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Estimation of the intake of milk by lambs, from the turnover of deuterium- or tritium-labelled water

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1. The total water turnovers of grazing ewes and their lambs were estimated on days 9, 23, 44, 69 and 86 of lactation from the dilution of tritiated water injected into the dam and deuterium oxide injected into the offspring. The contribution of milk to the total water turnover of the lambs was estimated at the same times from the accumulation of tritium in their body water.

2. Mean total water turnover in the ewes was 6·5 litres/d over the entire period. In lambs, total turnover rose from 1459 ml/d at 9 d to 2791 ml/d at 86 d, and was closely related to live weight (r^2 0·760, $P < 0{\cdot}001$). The corrections to total water turnover, which were required because of the increasing body water pool size of the lambs during each measurement period, fell from +10·6% at 9 d to +3·7% at 86 d. All corrections were significant ($P < 0{\cdot}001$).

3. The intake of water as milk fell throughout the study, from 1501 ml/d at 9 d to 471 ml/d at 86 d. Pool-size corrections were significant ($P < 0{\cdot}001$). Milk intakes calculated from these results were 1816, 1054, 862, 742 and 588 ml/d at 9, 23, 44, 69 and 86 d of lactation. The rapid decline in milk intake reflected undernutrition of the ewes in early lactation.

4. The level of live-weight gain in early lactation was closely related to, and at a level expected from, the estimated milk intakes. From comparisons of estimated milk intakes with published estimates, it is concluded that the combined use of deuterium oxide and tritiated water results in accurate estimates of milk intake by the lamb throughout the ewe's lactation.

Studies of the milk intake of preruminants have usually been based either on the 'test-weighing' of offspring before and after sucking (Coombe *et al.* 1960), or on hand- or machine-milking of the dam after oxytocin administration (Langlands, 1972, 1973; Geenty, 1979). The potential errors of these methods have been discussed by several authors (Coombe *et al.* 1960; Robinson *et al.* 1968; Dove & Freer, 1979). In general, test-weighing can underestimate intake, if the handling and disturbance of the animals interferes with normal sucking behaviour or milk ejection. Moreover, as the offspring grow and milk production declines, it becomes increasingly more difficult to detect the proportionately smaller weight gains after sucking. The milking of the dam can lead to an overestimate, particularly with single offspring, by resulting in a greater degree of udder emptying than is achieved by the offspring. Both methods have the disadvantage of involving the extrapolation, to the undisturbed grazing situation, of an estimate of intake obtained during a period of disturbance.

Macfarlane *et al.* (1969) suggested these potential errors could be avoided by estimating milk intake from the total water turnover, determined from the dilution of injected, isotopically labelled water. This 'single-isotope' method offers several advantages over other methods. First, milk intake is estimated in naturally sucking offspring between, rather than during, periods of disturbance, although the effects of any disturbance on lactation still need to be considered. Second, the method may give better estimates in twin offspring, by allowing sibling rivalry to have its effect on sucking behaviour. Third, the method also allows the monitoring of body-composition changes in the offspring, since body-water content is estimated as part of the method. The single-isotope method has since been applied to a range of species, using either tritiated water (TOH) or deuterium oxide (D_2O) as the tracer (Wright *et al.* 1974; Dove & Axelsen, 1979; Dove & Freer, 1979; Pettigrew *et al.* 1987). Provided changes in body water pool size are taken into account (Dove & Freer, 1979), the method gives accurate estimates of milk intake, but only when milk is the sole source of water intake.

To overcome this restriction, Holleman *et al.* (1975) described a 'double-isotope' method in which the total water turnover of the dam and offspring were measured using TOH and D_2O respectively, while the contribution of milk to the water turnover of the offspring was determined from its accumulation of TOH from the dam. The method has been applied to wild ruminants (Holleman *et al.* 1975, 1988; Carl & Robbins, 1988) and marsupials (Cork & Dove, 1986; Dove *et al.* 1987) but apart from one small study (Wright & Wolff, 1976) it has not been used with domestic species.

In the present study, the double-isotope method of Holleman *et al.* (1975) was used to estimate the milk intake of twenty-four lambs suckled by grazing ewes over the first 90 d of lactation.

MATERIALS AND METHODS

Animals and experimental protocol

Twenty-four Dorset Horn male × (Border Leicester male × Merino female) single lambs of mixed sex, with their dams, were selected at lambing (early August) from a separate study of the effects of nutrition from mating to mid-pregnancy on performance during late pregnancy and lactation (M. Freer and J. R. Donnelly, unpublished results). Details of the latter study will be reported elsewhere but briefly, the treatments involved the manipulation of grazing pressure to allow high (H), medium (M) or low (L) planes of nutrition from approximately 30 d before mating until day 100 of pregnancy. The aim was to generate gain (H), maintenance (M) or loss (L) of maternal weight over the treatment period. Observed weight changes over this 130 d period (including conceptus) were 13·5 (SE 1·31), 10·0 (SE 1·37) and 6·1 (SE 1·07) kg respectively. Ewes then grazed together, with *ad lib.* access to pasture, for the last 50 d of pregnancy. Weights immediately before lambing were 56·3 (SE 1·08), 52·1 (SE 1·14) and 50·1 (SE 2·09) kg respectively. The effect of treatments on birth weights was not marked ($0·10 > P > 0·05$). Very few twins were born. The mean weight of the twenty-four selected single lambs was 4·5 (SE 0·10) kg. Due to the spread of lambing dates, lambs ranged from 4 to 14 d of age at the start of the first intake estimate (mean 9 (SE 0·7) d).

The selected ewes and lambs grazed as a group on a pasture dominated by *Phalaris aquatica* and *Trifolium subterraneum*. Due to inadequate autumn and winter rain, pasture supply was sparse and pasture intake restricted after lambing, until rapid pasture growth followed the onset of spring rains on day 35 of lactation.

Estimates of milk intake were conducted over periods commencing when the mean age of the lambs was 9, 23, 44, 69 and 86 d. The protocol adopted was based on preliminary indoor work which indicated that, after injection of TOH into ewes: (a) the response in the specific activity of water extracted from blood or ewe's milk was identical, and unaffected by the presence or absence of milk in the udder at the time of injection; (b) consumption of the TOH-labelled milk by the lambs resulted in a shallow peak in the specific activity of their body water 4–5 d after the injection of their dams. This is similar to the observations of Holleman *et al.* (1975).

On the basis of these observations, the following protocol was adopted.

(1) The night before each estimate commenced, ewes and lambs were confined to a yard and denied access to food and water.

(2) The next morning, ewes were separated from lambs and given an intramuscular injection of approximately 8 MBq TOH. Actual doses were determined by weighing syringes before and after injection. After an equilibrium period of 6 h, the ewes were hand-

milked after the intravenous administration of 2 I.U. oxytocin, in order to determine milk composition and specific activity. In the second and later periods, milk samples were also obtained before TOH injection, to determine residual TOH from the previous injection. The ewes were weighed within the equilibration period.

(3) Within the above 6 h period and after a minimum separation of 2 h from their mothers, lambs were bled by jugular puncture if a background level of D_2O was needed. They were then injected with 1·5 g D_2O (99·7 atoms %)/kg live weight, based on their previous weight and an assumed growth rate. Actual doses were determined gravimetrically. Injections were given intramuscularly (at two sites for volumes between 5 and 10 ml). Volumes above 10 ml were given intraperitoneally (subsequent analysis of D_2O space as a proportion of live weight found no differences attributable to route of administration of D_2O). The lambs were re-bled after a 2 h equilibration period (Dove & Freer, 1979) during which period they were also weighed.

(4) After ewes were milked at the end of their 6 h equilibration period, they were re-united with their lambs and returned to the field.

(5) After 4 d (6 d in the fourth period, due to heavy rain), ewes and lambs were collected and separated for 2 h. The ewes were hand-milked as described previously, to obtain samples for water turnover and milk composition estimates. The turnover of D_2O in the lambs and their accumulation of TOH as a result of milk consumption were estimated by taking a further jugular blood sample. Lambs were then re-injected with D_2O (0·8 g/kg live weight) to establish the increase in D_2O space (Dove & Freer, 1979).

Chemical analyses

Milk dry-matter (DM) contents were determined by freeze-drying. Samples of body water were obtained from milk and blood serum by vacuum sublimation and were assayed for TOH specific activity by liquid-scintillation counting as described by Donnelly & Freer (1974).

Concentrations (w/v) of D_2O were estimated by infra-red spectroscopy using a Wilks Miran-1A fixed-filter spectrometer (wavelength 4 nm), essentially as described by Byers (1979). However, no attempt was made to control sample temperature during analysis, since this was found unnecessary if absorbance was read exactly 2 min after sample injection.

Calculations and statistical analysis

The water turnover of each ewe in each period was calculated as the product of its TOH space at the start of the period and its fractional water turnover during the period. No pool size corrections were needed.

In each lamb, total water turnover (T, ml/d), adjusted for the changing pool size, was calculated from the expression used by Dove & Freer (1979):

$$T = -\frac{(V_1 - V_0)}{t} \times \frac{\ln(C_1/C_0)}{\ln(V_1/V_0)}, \quad (1)$$

in which V_0 and V_1 are the initial and final D_2O spaces over the period t, and C_0 and C_1 are the initial and final concentrations of D_2O.

Estimates of milk intake by each lamb were obtained in two ways. In the first, equivalent to the single-isotope method, total water turnover was converted to apparent milk intake (AMI) by dividing by a factor reflecting the total contribution of milk to water turnover. Dove & Freer (1979) suggested that this factor was close to 0·95, but this assumes that, during metabolism, all the consumed milk fat and protein contributes to water turnover.

This is not the case, since fat and protein are deposited during growth. Estimates of AMI were therefore calculated by the method of Pettigrew *et al.* (1987) (eqn (2)):

$$\text{AMI} = \frac{T + 1{\cdot}07\ (\text{fat gain}) + 0{\cdot}41\ (\text{protein gain})}{0{\cdot}95}. \tag{2}$$

Estimates of the daily deposition of fat and protein by each lamb were made from its body composition at the start and finish of each period, calculated in turn from its D_2O space, using the prediction equations of Donnelly & Freer (1974).

The second estimate of milk intake was obtained by calculating the proportion of total water turnover contributed by the water of milk, using a modification of eqn (5) of Holleman *et al.* (1975):

$$m = \frac{Q(k_b - k_a)}{\alpha_0(\exp(-k_a t) - \exp(-k_b t))}, \tag{3}$$

where m is milk-water intake, Q is the amount of tracer (TOH) in the offspring as a result of consuming milk for t days (computed as the product of pool size V_t and concentration C_t), α_0 is TOH specific activity in the mother at the start of period t, k_a and k_b are fractional water turnover rates in mother and offspring respectively.

Eqn (3) makes no allowance for the increasing size of the body-water pool in the offspring. If k_b is calculated from an initial and final blood sample, as in the present study, it will underestimate the true fractional turnover rate, as earlier discussed (Dove & Freer, 1979). In order to obtain values for k_b adjusted for changing pool size, total water turnover calculated using eqn (1) (and thus adjusted for pool size changes) was divided by the initial pool size of the offspring in each period. The adjusted values for k_b were then used in eqn (3). Milk-water intake was then converted to milk intake by dividing by the water content of the milk.

All variates were examined by analysis of variance, for the effects of previous treatment of the ewes, time (i.e. stage of lactation) and their interaction, employing the procedures suggested by Rowell & Walters (1976) for the analysis of contrasts over time. The effect of time was further subdivided into linear and quadratic effects, tested against the corresponding component of the error term (Rowell & Walters, 1976). Heterogeneity of variances across the measurement periods was examined by plotting residuals *v.* fitted values. In the event, no heterogeneity was detected for any variate and no data transformations were required. Accordingly, means for the five measurement periods are quoted with a single standard error of the difference. The interaction term was not significant for any variate. Covariates used in the analyses of variance (see p. 380) were the initial spread in stage of lactation/lamb age (ewes, lambs) and birth weight (lambs).

Comparisons of the various estimates of water turnover and of milk intake were made using Student's t test for paired comparisons. Although AMI accurately estimated milk intake only in early lactation (see p. 381) it is retained in the Tables to allow an assessment of the single-isotope method and to allow comparison with earlier studies. Relations between water turnover or milk intake and live weight, and between live-weight gain and milk intake were established by regression analysis. Equations describing the milk-intake patterns were fitted using the OPTIMIZE routine in GENSTAT (GENSTAT, 1977).

RESULTS

Effects of previous treatment of the ewes

The treatment received by ewes before day 100 of pregnancy resulted in significant differences in the pattern of ewe live weights during lactation ($P < 0{\cdot}005$) and on daily

Table 1. *Changes in live weight, tritiated water (TOH) space and total water turnover in grazing ewes during lactation**†

Stage of lactation (d)	Live wt (kg)	TOH space (kg)	Total water turnover (ml/d)
9	48·1	32·2	7·01
23	47·2	30·9	5·20
44	45·4	32·5	6·50
69	43·4	28·8	7·18
86	44·2	30·1	6·55
Standard error of difference	1·25	0·90	0·337

* All values corrected by covariance analysis for the initial difference in stage of lactation.
† For details of experimental procedures, see pp. 376–377 and for calculations, see pp. 377–378.

Table 2. *Changes in live weight and deuterium oxide (D_2O) space in crossbred lambs during lactation*†

Stage of lactation (d)	Live wt‡ (kg)	D_2O space‡ (kg)	Regression parameters relating D_2O space to live weight*: Slope Mean	Slope SE	Intercept Mean	Intercept SE	r^2
9	6·1	4·4	0·65	0·016	0·40	0·099	0·988
23	9·3	6·4	0·63	0·037	0·57	0·343	0·931
44	13·1	8·9	0·53	0·042	1·88	0·561	0·878
69	18·6	11·9	0·37	0·054	4·94	1·020	0·684
86	22·1	14·2	0·60	0·048	1·02	1·060	0·877
Standard error of difference	0·38	0·24					

* All regressions highly significant ($P < 0.001$).
† For details of experimental procedures, see pp. 376–377 and for calculations, see pp. 377–378.
‡ After adjustment, by covariance, for initial difference in birth weight and stage of lactation.

water turnover ($P < 0.005$). There were also marked effects on lamb milk intake ($P < 0.001$), milk DM intake ($P < 0.001$), milk DM intake/kg live weight$^{0.75}$ ($W^{0.75}$) ($P < 0.001$), D_2O space ($P < 0.001$) and growth rate ($P < 0.05$). These responses will be examined in detail in a separate paper and, for the present paper, the twenty-four ewes and their lambs will be considered as a single group.

Effects of sex, birth weight and the range in initial ages

The spread of 10 d in the stage of lactation of the ewes when measurements commenced accounted for a small but significant proportion of the variance of ewe live weight ($P < 0.025$) but not that of total water turnover of the ewes. In the lambs, the spread of ages explained a small but significant proportion of the variance of total water turnover, AMI, milk intake and milk DM intake ($P < 0.005$ to $P < 0.001$).

The range in birth weights explained significant proportions of the variance in total water

Table 3. *Estimates of total water turnover, apparent milk intake (AMI), milk intake and milk dry matter (DM) intake in crossbred lambs**†

(Values in parentheses are pool size corrections (%))

Stage of lactation (d)	Total water turnover (ml/d)	Factor‡	AMI (ml/d)	Turnover due to milk water (ml/d)	Milk intake (ml/d)	Milk DM intake g/d	Milk DM intake g/d per kg live weight$^{0.75}$
9	1459 (+10·6)	0·909	1606	1501 (+6·2)	1816	315·4	78·2
23	1102 (+3·6)	0·918	1201	881 (+1·4)	1054	172·6	31·9
44	1421 (+3·2)	0·909	1565	717 (+1·1)	862	145·0	20·7
69	2567 (+5·3)	0·909	2827	598 (+3·1)	742	144·6	15·6
86	2791 (+3·7)	0·906	2082	471 (+1·3)	588	117·7	11·3
Standard error of difference	78·0	0·0043	87·4	56·6	67·8	11·95	2·52

* For details of experimental procedures, see pp. 376–377 and for calculations, see pp. 377–378.
† All values adjusted by covariance analysis for the initial differences in birth weight and stage of lactation.
‡ Total water turnover/AMI.

turnover ($P < 0{\cdot}001$), AMI ($P < 0{\cdot}001$), milk intake ($P < 0{\cdot}01$), milk DM intake ($P < 0{\cdot}05$) and live-weight gain ($P < 0{\cdot}05$).

Accordingly, both the spread of ages and birth weights were employed as covariates, as described previously. Sex had no effect on any variate and was not used as a covariate.

Changes in live weight and isotope-dilution space

Ewes' live weights and TOH spaces, shown in Table 1, differed in a significant linear fashion between periods ($P < 0{\cdot}001$). Over the five periods, TOH space in the ewes was 65–70% of live weight; this proportion did not change between periods. Total water turnover of the ewes was significantly lower ($P < 0{\cdot}05$) at day 23 of lactation, but by day 44 had increased to levels close to the original.

The increases in lamb live weight and D_2O space with age, shown in Table 2, were highly linear ($P < 0{\cdot}001$). The regression relations between D_2O space and live weight (Table 2) were highly significant at all stages ($P < 0{\cdot}001$), with D_2O space ranging from 72·1% (period 1) to 64·4% (period 5) of live weight. As a percentage of the initial D_2O space in each period, the daily rate of change of D_2O space fell from 5% in period 1 (equivalent to 215 ml/d) to 1·6% in period 5 (equivalent to 225 ml/d).

Water turnover and milk intake in the lambs

Total water turnover in the lambs (Table 3) fell significantly at day 23, but then rose significantly with each successive stage of lactation ($P < 0{\cdot}05$). As a consequence, both the linear and quadratic terms of the effect of stage of lactation were significant ($P < 0{\cdot}001$), although there were also significant deviations from this model ($P < 0{\cdot}001$).

The correction required to take account of changing pool size was large only for the first period (+10·6%), but was significantly different from zero at all stages ($P < 0{\cdot}001$). Over the entire period, total water turnover (T; ml/d) was related to lamb live weight (W, kg) by the expression

$$T = 102{\cdot}7\,(\text{SE }5{\cdot}34)\,\text{W} + 384{\cdot}1\,(\text{SE }84{\cdot}40) \quad (r^2\,0{\cdot}760, P < 0{\cdot}001).$$

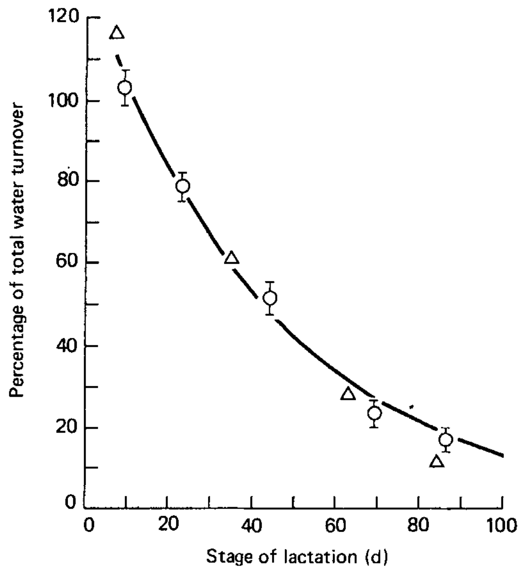

Fig. 1. Effect of stage of lactation (S; d) in grazing ewes on the percentage of the total water turnover (T%) in their lambs derived from the intake of water as milk. Points (○) are means with their standard errors represented by vertical bars.
Fitted relation is:

$$T\% = 129{\cdot}5\exp(-0{\cdot}0225S) \quad (r^2\ 0{\cdot}905,\ P < 0{\cdot}001).$$

(△) Values for single lamb from Wright & Wolff (1976).

By contrast, the water turnover attributable to milk fell in a curvilinear fashion throughout lactation (linear term $P < 0{\cdot}001$, quadratic term $P < 0{\cdot}001$, but deviations also $P < 0{\cdot}001$). Except at day 9, milk-water turnover was always significantly lower than total water turnover ($P < 0{\cdot}001$). The percentage of total water turnover (T%) derived from milk water fell from 103·7 at day 9 to 17·2 at day 86, as shown in Fig. 1, and was related to stage of lactation (S; d) by the expression

$$T\% = 129{\cdot}5\,(\text{SE}\ 3{\cdot}03)\exp(-0{\cdot}0225\,(\text{SE}\ 0{\cdot}000\,86)\,S) \quad (r^2\,0{\cdot}905, P < 0{\cdot}001).$$

AMI, calculated using eqns (1) and (2) and assuming no water was consumed in forms other than milk, closely followed the pattern of total water turnover, since the factor required to calculate one from the other was similar at all stages (see Table 3). Milk intake calculated from both isotopes (eqn (3)) and the milk-water contents, fell throughout lactation in a curvilinear fashion ($P < 0{\cdot}001$) similar to that observed with milk-water intake, as might be expected. Covariance regressions indicated that, for every 0·1 kg increase in birth weight, milk intake was increased by 16·1 ml/d ($P < 0{\cdot}01$) while for every extra day of age at the start of measurements, it was decreased 29·0 ml ($P < 0{\cdot}001$). At day 9 of lactation, milk intake was significantly higher than AMI ($P < 0{\cdot}001$), but thereafter it was always significantly lower ($P < 0{\cdot}001$).

Milk DM intakes, whether in g/d or g/d per kg $W^{0{\cdot}75}$, decreased throughout lactation in a curvilinear fashion ($P < 0{\cdot}001$), although there were still significant deviations ($P < 0{\cdot}001$) after fitting linear and quadratic terms.

Responses in lamb live-weight gain were also significantly curvilinear with time ($P < 0{\cdot}001$), falling significantly below the initial value (period 1) by periods 2 and 3, but then increasing to values close to the original.

DISCUSSION

The results presented extend those of Holleman *et al.* (1975) and indicate the usefulness, in field studies, of their double-isotope method for measuring milk intake.

Isotope-dilution spaces and total water turnovers

As a proportion of live weight, the TOH spaces of the ewes were consistent with earlier reports (Donnelly & Freer, 1974). Similarly, D_2O space as a proportion of lamb live weight was consistent with previous estimates based on D_2O (Wright & Wolff, 1976) or TOH (Searle, 1970; Donnelly & Freer, 1974; Dove & Freer, 1979). The use of body-composition prediction equations originally based on TOH space (Donnelly & Freer, 1974) therefore seems justified.

The total water turnovers in the lambs (Table 3) are in general agreement with earlier estimates (Dove & Freer, 1979). The sharp and significant fall in total turnover at the second measurement period is similar to that observed in the ewes, and in the milk intake and live-weight gain of the lambs (Tables 3, 4) and presumably reflects the low availability of pasture at this stage of lactation. The significant corrections, in all periods, for the effect of increasing pool size on estimates of water turnover, confirm the earlier study based on TOH turnover (Dove & Freer, 1979), although the size of the correction is generally smaller.

Total water turnover was converted to AMI using eqn (2), which discounts the metabolic water contribution of the milk solids for the tissue fat and protein depositions (Pettigrew *et al.* 1987). The net value of the factor required to convert total water turnover to AMI was close to 0·91. While this is lower than the factor of 0·95 used by Dove & Freer (1979), its relative similarity confirms the suggestion that milk intakes based on the single-isotope method are not markedly sensitive to changes in the rate of tissue protein and fat depositions (Pettigrew *et al.* 1987). However, the more important point is that, beyond the first two measurement periods, the single-isotope method gives an inaccurate estimate of milk intake, as suggested earlier (Dove & Freer, 1979).

Milk intake estimates based on the double-isotope method

As the results in Table 3 and Fig. 1 indicate, the use of the double-isotope method revealed that, as lactation proceeded, and pasture intake by the lambs increased, there was a marked decline in the percentage of total water turnover coming from milk. This is similar to the result found by Dove *et al.* (1987) with the tammar wallaby (*Macropus eugenii*). However, the only similar findings for sheep are those of Wright & Wolff (1976), and then only for one lamb in the field. The percentage of water turnover coming from milk, in that animal, is also shown in Fig. 1. The agreement with the present results is good, but it must be remembered that the relation between milk-water turnover and total water turnover will vary according to grazing conditions, since these, in part, determine the balance between milk and pasture intake in the lamb (Langlands, 1972, 1973; Treacher, 1983).

Live-weight gains in periods 1 and 2 averaged 175 and 161 g/d per litre milk consumed (Tables 3, 4). These values, and those implied by the equivalent regression equations in Table 4, lie within the expected range of 140–240 g/d per litre milk (Treacher, 1983). Similarly, the food conversion efficiencies in periods 1 and 2 can be calculated, from Tables 3 and 4, to be 1·01 and 1·06 g milk DM/g live-weight gain respectively, close to previously observed values in preruminant lambs (Hodge, 1974; Dove & Freer, 1979).

The results reviewed by the Agricultural Research Council (1980) suggest that, in early life, lambs could be expected to consume about 80 g milk DM/d per kg $W^{0.75}$. The mean intake in period 1 of the present study is very close to this, and close to the daily intake of

Table 4. *Lamb live-weight gains and the regression parameters relating these to the estimates of milk intake*†

Stage of lactation (d)	Live-wt gain‡ (g/d)	Regression parameters relating live-wt gain to milk intake: Slope Mean	Slope SE	Intercept Mean	Intercept SE	r^2
9	317	0·129	0·0264	81·8	48·70	0·533***
23	170	0·151	0·0263	11·2	28·71	0·600***
44	219	0·086	0·0909	145·2	81·09	0·039
69	315	0·045	0·0690	280·9	54·22	0·019
86	347	0·127	0·1459	272·7	89·73	0·033
Standard error of difference	26·0					

*** $P < 0·001$.
† For details of experimental procedures, see pp. 376–377 and for calculations, see pp. 377–378.
‡ Values adjusted by covariance for initial differences in birth weight and stage of lactation.

76·6 g milk DM/kg $W^{0·75}$ which can be calculated from the values of Hodge (1974) and that of 79·0 g milk DM/kg $W^{0·75}$ which can be calculated from the prediction of Graham *et al.* (1976) (their eqn (1)). After period 1 in the present study, milk intake/kg $W^{0·75}$ declined in a curvilinear fashion. The results of Hodge (1974) provide strong evidence that a hyperbolic model is the appropriate description of this response. In that study the voluntary intake of milk energy and milk DM (I) was linearly related to metabolic live weight (kg $W^{0·75}$), i.e. $I = a + bW^{0·75}$. It therefore follows that milk DM intake/kg $W^{0·75}$ must be related to $W^{0·75}$ in a hyperbolic fashion, i.e.

$$(I/W^{0·75}) = (a/W^{0·75}) + b.$$

Many studies, including the present one, have shown that in the young lamb, weight (W) is essentially a linear function of age (A) (Hodge, 1974; Dove & Freer, 1979), i.e.

$$W = c + dA.$$

Hence, the relation between milk DM intake/kg $W^{0·75}$ (I') and age (A) would be expected to be a hyperbola of the form:

$$I' = (I/W^{0·75}) = b + (a/(c + dA)^{0·75}).$$

Under field conditions, the pattern of voluntary intake/kg $W^{0·75}$ will also be influenced by the level and timing of the onset of pasture consumption (Langlands, 1972). Dove & Freer (1979) used the single-isotope method to measure milk intake in single and twin lambs from well-nourished ewes and presented evidence that intakes were accurately estimated up to day 28 of lactation. These findings have been re-calculated in terms of milk DM intake/kg $W^{0·75}$ and are shown in Fig. 2(*a*), together with the hyperbolic functions, fitted separately for single and twin lambs. In single lambs, milk DM intake/kg $W^{0·75}$ (I'; g) was related to age (A; d) by the expression:

$$I' = 22·53\,(\text{SE}\,9·951) + (146·35\,(\text{SE}\,39·310)/(1 + 0·2711\,(\text{SE}\,0·225\,16)\,A)^{0·75}),$$
$$(r^2\,0·865, P < 0·001;\ \text{residual standard deviation } 6·20).$$

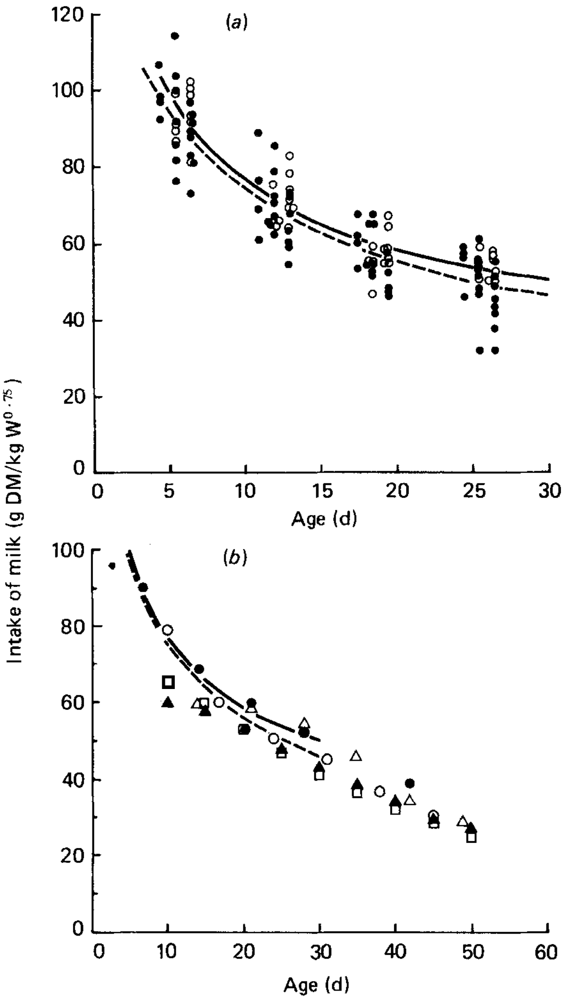

Fig. 2. Relations describing the change, with age of lamb (A; d), in milk dry matter (DM) intake (g) per kg metabolic live weight ($W^{0·75}$) (I').

(a) Recalculated from the values of Dove & Freer (1979) for single lambs (○), for which the fitted curve (——) is

$$I' = 22·53 + (146·35/(1 + 0·2771A)^{0·75}),$$

and for twin lambs (●), for which the fitted curve (-----) is

$$I' = 12·98 + (131·92/(1 + 0·1773A)^{0·75}).$$

For standard errors of regression parameters, see pp. 383 and 385.

(b) Recalculated values from milk intakes originally based on either test-weighing or ewe milking. (○), Recalculated from Langlands (1973); values are means of all treatments using single lambs. (●), Recalculated from Geenty (1979), 1973 experiment; values are means for all breeds and crosses using single lambs. (△), Recalculated from Maxwell *et al.* (1979) for single lambs. (▲, □), Recalculated from the equations given by Gibb & Treacher (1982) for single and twin lambs respectively. The fitted curves from Fig. 2(a) are included for comparison ((——), singles; (– – –), twins).

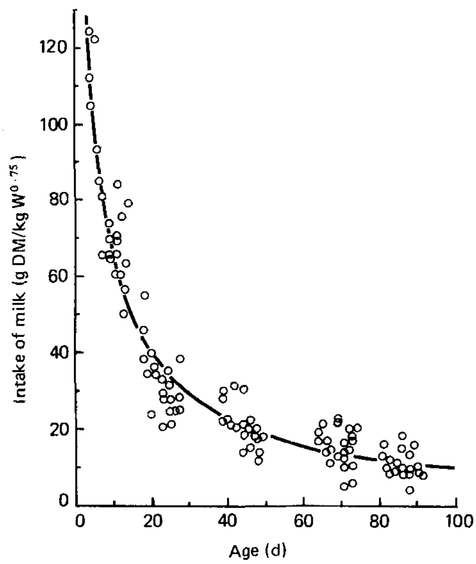

Fig. 3. Change in the daily intake (g) of milk dry matter (DM) per kg metabolic live weight (W$^{0.75}$) (I') with age (A; d), in the single lambs in the present study. The fitted curve is:

$$I' = -4.49+(303.11/(1+0.5956A)^{0.75}), \quad (r^2\,0.933, P < 0.001).$$

For standard errors of regression parameters, see p. 385.

The equivalent expression for twin lambs was:

$$I' = 12.98\,(\text{SE}\,10.968)+(131.92\,(\text{SE}\,13.525)/(1+0.1773\,(\text{SE}\,0.10258)\,A)^{0.75}),$$
$$(r^2\,0.817, P < 0.001;\ \text{residual standard deviation } 7.71).$$

From the equation for single lambs, predicted daily intakes at 5, 10 and 25 d of age are 99·5, 77·3 and 54·0 g milk DM/kg W$^{0.75}$ respectively. Equivalent values for twins are 94·9, 74·4 and 50·0 g milk DM/kg W$^{0.75}$. This rapidly decreasing intake is in marked contrast to the constant value of 80 g milk DM/kg W$^{0.75}$ suggested by the Agricultural Research Council (1980) for lambs in their first month of life.

Previous estimates of milk intake, based on test-weighing or ewe milking also conform well to the hyperbolic form, as shown in Fig. 2(*b*), which presents re-calculated values for lambs from well-nourished ewes, over a wide range of breeds and environments (Langlands, 1973; Geenty, 1979; Maxwell *et al.* 1979; Gibb & Treacher, 1982). The previous equations from the findings of Dove & Freer (1979) are superimposed for comparison and it is clear that there is good agreement over the first 30 d of lactation.

In the present study, the severe grazing pressure led to a sharper drop in milk DM intake/kg W$^{0.75}$ than in the previous studies, but the pattern of intake still conformed extremely well to the hyperbolic model, as show in Fig. 3. The results were described by the equation:

$$I' = -4.49\,(\text{SE}\,1.718)+(303.11\,(\text{SE}\,57.117)/(1+0.5956\,(\text{SE}\,0.19989)\,A)^{0.75}),$$
$$(r^2\,0.933, P < 0.001;\ \text{residual standard deviation } 6.84).$$

In young lambs aged 5 d, this equation predicts an intake of 103·1 g milk DM/kg W$^{0.75}$, close to that predicted from the equation for the single lambs of Dove & Freer (1979) (see p. 383). In fact, for all lambs of less than 7 d of age, predicted intakes are again greater than the constant level of 80 g milk DM/kg $W^{0.75}$ suggested by the Agricultural Research Council (1980) for lambs up to 1 month of age.

Comparison of the double-marker and other methods

A major concern in evaluating any method for estimating milk intake is the extent to which the method itself causes disturbance to the animals and perturbs either normal sucking behaviour, milk ejection or, in the extreme, interferes with milk synthesis over longer periods.

As can be seen in Fig. 2(*b*), apparently similar estimates of milk intake have been obtained by test-weighing or ewe milking (Langlands, 1973; Geenty, 1979; Maxwell *et al.* 1979; Gibb & Treacher, 1982) but, as mentioned, a potential major disadvantage of these methods is that the accuracy can be affected by the disturbance caused during the measurement period. Moreover, the estimate, obtained over a period of 4–6 h, is then scaled up to provide an estimate of daily intake or production.

Isotope-dilution methods, including the present one, avoid these potential disadvantages, as discussed earlier. Nevertheless, the extent to which some of the procedures of the double-marker method might perturb lactation must also be evaluated. The issue of greatest concern here is the possible effects, on the ewe's milk synthesis, of the denial of access to pasture overnight before TOH injection. In the present study, this was done to reduce the effects of gut contents on the accuracy of estimating TOH space and, ultimately, body composition. However, the overnight starvation is probably unnecessary on two grounds. (1) There is good evidence that accurate estimates of the body-water space and body composition can be obtained without previous starvation, provided ewes are denied access to food and water during the 6 h equilibration period (Foot & Greenhalgh, 1970). (2) Although an accurate assessment of TOH space, and thus body composition, is a useful adjunct to this method, all that is actually required for eqn (3) is a knowledge of the initial specific activity of milk water (α_0) and the fractional water turnover rate of the ewe (k_α). These can validly be obtained without starvation. Accordingly, later studies with this method, involving a range of species, have not used overnight starvation (Cork & Dove, 1986; Dove *et al.* 1987; Holleman *et al.* 1988; Carl & Robbins, 1988).

There is also evidence, based on lamb live-weight gains, that the overnight starvation did not affect milk supply over the measurement periods. The live weights (Table 2) and live-weight gains (Table 4), which closely reflected milk intake in periods 1 and 2 (Table 4) indicate that, in undisturbed animals between periods 1 and 2, or between periods 2 and 3, the live-weight gains were very close to the averages of the gains measured during each of the two periods concerned.

Disturbance can be further reduced in the double-isotope method by not re-injecting the offspring, 4–5 d later, to obtain the estimate of the increase in pool size. In practice, little accuracy is lost if the increase is estimated from live weight by assuming that D_2O space is the same proportion of live weight as measured 4–5 d earlier (Dove & Freer, 1979; Pettigrew *et al.* 1987). Improvements in the sensitivity of D_2O analysis, as described by Byers (1979) and in the present study, plus oral administration of D_2O (Holleman *et al.* 1988; Carl & Robbins, 1988) also allow smaller doses of D_2O (e.g. 0·7 g/kg live weight) and further reductions in disturbance.

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