Original article

Isotopic estimation of milk intake in sucking lambs with deuterium oxide space: effects of milk concentration and level of milk intake

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Summary — Twenty-three artificially-reared lambs were injected at 23 and 35 d after birth with deuterium oxide (D_2O) used as a marker of body water. During both periods they were divided into 3 experimental groups. During the first period they received milk replacers *ad libitum* at different dry matter (DM) concentrations: 13.5, 15.7 or 17.7% (w/w). During the second period, milk was supplied at 3 levels of intake: 7.8, 8.7 and 9.6% of live-weight. Suggestions put forward by different authors to take into account non-steady states of water pool content in growing animals were confirmed as adequate. Under both diet conditions, the D_2O dilution technique was found to be efficient for prediction of total water intake of milk-fed lambs ($R^2 = 0.975$; RSD = ± 36 g/d). A brief survey of milk yield and milk DM content variations observed in nutritional trials with suckling ewes showed that milk yields were far more affected by experimental treatments than by milk DM variations. In this case, choice of a mean DM content is acceptable for the calculation of metabolic water contribution. The use of deuterium oxide as a non-radioactive marker with minimal handling procedure appears useful for estimation of milk yield in suckling ewes. It can also be used to simultaneously estimate the body composition of lambs.

ewe / milk yield / lamb / marker

Résumé — Estimation de la quantité de lait consommée par les agneaux avec la méthode de l'espace de diffusion de l'eau lourde (D_2O) : effets de la concentration et de la disponibilité en lait. *L'eau lourde (D_2O), utilisée comme marqueur de l'eau corporelle, a été injectée à 23 agneaux en allaitement artificiel répartis en 3 groupes expérimentaux dont on a mesuré les consommations. En première période (23 j après la naissance), ils étaient ad libitum et recevaient des laits de différentes concentrations : 13,5, 15,7 ou 17,7% de MS. En seconde période (+ 35 j) 3 niveaux d'apports en lait ont été imposés : 7,8, 8,7 et 9,6% du poids vif. L'espace de diffusion de l'eau lourde ainsi que le taux de renouvellement de l'eau corporelle ont été calculés d'après les teneurs sanguines en D_2O mesurées pendant 4 jours.*
Il existe une relation étroite entre le taux de renouvellement de l'eau corporelle et l'eau bue par les agneaux. Les corrections proposées pour tenir compte de l'accroissement du volume hydrique pendant la mesure (4 j) se sont avérées efficaces pour prévoir l'eau bue ($R^2 = 0.975$; RSD = ± 36 g/j). La méthode est fiable quels que soient les concentrations en MS du lait (ns) et les niveaux de consommation (ns). L'estimation de l'eau métabolique, provenant de la transformation des nutriments, repose sur des hypothèses qui sont discutées. Les résultats de la bibliographie montrent que le choix d'une teneur moyenne en MS du lait de brebis entraîne des erreurs minimes dans l'estimation

du lait bu. Cette méthode est donc précise et fiable pour estimer les consommations de lait par les agneaux pendant l'allaitement. De plus, elle permet d'estimer simultanément la composition corporelle des agneaux.

brebis / production laitière / agneau / marqueur

INTRODUCTION

It is of particular importance to find reliable methods for the estimation of milk intake by lambs, either for explanation of differences in lamb growth rates or for analysis of the effects of nutrition on the distribution of available nutrients between milk output and other requirements (body reserves and/or wool growth) in the ewe. Available methods (Doney *et al*, 1979) should be adapted to the design of the experiment in respect of required accuracy. General relationships between lamb growth rate and milk intake (Ricordeau *et al*, 1960) are of poor precision in nutrition experiments. Milking procedures must be applied carefully and results should be viewed with caution, mainly because suckled ewes are not accustomed to milking (machine or manual) and use of exogenous oxytocin affects both volume and milk composition. The test-weighing method is debatable in sheep unaccustomed to frequent handling.

Under these conditions, methods based on single tracer injection to determine body-water turnover in normally-sucking lambs might be an adapted solution. However, these techniques should be used only for a limited period when milk is the sole source of water and/or when water intake is controlled. This limitation is not serious since ewe peak milk yields appear around 3rd wk of lactation when lamb water intake is nil or low. On the other hand, the main advantage of this technique is that the degree of udder emptying only depends on lamb activity which might not be changed with limited handling procedures.

Body-water dilution techniques are based on the use of water isotopes; tritiated water (TOH: MacFarlane *et al*, 1969; Dove and Freer, 1979) or deuterium oxide (D_2O; in foal: Doreau and Dussap, 1980; and in piglets: Pettigrew *et al*, 1987). Deuterium oxide was seldom used due to its cost and difficulty of estimation in blood, but this is no longer the case (Tissier *et al*, 1978; Byers, 1979) and the use of non-radioactive tracer is of substantial advantage compared to TOH when applied to farm animals. We did not follow the procedure used by Dove (1988) using a double isotope dilution technique, TOH in ewe, D_2O in lambs, because this method was shown to be adequate when milk is not the major source of water.

The first aim of this experiment was to predict milk intake of lambs with a specific equation using D_2O as a marker. The second objective was to measure the practical consequences of differences in milk concentration and to third was to assess the practical use of this method in conditions of limited milk supply.

MATERIALS AND METHODS

Animals and experimental diets

Twenty-three lambs, pure Limousine (11) or Limousine crossed (12) were individually penned indoors and artificially fed from lambing with a milk replacer. Lambs were twice infused with D_2O at d 23 and 35 after birth (see below).

During the first period, lambs were offered the same milk replacer *ad libitum* (15% refusals)

at 3 DM (w/w) concentrations: 13.5 ($n = 8$ lambs), 15.7 ($n = 7$) and 17.7% ($n = 8$) to assess the effect of variability in nutrient concentration of ewes' milk on estimation of milk intake.

After completion of the first period, the DM of milk replacer was standardized (16.0% w/w) and milk availability was progressively reduced to simulate the observed decline in milk yield (Bocquier et al, 1987). During the second period the same lambs were offered 1 of the 3 levels of milk replacer at: 7.82 ($n = 8$), 8.74 ($n = 8$) and 9.62% ($n = 7$) of bodyweight per d and had free access to drinking water and concentrate.

At both periods milk was given in 2 equal meals (09.00 and 13.00 h).

Measurements and analysis

Milk intake and liveweight

Intake was measured daily and samples of offered and refused milk were taken for DM determination (48 h at 80 °C). Commercial milk powder was composed of 27% crude protein, 38% lactose, 25% fat and 10% ash in DM. Concentrate and water intake were also measured daily in the second period. Lambs were weighed weekly (13.00 h) during the entire experiment and daily during marker collections.

Dilution procedure

Lambs were infused with D$_2$O (1 g D$_2$O/kg BW) with a catheter inserted in the jugular vein. Injected D$_2$O was measured by difference between pre- and post infusion weight of syringes (nearest mg). For both periods, lambs were injected at 09.00 h and blood samples were taken (by venipuncture) at 13.00 h, on the day of infusion and at 13.00 h during the following 3 days. Concentrations of D$_2$O were determined by infrared spectrophotometry after water extraction by vacuum sublimation (Tissier et al, 1978). Water space diffusion (SPACE, g) was calculated by back extrapolation after log_N transformation of blood concentrations of D$_2$O (Robelin, 1983). Apparent water turnover rate (TURNapp, g/d) was determined by multiplying fractional turnover rate (ie slope) with space:

$$TURNapp = slope \times SPACE$$

The knowledge of D$_2$O diffusion space also allowed the calculation (Robelin, 1977) of body composition of each lamb.

Estimation of metabolic water formation from solid milk nutrients was calculated from digestibility trials (Villette and Thériez, 1983). With the same type of milk replacer the digestibility coefficients were 0.96 for fat, 0.97 for protein and 0.99 for lactose. We assumed catabolism to be the difference between body chemical deposits (Robelin, 1977) and metabolizable nutrient intake. These hypotheses led to a conversion factor (Cm) of 0.45 g metabolic water per g milk OM.

To convert the measured total water intake (TWI, g/d) into milk yield (MY, g/d), dry matter content (DMm, %) of milk should be known and other water sources substracted, ie liquid water intake and water from solid food intake (SFI):

$$TWI = \text{Water from milk + other sources} \qquad (1)$$

ie : Water from milk = (1 – DMm) x MY
+ Metabolic water related to milk DMm intake:
DMm x Cm x MY
+ Water intake (if any)
+ Water from solid food: (1 – DMs) x SFI
+ Metabolic water from solid food related
to nutrient intake: DMs x SFI x Cs

In the first period, the main water supply was milk:

$$ie \quad TWI = MY \times ((1 - DMm) + DMm \times Cm) \qquad (2)$$
$$MY = TWI / (1 - (DMm \times (1 - Cm))) \qquad (3)$$

Data were analyzed by linear regression and by analysis of variance and covariance.

RESULTS

Lamb growth

Mean bodyweights of lambs were respectively 5.6 and 12.0 kg at the first and second infusions with an average daily gain (ADG) of 283 and 186 g/d (tables I and II). During the ad libitum period milk intakes were very variable either between lambs

F Bocquier *et al*

Table I. Mean characteristics of lambs during the first part of experiment. Effect of milk DM content.

Milk replacer	DM content (%)			Overall
	13.5	*15.7*	*17.7*	*SD (1)*
Intake (g/d)				
Milk intake	2 007	1 784	1 807	454
Milk DM intake	276	282	315	69
Total water intake	1 918	1 629	1 634	420
Lamb liveweight (kg)				
At birth	4.29	4.49	4.39	0.70
At injection (BW0)	5.38	5.72	5.88	0.91
Injection + 4 d (BW4)	6.49	6.87	7.12	1.00
Chemical composition (kg)				
Body water	4.19	4.54	4.64	0.66
Body lipid	0.33	0.34	0.37	0.08
Body protein	0.82	0.87	0.89	0.14
Body energy (MJ)	30.7	32.1	33.4	6.27

(1) None of these differences were significant ($P > 0.05$).

(615–2 860 g/d) or from day to day. During the restricted period milk intake was limited and ADG were reduced. On both periods, infusion of $\approx$ 5–12 g D_2O per lamb caused little disturbance (only 5 of over 46 injected lambs had reduced ADG following infusion).

Relationship between milk intake and lamb growth

During the first part of the experiment, the relationship between total milk intake and ADG was poor ($R^2 = 0.43$, $P < 0.01$) with a residual standard deviation of ± 383 g/d (CV = 18.4%, fig 1). This was due to difference in milk DM content ($P < 0.01$), water intake increased as DM content decreased

and lamb growth rate was better related to milk DM intake ($R^2 = 0.75$, $P < 0.001$; CV = 12.2%). During the restricted period, lamb
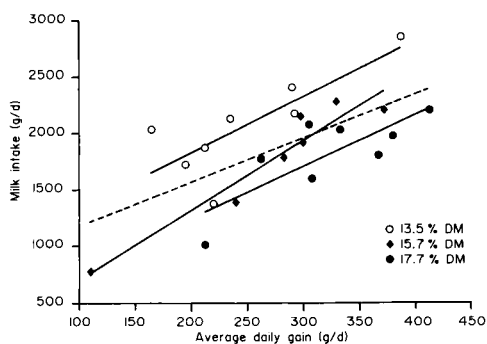

Fig 1. Relationship between lamb weight gain and milk intake: effect of milk dry matter concentration.

Table II. Mean characteristics of lambs during the second part of experiment: Effect of milk restriction.

Milk replacer	Milk availability (g/kg/d)			Overall
	Low (78.2)	Medium (87.3)	High (96.2)	SD (1)
Intake (g/d)				
Milk intake	945	1091	1115	180
Milk DM intake	137	158	162	26
Concentrate DM	69	59	34	36
Fresh water intake	7	6	7	4
Total water intake	857	977	961	155
Lamb liveweight (kg)				
At injection (BW0)	11.6	11.6	10.9	1.87
Inject + 4 d (BW4)	12.6	13.0	12.2	1.98
Chemical composition (kg)				
Body water	8.46	8.68	8.11	1.28
Body lipid	1.20	1.25	1.20	0.33
Body protein	1.86	1.91	2.81	0.32
Body energy (MJ)	89.1	92.2	87.8	20.4

(1) Nine of these differences were significant ($P > 0.05$).

growth was poorly (ns) related to milk intake. The concentrate intake was low (53 g DM/d, *ie* 8 g of water) as was water intake (7.5 g/d, table II). Under our conditions, water supply from milk replacer was the most important (720 g/d), compared to 15.5 g/d from other sources.

Estimation of milk intake

A limitation of this method might be connected with the increase in body water pool (non-steady state) during the measurement period (4 d). This bias might lead to an overestimation of milk intake (Holleman and White, 1988) but appropriate corrections have been proposed by Dove and Freer (1979) for lambs (formula (4)) and by Doreau and Dussap (1980) for foals. The Dove's correction had been applied to our data:

$$TURNcor = TURNapp \times ((V_4 - V_0)/V_0)/(LogN(V_4/V_0))) \qquad (4)$$

where V_0 is initial body water and V_4 water volume after 4 d. Assuming that the water volume *(V)* to body weight *(BW)* ratio was constant during measurement period we replaced V_i by BW_i in formula (4).

Corrected turnover was plotted against total water intake (*ie* milk water + metabolic water + water from other sources; see fig 2). Relationship of prediction of total water intake (*TWI*, g/d) was not different
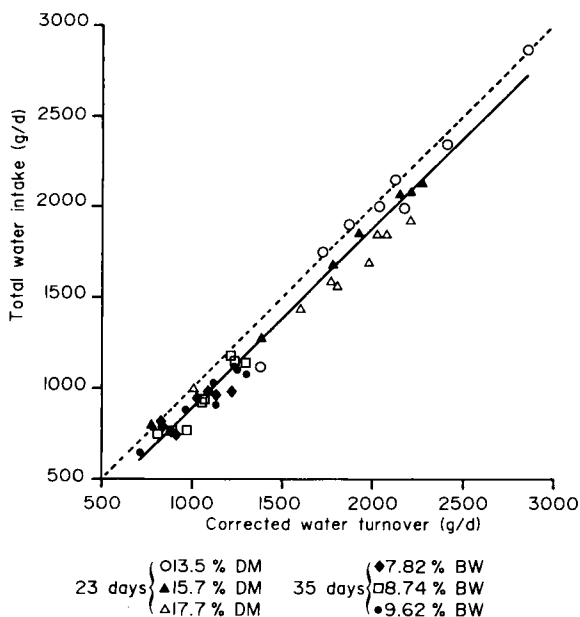
F Bocquier *et al*

Fig 2. Relationship between corrected water turnover and total water intake of lambs.

on overall data between *ad libitum* (first period) and restricted period (second part) when tested by covariance analysis:

$$TWI = 0.987 \times TURNcor - 97 \quad (5)$$
$$g/d \pm 0.023 \ g/d \quad (RSD = \pm 36, \ n = 46)$$
$$(R^2 = 0.975, \ P < 0.001)$$

The estimated chemical body composition of lambs was not significantly (ns) affected by treatments within each period (tables I and II). No direct comparisons can be made between first and second period treatments since lambs were reallocated.

DISCUSSION

Lamb growth and milk intake

Lamb growth rates during the first period were within the observed range for this breed of sheep. During the second period reduced ADG were due to the restriction of milk availability and probably to a slow adaptation to concentrate. This might be due to isolation of lambs in individual pens. In normal conditions social facilitation might help the transition from liquid to solid food with no reduction of ADG.

Many relationships between live-weight gain and ewe's milk yield have been reported in the literature; our results ($R = 0.65$) are within the wide range of observed correlation coefficients, from 0–0.91 (Ricordeau et al, 1960; Robinson et al, 1969; Torres-Hernandez and Hohenboken, 1980). Torres-Hernandez and Hohenboken (1980) found no direct effects of milk DM content on the relationship, but inclusion of protein content as a second variable increased the correlation coefficient from 0.73 to 0.86. In our experiment, the relationship between ADG and milk consumption was poor because, under ad libitum conditions, lambs partly adjusted their milk consumption to nutrient intake, so that when DM increased total milk intake decreased. This was confirmed by a better relationship observed between DM intake and ADG ($R = 0.86$), as previously shown by Large (1965) and Molénat and Thériez (1974).

Corrected turnover rate and water intake

Mean correction of water turnover for increase in body water content was more important in the first period (9.7%) than in the second (2.6%). This is directly related to the ADG differences between periods; in the second period, water space variations due to non-steady state were small. These values are in agreement with those observed by Dove (1988) in early lactation. Even if this correction only slightly improves the prediction of water intake, it has been kept since it may be more general and might be necessary for higher ADG or for longer periods of measurements (Holleman and White, 1988). The small overestimation of body water turnover (constant

term: – 97 g/d ($P < 0.05$), in equation (5) might be due to the well-known overestimation of total body water by water space diffusion (Robelin, 1977).

Metabolic water and its estimation

Estimation of metabolic water production using nutrient intake appears reliable because the slope between corrected turnover and total water intake is near 1 (0.987). The coefficient of conversion ($Cm = 0.45$) is lower than that assumed by Dove and Freer (1979), but in good agreement with that proposed by Doreau and Dussap (1980) in foals using another method. Metabolic water from concentrate was neglected due to low intake. Milk DM content can be directly measured by milking techniques or assumed from the literature; the overall mean value of DM content of ewe milk is 189 g/kg with a ± 4.0% range of variation (authors: mean ± range %; Cowan et al, 1980: 199 ± 2.3%; Cowan et al, 1981: 210 ± 5.5%; Geenty and Sykes, 1986 a,b: 182 ± 3.8%; 171 ± 5.8% and 183 ± 2.7%). The consequence of DM variation within the observed range (4%) lead to 25 to 30 g/ewe/d bias on milk yield (ie 1%). Compared to the direct effects of feeding treatments on milk yield in those nutritional trials (Cowan et al, 1980: 900 g/d; Cowan et al, 1981: 982 g/d; Geenty and Sykes, 1986 a,b: 490 g/d, 742 and 166 g/d), the effects of unknown milk DM variations on milk yield estimations are far below induced milk yield variations.

Corrected turnover rate and milk yield

From milk water intake of lamb, estimation of the actual ewe's milk yield can be calcu-

lated with little bias assuming $Dm = 0.189$ and $Cm = 0.45$ from equation 3:

Milk yield = 1.116 x (TWI − other water sources)   (6)

Water turnover measurements in sucked lambs using the procedure described here cause little disturbance to the lambs: a double weighing; at injection and at last sampling, with a limited number of blood samples. This technique improves the accuracy with which milk intake may be predicted from other methods; namely test-weighing or oxytocin milking techniques. Test-weighing is presumed to estimate the milk consumption by the lamb(s) while the oxytocin measures the rate of milk secretion by the ewe (Doney *et al*, 1979). During test-weighing measurements, particularly in early lactation, lamb milk intake is reduced by animal disturbances; probably due to lamb-ewe isolation and inadequate suckling frequency. With the oxytocin procedure, responsiveness to oxytocin injection before milking is very variable between ewes, and milking interval has a strong effect on milk secretion rate (unpublished observations). Comparisons reported by Doney *et al* (1979) revealed that oxytocin gave an overestimate of 13–23% when compared to test-weighing. When milk is the only source of water (or if extra milk water is controlled), the dilution technique seems to be the best method ensuring that milk rate secretion of the ewe and milk extraction by lambs are equal.

CONCLUSION

Reliable estimates of milk output are needed to calculate nutrient balances in ewe or lambs. This method can be applied several times consecutively to the same lamb with a preinfusion D_2O concentration subtracted. Deuterium oxide, as a stable isotope, could be preferred to TOH which is radioactive with biological hazard, together with the major advantage that it does not disrupt normal maternal-offspring relationship during measurements. We confirmed here the suitability of this method in both cases of DM content variation and reduced milk availability. Meanwhile, this method is better adapted for indoor experiments (winter) than for grazing trials (see Dove, 1988).

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