New Isotope Methods for Estimating Milk Intake and Yield

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ABSTRACT

Two isotope tracer methods, based on the transfer of isotopic tracer via milk from a female to offspring, were developed for estimating milk intake of nursing offspring. One method depended upon the transfer of tritiated water from female to offspring in milk and simultaneous determination of water turnover of the offspring by deuterium oxide. The second method was based on the transfer of radiocesium from female to offspring in milk and required the use of whole body counting techniques for evaluating the radiocesium body burden of the offspring. Experiments evaluating the two methods were with hand-reared Holstein calves given fixed milk intake. The methods gave valid estimates of milk intake; estimates were within \pm 2.5\% of the actual intakes. The application and limitations of these methods for estimating milk intake and yield of other species are discussed.

INTRODUCTION

Knowledge of milk yield of lactating females and intake by nursing offspring frequently is required in ecological, nutritional, and husbandry studies. Several recent reviews stress the importance of milk in sustaining life processes and point to variations between species in yield and composition of milk and its constituents (2, 8, 9, 10, 11, 15, 16). Estimation of milk intake and yield has been attempted by measuring prenursing/postnursing weight changes of the offspring (5) and by exhaustive milking of the mother (4, 12, 21). These conventional methods require frequent and often prolonged separation of the female and offspring which may preclude their application to semidomesticated and nontractable animals. Macfarlane and co-workers (13) recently proposed a method based on estimating body water turnover of the nursing offspring with tritiated water (HTO) and equating body water turnover to milk intake. Although the HTO method minimized disturbance of the experimental animals, it required that the only source of exogenous water to the nursing offspring was that contained in milk. This requirement restricted the application of the HTO method to nursing animals before they commence to forage for themselves. The HTO method has been applied to sheep (13), cattle (23), reindeer and caribou (14), and baboons (3).

Since the HTO method may overestimate milk intake from as early as 3 wk of age in young ruminants which have access to forage, two new methods for the estimation of milk intake were developed. The two new methods, which are applicable to the entire lactation period, were based on injection of a tracer into the lactating female and subsequent monitoring of tracer in milk and nursing offspring. New methods were evaluated in hand-reared Holstein calves. Preliminary accounts of both methods have been reported (7, 22).

EXPERIMENTAL ANIMALS

Four Holstein calves were used to evaluate methods of measuring milk intake. Calves were maintained on milk intakes of approximately 4.25, 5.5, 7.0, and 8.5 liters/day for 10 wk (4 wk preconditioning and 6 wk of experiments). Milk replacer (Calf Milk Replacer, Land O'Lakes, Inc., Minneapolis) was given twice daily while water and starter (Ralston Purina Cattle Starter No. 1) were offered ad libitum. Calves were weighed twice weekly and immediately prior to starting the experiments. Milk replacer, water, and starter intake was measured twice daily.

MATERIALS AND METHODS

A model illustrating the transfer of tracer from a lactating female to the nursing offspring is in Fig. 1. Rate of tracer intake by the nursing offspring equals rate of milk intake (m) times

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FIG. 1. A model illustrating the method for estimating milk intake by a nursing offspring based on the injection of tracer into the lactating female. Arrows indicate the transfer of tracer. An explanation of the symbols appears in the text.

The concentration of tracer in the milk ($\alpha$), where the tracer concentration in milk is a function of time (equation 1).

$$\alpha = \alpha_0 e^{-k_at} \quad [1]$$

($\alpha_0$ = concentration of tracer in milk at zero time, $k_a$ = fractional loss of tracer per unit time from the lactating female, $t$ = time).

The quantity of tracer in the nursing offspring ($Q$) is also a function of time. The rate of tracer loss from the offspring is the product of $Q$ and the fractional loss of $Q$ per unit time ($k_b$). The differential equation which describes the change in $Q$ with time may be written as the difference between tracer intake and tracer loss, i.e.,

$$\frac{dQ}{dt} = \text{intake} - \text{loss} = m\alpha - k_bQ \quad [2]$$

then substituting equation 1 into the above equation,

$$\frac{dQ}{dt} = m\alpha_0 e^{-k_at} - k_bQ \quad [3]$$

The solution to this equation is

$$Q = \frac{m\alpha_0 (e^{-k_at} - e^{-k_bt})}{(k_b - k_a)} \quad [4]$$

or rearranging,

$$m = Q(k_b - k_a)/[\alpha_0 (e^{-k_at} - e^{-k_bt})] \quad [5]$$

If $k_b$ equals $k_a$, then

$$m = Q e^{k_at}/(\alpha_0 t) \quad [6]$$

Hence, by incorporating the appropriate values into equations 5 or 6, the rate of milk intake ($m$) may be calculated from a single measurement of $Q$ at a given time $t$ following the tracer injection into the lactating female. However, a more precise procedure is to simulate $Q$ with equation 4 while varying the rate of milk intake until the simulation approaches a best fit of the data. Both procedures were used in our study.

HTO and cesium-134 (Cs) were added to the milk replacer given the Holstein calves to produce tracer concentrations similar to those expected for females that had received a single intravenous injection of tracers. The build-up of radiotracers and the kinetics of the tracers in the calves were determined, milk replacer intakes were calculated from these data, and results were compared with actual milk intakes to establish the reliability of methods.

HTO/D$_2$O Method

Simultaneous to the initial addition of HTO to the milk replacer, a single oral dose of deuterium oxide ($D_2O$) (99.8 atoms %, 3 ml/kg body weight) was given to each calf. $D_2O$ was used to establish the kinetics of body water in the calf. At periodic intervals, blood samples from each calf were obtained by venipuncture. Subsamples of milk were also taken for determination of HTO concentration. Blood samples were centrifuged, and plasma was separated and stored at -3 C. Water was separated from plasma and milk replacer by vacuum sublimation. Samples (1 ml) of the sublimated water were prepared for radioassay of tritium in 5 ml of scintillation solution (9/4 triton X-100/toluene, 5 g/liter PPO, and .1 g/liter POPOP) and counted in a Nuclear Chicago, Mark I, Liquid Scintillation System. $D_2O$ concentration in plasma water was determined in a double beam Perkin-Elmer 621 Infrared Spectrophotometer (18, 19).

The HTO concentration in the milk replacer was described by equation 1. Similarly, the retention of $D_2O$ in the calf also was given by a single exponential equation:

$$\beta = \beta_0 e^{-k_bt} \quad [7]$$

($\beta$ = concentration of $D_2O$ in plasma water at time $t$ after the initial intake of $D_2O$ in the milk, $\beta_0$ = the concentration of $D_2O$ in the calf at zero time, $k_b$ = fractional loss of $D_2O$ per unit time from the calf).

Total body water (TBW) for the calf was calculated from the $D_2O$ dose ($D_{inj}$) and the equilibrium concentration of $D_2O$ ($\beta_0$), i.e.,

$$\text{TBW} = \frac{D_{inj}}{\beta_0} \quad [8]$$

The HTO body burden of the calf ($Q$) at a particular time was calculated as the product of
the HTO concentration in plasma water at that time and the TBW, Q was used in computing the rate of water intake via milk replacer for the calf (see equation 5). The rate of milk intake was calculated from estimates of water intake via milk and percent solids in milk.

Water transport rate \( w \) represents the flow of water through the body water pool and for the calf was calculated from the equation,

\[
w = k_b \cdot (TBW) \tag{9}
\]

The total preformed water intake for the calf was estimated by subtracting metabolic water from the water transport rate where metabolic water was water produced by catabolism of milk solids and feed and was estimated from the chemical composition of the diet by the equation of Van Es (20). It was assumed that 30% of the milk solids and food were diverted to body weight gain of the calf, and of the 70% metabolized, half was assumed to be protein and half fat. All carbohydrate was assumed to be oxidized to water. Intake of water from nonmilk sources was calculated as the difference, preformed water intake minus water intake via milk.

Cs Method

Cs (cesium-134 chloride, New England Nuclear) was added to the milk replacer simultaneously with the HTO. Milk replacer samples were obtained periodically and radioassayed for Cs

![FIG. 2. HTO concentration in water obtained from milk and a calf following HTO spiking of the milk. Also indicated is the change in D₂O concentration in plasma water of the calf following D₂O ingestion in a single milk meal.](image)

**TABLE 1. Water kinetics in hand-reared Holstein calves estimated with D₂O.**

<table>
<thead>
<tr>
<th>Calf no.</th>
<th>Age (days)</th>
<th>Body weight (kg)</th>
<th>( k_b^* ) (day⁻¹)</th>
<th>TBW** (liters)</th>
<th>Water transport rate (liters/day)</th>
<th>(ml/day/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation Trial 1</td>
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<tr>
<td>1</td>
<td>51</td>
<td>73.9</td>
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<td>54.2</td>
<td>7.37</td>
<td>99.7</td>
</tr>
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<td>2</td>
<td>49</td>
<td>83.9</td>
<td>.139</td>
<td>57.0</td>
<td>7.90</td>
<td>94.2</td>
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<td>44</td>
<td>72.6</td>
<td>.182</td>
<td>51.3</td>
<td>9.36</td>
<td>128.9</td>
</tr>
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<td>4</td>
<td>54</td>
<td>72.6</td>
<td>.139</td>
<td>50.3</td>
<td>10.56</td>
<td>145.5</td>
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<tr>
<td>Evaluation Trial 2</td>
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<td>1</td>
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<td>76.1</td>
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<tr>
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<td>101.2</td>
<td>.147</td>
<td>76.1</td>
<td>11.01</td>
<td>108.8</td>
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</table>

*\( k_b \), fraction of TBW replaced per day.

**TBW, total body water.
by gamma spectroscopy (7). The Cs body burden of the calves was monitored with a whole body counting technique (6, 7). When Cs build-up in the calf was documented adequately for kinetic analysis (10 to 15 days), Cs (3 μCi/kg body weight, intravenous) was given to the calf, and the body burden of the calf was monitored for an additional 10 to 15 days.

The Cs body burden of the calf depended upon the accumulation and loss of tracer by the calf. These data could then be converted to milk intake from the concentration of Cs in milk. In large animals such as cattle, a two compartment model is necessary to describe adequately the kinetics of Cs. Such a system does not lend itself readily to a direct calculation of milk intake. Therefore, estimates of milk intake were obtained by simulation on an analog computer (Electronics Association Inc., TR-20).

RESULTS

The concentration of HTO in plasma water increased to a peak at 4 to 6 days and declined from day 7 (Fig. 2). The concentration of D₂O in plasma water declined exponentially (Fig. 2). A summary of the kinetics of water metabolism in the Holstein calves is shown in Table 1. Mean TBW calculated from D₂O dilution was 74.2 ± .9% body weight and did not change significantly between trials. The biological half-time for TBW was between 3.3 and 6.1 days. The biological half-time of HTO in milk replacer was 3.8 to 3.9 days (Fig. 2).

A sample calculation for the milk intake of calf 3 (Fig. 2) can be made from the following parameters for this experiment,

\[ \alpha_o = 37.6 \text{ μCi/liter; } k_a = .178 \text{ day}^{-1}, \quad k_b = .182 \text{ day}^{-1}; \quad \text{TBW} = 51.3 \text{ liters; } Q = \text{TBW (} \beta_t \text{)} \]

Thus, from equation (5) milk water intake equals

\[ m = \text{TBW (} \beta_t \text{)} (k_b - k_a)/[\alpha_o(e^{-k_a t} - e^{-k_b t})] \]

for \( t = 5.2 \text{ days and } \beta = 9.14 \text{ μCi/liter, then} \]

\[ m = 6.11 \text{ liters/day} \]

and for \( t = 6.2 \text{ days and } \beta = 8.94 \text{ μCi/liter then} \]

\[ m = 6.01 \text{ liters/day} \]

Milk replacer intake was calculated as milk replacer water intake divided by percent water (vol/vol) in milk (i.e., m/.905). Thus, in the two examples, milk replacer intake was 6.75 and 6.64 liter/day. When calculated from HTO concentrations between 0 and 2 days, estimates of milk intake based on this method were invariably higher than the actual milk intakes. After this time, results were in close agreement until day 10 when experiments were terminated. Mean estimates of milk intake based on 4 to 6 estimates of HTO concentration in plasma water are in Table 2.

An example of the line of best fit generated from the analog computer is shown in Fig. 2 for the build-up of HTO in the body water of calf 3 (age 44 days, Table 1). The dashed line corresponds to an intake of 6.83 liters/day. Estimates of milk replacer intake generated by the analog computer technique using the best fit to the HTO/D₂O data are in Table 2.

Data for calf 3 (age 44 days) with Cs as the tracer are in Fig. 3. The line of best fit to the Cs concentration in milk is indicated. The theoretical curve for the Cs body burden of the calf corresponding to a milk intake of 6.80 liters/day is shown for comparison with the measured cesium body burden. Also shown are the kinetics of Cs following a single injection of Cs at day 15. A summary of milk replacer intakes of the Holstein calves by the Cs method is in Table 2.
### TABLE 2. Pertinent data related to the HTO/D₂O and Cs method evaluation experiments with Holstein calves.

<table>
<thead>
<tr>
<th>Calf no.</th>
<th>Food intake (kg/day)</th>
<th>&quot;Preformed&quot; water intake (liters/day)</th>
<th>Milk intake (liters/day)</th>
<th>Calculated milk intake (liters/day)</th>
<th>HTO/D₂O method</th>
<th>Cs method</th>
<th>Water intake via milk (%)</th>
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<td></td>
<td>Equation simulation analysis</td>
<td>Computer simulation analysis</td>
<td>Measured HTO/D₂O method</td>
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<td>5</td>
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</tr>
<tr>
<td>1</td>
<td>1.38</td>
<td>7.33</td>
<td>4.25</td>
<td>4.18 (.20)</td>
<td>4.08</td>
<td>4.15</td>
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<td>1.95</td>
<td>7.81</td>
<td>5.62</td>
<td>5.43 (.11)</td>
<td>5.48</td>
<td>5.33</td>
<td>65.2</td>
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<tr>
<td>3</td>
<td>1.08</td>
<td>8.29</td>
<td>6.91</td>
<td>6.74 (.18)</td>
<td>6.83</td>
<td>6.80</td>
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<tr>
<td>4</td>
<td>.4</td>
<td>9.77</td>
<td>8.70</td>
<td>8.88 (.21)</td>
<td>8.88</td>
<td>8.75</td>
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<td>Trial 2</td>
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<td>1</td>
<td>2.37</td>
<td>9.92</td>
<td>4.37</td>
<td>4.65 (.05)</td>
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<td>2.43</td>
<td>9.67</td>
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<td>9.46 (.13)</td>
<td>8.63</td>
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<td>72.4</td>
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</table>

*a* Water obtained from milk as a percentage of total water intake.

*b* "Preformed water" = drinking water, food water plus milk water.

*c* Milk solids = 13.0% by weight.

*d* Values in parentheses are standard errors for 4 to 6 estimates.

*e* Not determined.
Milk replacer intake estimates were made simultaneously by the Cs and HTO/D₂O methods in trial 1 (Table 2). Methods Cs and HTO/D₂O agreed. From the computer simulation methods of analysis, the largest and smallest deviations were 130 and 30 ml/day for milk replacer intakes of between 4 and 8.9 liters/day. Table 2 also shows the mean daily intake of food, preformed water, and milk replacer. Estimates of milk intake by the HTO/D₂O or Cs methods were similar to actual milk replacer intake. For further evaluation of the HTO/D₂O method, the water intake via milk replacer expressed as a percent of the water transport rate was compared with measured values of water intake via milk replacer as a percent of preformed water intake (Table 2). The largest difference between these estimates was 5% indicating that the HTO/D₂O isotopes were giving close estimates of the contribution of water intake via milk replacer to the body water transport rate.

Correlation was large between milk replacer intake measured by the Cs method (Cs, liter/day) and the HTO/D₂O method (T, liter/day):

\[ Cs = 0.131 + 0.970 T \quad (r = .999, n = 4, \ P < .001) \]  \[10\]

The intercept was not significantly different from zero (P<.05). The mean slope for the relationship was .993 ± .009. For the eight observations on the HTO/D₂O method (T, liter/day), correlation was high with actual milk replacer intake (m, liter/day).

\[ T = 0.37 + 1.046 m \quad (r = .998, n = 8, \ P < .001) \]  \[11\]

The intercept was not significantly different from zero, the slope of the line was not significantly different from unity (P<.001), and the mean ratio of calculated intake to measured intake was .984 ± .008.

**DISCUSSION**

Based on the observations that the HTO/D₂O and Cs methods were highly correlated (equation 10) and that the HTO/D₂O method was highly correlated with static estimates of milk replacer intake (equation 11), these are valid methods for estimating milk intake. In the first trial with Holstein calves, the largest difference between estimated and actual milk replacer intake was less than 5% of the milk intake. Other studies (13, 14) with bottle reared lambs, in which milk was the only source of water, also have shown close agreement between milk intake estimated from HTO turnover and actual milk intake: the mean difference between estimated and actual intake was approximately 2.5% compared with 2.4% in this study. This agrees especially considering that two isotopes were used to estimate milk intake by the HTO/D₂O technique. Probably the main reason for the close agreement by this technique is that water transport estimated by D₂O dilution (W, liters/day), see also equation 9, accurately measures preformed water intake (i.e., water in drinking water, food, and milk) (Wₚf, liter/day).

\[ W = 0.26 + 1.086 Wₚf \quad (r = .940, n = 8, \ P < .001) \]  \[12\]

A critical consideration in the application of this technique to routine studies is the time taken to complete an experiment. A minimum of 2 days was required before estimates of milk intake could be made accurate by equation 5. From a theoretical consideration (17) and based on subjective assessment of curve fitting with the analog computer, the experiment also should last until the concentration of isotope in the offspring is at or just beyond its maximum value. The time \( t' \) required for nursing offspring to reach maximum tracer body burden depends upon the tracer concentration in milk as a function of time and on the elimination kinetics in vivo of the tracer in the nursing offspring and is given by equation 13 (17).

\[ t' = \frac{\ln(k_b/k_a)}{k_b - k_a} \]  \[13\]

If \( k_b \) equals \( k_a \), then

\[ t' = \frac{1}{k_a} \]  \[14\]

The turnover of body water was relatively rapid in Holstein calves, and as a result the maximum body burden of tracer in the calf was reached quickly, e.g., approximately the 5th day for calf 3 (Fig. 2). Generally, a reliable estimate of milk intake by the HTO/D₂O method could be made in 5 to 8 days after injection of radioisotope. An early determination is desirable as the tracer methods assume a constant milk intake rate during the experimental period. This may not be a valid assumption if the experimental period is long. The simultaneous use of D₂O to
evaluate body water kinetics of the calf minimizes the duration of the experimental period.

The turnover of cesium was slower than that of body water. Therefore, the time required to reach maximum Cs body burden of the calf was also longer (8 to 10 days vs. 5 days, Fig. 3). This extended period as well as the time required to evaluate the Cs kinetics of the calf resulted in an experimental period of approximately 15 to 25 days. Although the Cs method has the disadvantage of requiring a long experimental period, blood samples are not required. Thus, the method may be particularly advantageous in small mammals where blood sampling is difficult or impossible. Also, the Cs body burden is determined directly whereas in the HTO/D2O method tracer body burden is calculated from specific HTO radioactivity and a pool size (TBW).

The primary advantage of the Macfarlane HTO method (13) is its simplicity and minimal disturbance of the animals. However, these simplifications may lead to unreliable estimates of milk intake because the HTO method equates body water transport of the nursing offspring to its milk intake. Body water transport is greater than water intake by the amount of water produced by oxidative processes. The Cs and HTO/D2O methods do not require an estimate of the metabolic water production since milk intake is estimated from the transfer of tracer (Cs or HTO) from the lactating female to the nursing offspring. More important, Macfarlane's HTO method can only be applied when milk is the only source of preformed water for the nursing offspring; i.e., the method cannot distinguish between preformed water obtained from milk and water obtained from nonmilk sources (e.g., forage, free water, ice, snow). The HTO/D2O method may be used to estimate the percentage of the total water intake obtained from milk to nonmilk sources but cannot be used, without modification, for species in which the female ingests a high proportion of the urine and feces of the offspring and thus recycles tracer (1).

These new tracer methods may be used to estimate milk intake and yield in a wide variety of mammalian species. Animal size should not be a major problem except for extremely small mammals in which it may be difficult to obtain milk and blood samples. Although the methods actually measure milk intake of nursing offspring, milk production of the lactating female can also be determined provided consideration is given to certain experimental conditions. In multiple suckling, the methods can be used to determine milk intake by individual members and, therefore, the total yield by the mother. The methods also have been used to estimate the extent of cross-nursing between reindeer calves and reindeer cows that were not their mothers (7). If cross-nursing is suspected, then separation of female/offspring pairs may be necessary for reliable estimates of milk intake and yield.

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ESTIMATING MILK INTAKE AND YIELD