean of 45.0 Mcal [SD 10.0]) and represented between 1.7 and 2.6 Mcal/kg (mean of 2.1 Mcal/kg [SD 0.2]) of EB weight. Such body composition was similar to that observed in Alpine male goat kids at 22 wk of age (Sousa et al., 1998), with a slaughter BM of 26 kg and 64.9, 15.2, 14.2, and 5.7% of EB weight as water, protein, lipids, and ash, respectively, and where

Table 2. Anatomical measurements and body chemical composition of male goat kids receiving medium- or high-energy diets and slaughtered at 14, 17, or 21 wk of age

Item	Age 14 wk Medium diet	Age[1]		Diet[2]		SEM[3]	*P*-value[3]	
		17	21	Medium	High		Age	Diet
Anatomical measurements, kg								
Infusion body mass (BM)	28.0	27.6	30.9	26.6	31.9	1.04	0.04	<0.01
Slaughter BM	28.3	27.4	30.2	26.2	31.4	1.03	0.07	<0.01
Gut contents	6.6	6.6	7.6	7.1	7.1	0.41	0.13	0.90
Empty body weight	21.6	20.6	22.4	18.9	24.1	0.70	0.08	<0.001
Perirenal adipose tissue, g	70	164	223	128	259	34.3	0.38	0.16
Chemical composition, kg								
Total body water	20.4	19.5	21.2	18.9	21.8	0.71	0.11	0.01
Gut water content	5.5	5.6	6.3	6.0	5.9	0.36	0.20	0.82
Empty body[4] water	14.8	13.9	14.8	12.8	15.8	0.44	0.19	<0.001
Empty body lipids	2.1	2.4	2.9	2.1	3.2	0.20	0.07	<0.01
Empty body protein	3.8	3.5	3.9	3.3	4.1	0.11	0.03	<0.001
Empty body ash	0.8	0.8	0.9	0.7	0.9	0.03	0.05	<0.01
Empty body energy, Mcal	40	42	49	38	52	2.3	0.04	<0.001
Proportions of body components in empty body weight,[4] %								
Water	69.6	67.8	66.0	67.9	65.9	0.57	0.05	0.03
Lipids	9.6	11.4	12.8	11.1	13.1	0.67	0.15	0.06
Protein	17.9	17.2	17.4	17.4	17.2	0.12	0.27	0.34
Ash	3.8	3.8	3.9	3.8	3.8	0.09	0.45	0.57
Energy, Mcal/kg	1.89	2.02	2.16	2.00	2.17	0.058	0.10	0.06
Proportions of body components in fat-free empty body weight,[5] %								
Water	75.8	76.5	75.7	76.4	75.8	0.15	<0.01	<0.01
Protein	19.5	19.4	20.0	19.6	19.8	0.12	<0.01	0.18
Ash	4.2	4.2	4.4	4.3	4.3	0.10	0.21	0.94

[1]Mean of 17.2 (SD 0.4; $n = 8$) and 21.4 wk (SD 0.4; $n = 8$).

[2]Male goat kids received a diet composed of hay ad libitum, complemented with 20 g DM·kg BM–1·d–1 of a concentrate mixture (Fluvial Junior, Sanders, Einville au Jard, France) alone (medium-energy diet; $n = 9$) or with an additional supply of 13 g DM·kg BM–1·d–1 of corn grain (high-energy diet; $n = 8$).

[3]Statistical model was applied to data only for the 17- and 21-wk-old male goat kids. Interaction of age × diet was not significant ($P > 0.10$). Least squares means are reported for those groups, whereas individual raw data are provided for the 14-wk-old kid ($n = 1$).

[4]Empty body: total body minus gut contents, hair, and horns.

[5]Fat-free empty body: empty body minus empty body lipids.

energy represented 2.4 Mcal/kg of EB weight (Sousa et al., 1998). Our results are also in accordance with a previous report on Saanen male goat kids at a slaughter BM of 24.5 kg, where lipids represented 11.4% of EB weight (Mtenga et al., 1996).

Due to growth, slaughter BM and EB weight tended to be higher ($P \leq 0.08$) at 21 than at 17 wk of age. Such EB weight changes are mostly due to the increase of lipids (+0.5 kg from 17 to 21 wk old; $P = 0.07$) followed by protein and ash (+0.4 and +0.1 kg, respectively; $P \leq 0.05$), whereas EB water was not significantly affected by age ($P = 0.19$; Table 2). As expected, when comparing medium- and high-energy diets, kids supplemented with of 26 kcal of NE·kg $BM^{-1}·d^{-1}$ over a period of 8 to 12 wk after weaning had greater BM and altered body composition. Both slaughter BM and empty BM were higher ($P < 0.01$) for kids on the high-energy diet compared with the medium-energy diet. The diet effects on body component weights were greatest for water (+3.0 kg from medium- to high-energy diets; $P < 0.001$) followed by lipids (+1.1 kg; $P < 0.01$), protein (+0.8 kg; $P < 0.001$), and ash (+0.2 kg; $P < 0.01$; Table 2). Moreover, EB energy was greater for the high-energy diet compared with the medium-energy diet (+14 Mcal; $P < 0.001$). Age and diet effects were much less marked when expressed as proportions of EB weight or fat-free EB. Water proportion in the EB and fat-free EB decreased ($P \leq 0.05$) from 17 to 21 wks (–1.8 and –0.8%, respectively), whereas this proportion was lower (–2.0% and –0.6%, respectively; $P \leq 0.03$) and EB lipids tended to be higher (+2.0%; $P = 0.06$) for the kids on the high-energy diet compared with kids receiving the medium-energy diet (Table 2). A decreasing effect of growth on the proportion of EB water has been previously described in lambs growing from 1 to 16 wk of age (i.e., the water proportion in fat-free EB varied from 78.8% at 1 wk old to 75.2% at 16 wk old; Robelin et al., 1977). Nevertheless, in the present study, variations in the proportion of EB water and lipid according to diet was dissimilar to that observed when compared with

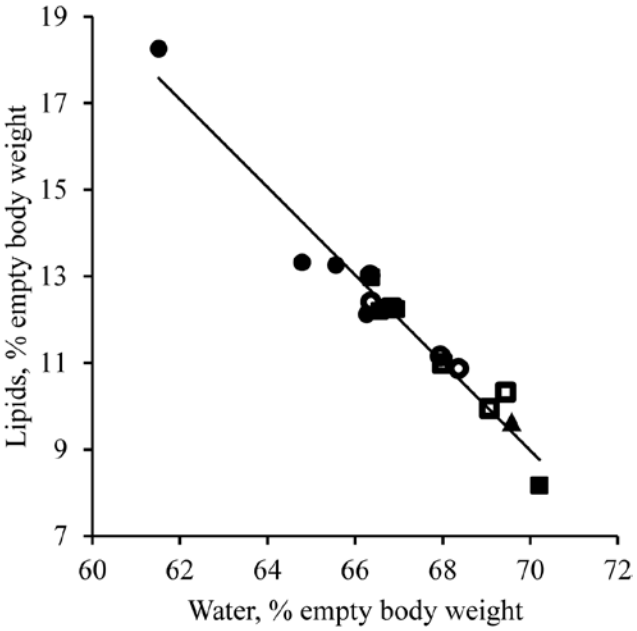

Figure 1. Relationship between empty body water and lipid content of male goat kids of 14 (▲; $n = 1$), 17 (○ and □; $n = 8$), or 21 wk (● and ■; $n = 8$) of age and receiving medium- (▲, □, and ■; $n = 9$) or high-energy (○ and ●; $n = 8$) diets.

adult Alpine and Saanen goats at different physiological stages (between dry and lactating goats at 60 d of lactation: +5.4% and –6.4% of EB water and lipids proportions, respectively; Schmidely et al., 1995).

Relationship between Lipid and Water Proportions

Water and lipid proportions in the EB were closely and negatively related ($P < 0.001$, $r = –0.98$; Fig. 1). The relationship is defined by the following equation, in which neither slope nor intercept were affected by age or diet ($P > 0.10$): lipids (% EB weight) = –1.013 (estimate; SE 0.056) × water (% EB weight) +79.89 (estimate; SE 3.74; residual SD [**rSD**] = 0.47%, residual CV [**rCV**] = 3.9%, $R^2 = 0.957$, $n = 17$).

A strong linear relationship between EB water and lipid proportions was expected, as it is widely reported in mammals (Robelin, 1973; Speakman et al., 2001) and more specifically in small ruminants (for example, in dairy goats, rCV = 9.0% [Schmidely et al., 1995], and in dairy ewes, rCV = 4.0% [Castrillo et al., 1995] and rCV = 3.2% [Bocquier et al., 1999]).

Postmortem Predictions of Body Components from Body Mass and Body Water or Perirenal Adipose Tissue Weight

Our results confirm the principle of body composition prediction from BM and body water (Robelin, 1973; Speakman et al., 2001) because 1) the proportions of water and lipids in the EB are closely related and 2) the fat-free EB composition is practically constant (see above).

All postmortem body component weights were correlated with slaughter BM ($P < 0.001$; $r = +0.93$, $r = +0.87$, $r = +0.95$, $r = +0.92$, and $r = +0.93$ for water, lipids, protein, ash, and energy, respectively; Table 3). When including total body water as a second independent variable, the precision of lipids and energy predictions was improved ($P < 0.001$) and rCV decreased from 15.3 to 6.6% for lipids and from 8.3 to 2.3% for energy (Table 3). This improvement of rCV by using both BM and total body water is comparable with the literature on goat kids at 7 wk of age (rCV was reduced from 13.3 to 5.7% for lipids; Schmidely et al., 1992) and fat-tailed ewes (rCV was reduced from 21.1 to 5.7% for lipids and from 16.3 to 2.0% for energy; Atti et al., 2000) Conversely, the predictions of EB protein and ash weights were not improved ($P > 0.10$) when total body water was included together with slaughter BM (rCV from 4.4 to 4.4% for protein and from 6.2 to 6.3% for ash; Table 3), as in fat-tailed ewes (rCV from 10.4 to 10.2% for protein; $P > 0.10$; Atti et al., 2000).

Prediction of EB lipids weight from perirenal adipose tissue weight showed good accuracy (rCV = 12.3%), and it was improved when perirenal adipose tissue weight was included with slaughter BM (rCV = 5.6%; Table 3). In Creole dry goats, total dissected adipose tissue weight could also be predicted with high accuracy using perirenal adipose tissue weight (rCV = 4.3%; Aumont et al., 1994).

In Vivo Predictions of Body Components from Body Mass and Deuterium Oxide Dilution Space

Kinetics of Deuterium Oxide Dilution. Among the 5 blood samples taken after D_2O injection (+5, +7, +29, +31, and +48 h after injection), all combinations of 4 and 5 sampling times were tested to minimize the CV attached to the determination of the C0 of the D_2O dilution regression. The use of all 5 samples, or of 1 of the 4 combinations of 4 samples including the +48-h sample, led to a higher C0 CV (≥2.8%) than when using the combination of the 4 first samples (at +5, +7, +29, and +31 h; CV = 1.1%). Such drawback could be explained by the fact that the 4 first samples correspond to the afternoon prefeeding times (+5 and +29 h) and to proximate afternoon postfeeding times (+7 and +31 h) over 2 d, whereas sampling at +48 h was not linked to such regular timing of sampling. Because D_2O dilution is dependent on body water fluxes (inputs: drinking and eating; outputs: urine, feces, transpiration, and respiration) and is, therefore, variable within the day, indicates that a specific and reproducible time frame of sampling should be applied to reduce the intercept prediction error of D_2O dilution ki-

Table 3. Postmortem prediction equations of male goat kids empty body components from slaughter body mass (BM) and measured total body water or perirenal adipose tissue weight[1]

Empty body[2] component	Regression coefficient mean (SE)[3]			Intercept mean (SE)[5]	Statistics		
	Slaughter BM, kg	Total body water[4], kg	Perirenal adipose tissue, kg		rSD[6]	rCV,[6] %	R^2
Lipids, kg	+0.174 (±0.025)	–	–	–2.396 (±0.732)	0.399	15.3	0.760
	+0.704 (±0.055)	–0.867 (±0.078)	–	NS	0.173	6.6	0.955
	–	–	+6.459 (±0.712)	+1.407 (±0.154)	0.321	12.3	0.846
	+0.094 (±0.012)	–	+4.281 (±0.434)	–0.881 (±0.309)	0.147	5.6	0.970
Protein, kg	+0.130 (±0.001)	–	–	NS	0.163	4.4	0.909
	+0.164 (±0.053)	–0.049 (±0.075) NS	–	NS	0.166	4.4	0.912
Ash, kg	+0.028 (±0.0004)	–	–	NS	0.050	6.2	0.852
	+0.034 (±0.017)	–0.008 (±0.023) NS	–	NS	0.052	6.3	0.853
Energy, Mcal	+2.35 (±0.24)	–	–	–22.63 (±6.82)	3.72	8.3	0.870
	+7.52 (±0.33)	–8.42 (±0.46)	–	NS	1.02	2.3	0.990

[1]All regressions were highly significant ($P < 0.001$). Equations were developed from data from 17 male goat kids of 14 ($n = 1$), 17 ($n = 8$), and 21 wk ($n = 8$) of age receiving medium- ($n = 9$) or high-energy ($n = 8$) diets. Age and diet had no effect on regression coefficients or the intercept ($P > 0.10$).

[2]Empty body: total body minus gut contents, hair, and horns.

[3]Predictive variables were included alone (slaughter BM or perirenal adipose tissue weight) or together (slaughter BM and perirenal adipose tissue weight, or slaughter BM and total body water weight) in the regression analyses. The – symbol indicates that the corresponding variable was not included in the resultant model. Regression coefficients were always significantly different from 0 ($P < 0.05$), unless when stated (nonsignificant [NS]; $P > 0.10$).

[4]Total body water: empty body water plus gut water content.

[5]Intercepts were always significantly different from 0 ($P < 0.05$), unless when stated (NS; $P > 0.10$). In these cases, intercepts were removed from the model (forced to 0).

[6]rSD = residual SD; rCV = residual CV.

netics. Similar observations and conclusions previously were made in goat kids (Schmidely et al., 1989) and ewes (Tissier et al., 1983; Bocquier et al., 1999). Therefore, we chose to determine intercept and then D_2OS only from the 4 samples +5, +7, +29 and +31 h after injection.

When examining the individual kinetics of D_2O dilution, the water turnover (i.e., the slope of the regression) was extremely variable across the 17 kids, with a range in half-life (i.e., ln(2)/slope) for water elimination varying from 3.5 to 7.1 d (mean of 5.5 d [SD 1.0]). Water half-life of the same order of magnitude was found in male goat kids of 7 to 20 wk old and was also highly variable across individuals (1.9 to 14.4 d; Schmidely et al., 1989). Such high variability in water turnover rate confirms that using a single measurement of D_2O at a supposed equilibration time is not suitable for the prediction of body water in ruminants, as previously observed in lactating ewes (Cowan et al., 1980).

Prediction of Body Water. The relationship between total body water and D_2OS was highly significant ($P < 0.001$; Fig. 2) and is defined by the following equation, in which the intercept was not significantly different from 0 ($P = 0.73$) and neither slope nor intercept were affected by age or diet ($P > 0.10$): total body water (kg) = 0.945 (SE 0.006) × D_2OS (kg; rSD = 0.58 kg, rCV = 2.9%, $R^2 = 0.944$, $n = 17$).

The accuracy of the prediction of total body water from D_2OS determined by IRMS obtained in the present study was similar to those previously observed when determining D_2OS by infrared spectroscopy in goat kids (rCV = 3.3% [Schmidely et al., 1988] and rCV = 3.0% [Schmidely et al., 1989]). Deuterium oxide space overestimated total body water from +5.8%, which is close to what was observed previously (+4.5 [Schmidely et al., 1988] and +2.2% [Schmidely et al., 1989]). In fact, overestimation of total body water by D_2OS is classically described, and in most cases, this observation was attributed by authors to the exchange of deuterium from injected D_2O with hydrogen from OM molecules of the EB and gut contents (Robelin, 1973; Speakman et al., 2001).

The precision of the relationship between EB water weight and D_2OS (Table 4) was lower than between total body water and D_2OS (rCV increased from 2.9 to

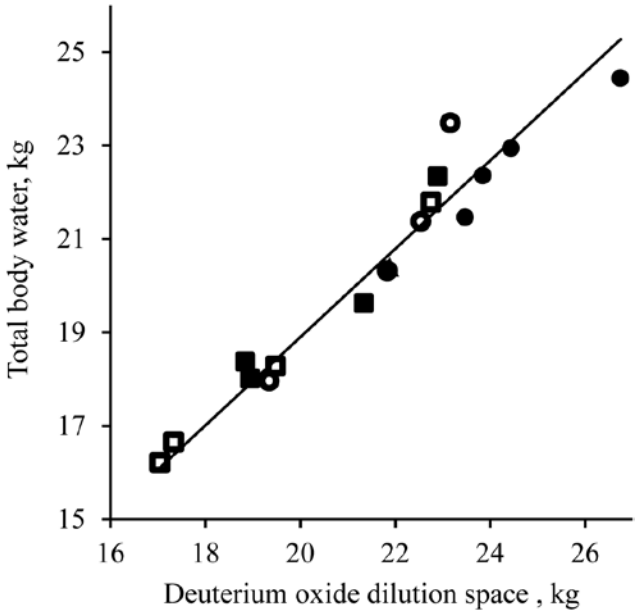

Figure 2. Relationship between deuterium oxide dilution space and total body water of male goat kids of 14 (▲; $n = 1$), 17 (○ and □; $n = 8$), or 21 wk (● and ■; $n = 8$) age and receiving medium- (▲, □, and ■; $n = 9$) or high-energy (○ and ●; $n = 8$) diets.

5.4% for total body water and EB water, respectively), in accordance with previous reports on goat kids (rCV increase from 3.3% to 5.4% [Schmidely et al., 1988], and from 3.0% to 5.7% [Schmidely et al., 1989], for total body water and EB water, respectively). This discrepancy could be attributed to the variable proportion of water in gut contents in total body water across goat kids (in the present study, water in gut contents represented between 23.8 and 35.8% of total body water).

Prediction of Body Components. This study indicates that 1) DM body component weights (especially lipids and energy) can be predicted with high precision when slaughter BM is associated with total body water measured postmortem and that 2) D_2OS is closely related to total body water (see above), thus confirming the principle of in vivo body composition prediction from BM and D_2OS (Robelin, 1973; Speakman et al., 2001).

To our knowledge, the only available equations predicting body components in goat kid from BM and D_2OS concern body water (Schmidely et al., 1988, 1989). For all body components, the intercept was never significantly different from 0 ($P > 0.10$), and regression coefficients were not affected by age or diet ($P > 0.10$). Therefore, only generic equations developed with all the goat kids ($n = 17$) with intercepts equal to 0 are provided in Table 4. Including D_2OS together with the infusion BM improved ($P < 0.01$) the predictions of EB lipids and energy compared with slaughter BM alone (Table 4). The accuracy of in vivo predictions from the infusion BM and D_2OS was lower than postmortem predictions from slaughter BM and total body water (rCV = 13.1% and rCV = 6.6% for lipids for in vivo and postmortem predictions, respectively, and rCV = 7.9% and rCV = 2.3% for energy for in vivo and postmortem predictions, respectively; Tables 3 and 4). Moreover, rCV for in vivo predictions were slightly higher than those reported in lambs at 10 to 16 wk of age (rCV = 10.5% and rCV = 4.2% for lipids and energy, respectively; Robelin, 1977) but slightly lower for lipids than those obtained in adult dry or lactating goats (rCV = 14.2% [Brown and Taylor, 1986], rCV = 16.1% [Dunshea et al., 1988], and rCV = 15.5% [Schmidely et al., 1995]). For both lipids and energy, regression coefficients associated with the infusion BM and D_2OS were almost identical but of opposite sign (+0.523 and –0.588 for lipids for infusion BM and D_2OS, respectively, and +5.27 and –5.06 for energy for infusion BM and D_2OS, respectively; Table 4), which is in agreement with the literature on adult goats (+0.809 and –0.867 [Dunshea et al., 1988], +0.708 and –0.621 [Brown and Taylor, 1986], and +0.65 and –0.75 [Schmidely et al., 1995] for infusion BM and D_2OS, respectively).

In contrast to the results for lipids and energy, D_2OS did not improve ($P > 0.10$) predictions for protein and ash (Table 4), as in 10- to 16-wk-old lambs (Robelin, 1977). This could be explained by the fact that in growing small ruminants of similar age, BM alone was already a good estimator of protein weight (rCV = 4.4% and rCV = 2.4% in the present study and in Robelin [1977], respectively).

Conclusions

Prediction equations of body composition of growing goat kids from postmortem and in vivo parameters are reported in the present paper. For all variables tested, the simultaneous inclusion of slaughter BM and perirenal adipose tissue weight in prediction equations provided the most convenient and precise estimates of body fatness in goat kids postmortem. Deuterium oxide dilution space determined by IRMS was closely correlated with total body water, despite an overestimation of 5.8%. Compared with BM alone, including D_2OS and BM improved the in vivo predictions of EB lipids and energy but not those of protein and ash. Accuracy of the prediction equations obtained was similar to those observed in studies predicting ruminant body composition from D_2OS determined by infrared spectroscopy. Therefore, when one aims to estimate in vivo body composition with a high degree of accuracy, using the D_2O dilution technique together with D_2O quantification by IRMS rather than infrared spectroscopy could help to significantly reduce the time needed to prepare the blood samples (150 vs. 20 samples/d for IRMS and infrared spectroscopy

Table 4. In vivo prediction equations of male goat kids empty body components from injection body mass (BM) and deuterium oxide dilution space (D_2OS)[1]

Empty body[2] component	Regression coefficient mean (SE)[3]		Statistics		
	Infusion BM, kg	D_2OS, kg[4]	rSD[5]	rCV,[5] %	R^2
Water, kg	–	+0.668 (±0.009)	0.773	5.4	0.837
Lipids, kg	+0.523 (±0.098)	–0.588 (±0.133)	0.341	13.1	0.825
Protein, kg	+0.066 (±0.052) NS	+0.084 (±0.071) NS	0.183	4.9	0.893
Ash, kg	+0.028 (±0.014)	$-6.2.10^{-6}$ (±0.019) NS	0.050	6.2	0.852
Energy, Mcal	+5.27 (±1.01)	–5.06 (±1.37)	3.54	7.9	0.883

[1]All regressions were highly significant ($P < 0.001$) and intercepts were never significantly different from 0 ($P > 0.10$) and, therefore, were removed from the model (forced to 0). Equations were elaborated from data originated from 17 male goat kids of 14 ($n = 1$), 17 ($n = 8$), and 21 wk ($n = 8$) of age receiving medium- ($n = 9$) or high-energy ($n = 8$) diets. Age and diet had no effect on regression coefficients or intercept ($P > 0.10$).

[2]Empty body: total body minus gut contents, hair, and horns.

[3]Regression coefficients were significantly different from 0 ($P < 0.05$), unless when stated (nonsignificant [NS]; $P > 0.10$).

[4]Determined from blood samples collected at +5, +7, +29, and +31 h after injection.

[5]rSD = residual SD; rCV = residual CV.

analyses, respectively) and thus counteract this main limitation. Compared with infrared spectroscopy, the advantage of using the D_2OS method in conjunction with IRMS may be even greater when studying larger animals (e.g., adult cattle), as the use of IRMS could significantly reduce the amount of D_2O injected (0.2 vs. 0.5 g/kg BM) and lower the cost. Nevertheless, IRMS requires specific equipment and expertise.

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