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Lithium salts as a marker of intake of supplements by cattle

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Abstract. In a series of experiments the appearance of lithium cation in plasma following ingestion or intravenous administration of lithium salts was measured in order to examine the suitability of lithium as a marker of supplement intake by cattle. In experiment 1, cattle were offered low quality hay *ad libitum* and 0.15, 0.4, 1.0 and 3.0 kg cottonseed meal (CSM) supplement per day. Following ingestion of lithium-labelled CSM, the lithium concentration in plasma reached a maximum after about 24 h and the subsequent decrease in concentration appeared to follow a single exponential relationship. The rate constant of this disappearance of lithium from plasma was greater ($P<0.05$) when 3.0 kg than when lesser amounts of lithium-labelled CSM were consumed. Plasma lithium concentrations 24 and 32 h after ingestion of lithium-labelled CSM were both linearly related to the amount of supplement consumed ($r = 0.86$ and 0.87, respectively, $n = 32$, $P<0.001$), indicating that the plasma lithium concentrations could be used to measure intakes of supplement by individual animals. In experiment 2, following intravenous administration of lithium chloride the disappearance of lithium from plasma over 96 h appeared to be best described by 3 exponential compartments. In experiment 3, cattle were offered molasses-based or loose mineral mix (LMM) supplement, or water, labelled with lithium and the rate constant of disappearance of lithium from plasma between 32 and 96 h was determined. The pattern of the decrease in plasma lithium concentration was similar to that observed in experiment 1. In experiment 4, the variation about the estimates of supplement intake was measured in heifers ($n = 24$) offered hay and water *ad libitum* and in addition a meal of 60 g of lithium-labelled molasses–urea supplement; between 20 and 28 h after ingestion of the lithium-labelled supplement the coefficient of variation (CV) among animals in plasma lithium concentration was about 15%. The likely errors associated with the use of lithium salts to measure intake of supplement are discussed. These experiments confirm that lithium salts can be used as a marker to estimate intake of supplement by individual animals.

Introduction

Supplements are often provided to grazing ruminants to supply nutrients that are likely to be insufficient in pasture, but it is often difficult to achieve intended intakes of supplement by all individual animals in a herd or flock. Many animals may not consume any supplement, while among those that do consume there may be high variability in supplement intake (Arnold and Maller 1974; Nolan *et al.* 1975; Murray *et al.* 1978; Eggington *et al.* 1990; Bowman and Sowell 1997). Variability in intake of supplements is likely to be particularly high when mineral and non-protein nitrogen supplements are provided *ad libitum* as loose mineral mix or solidified feed blocks to grazing animals (Dixon and Petherick 1996; Dixon and Smith 2000; Dixon *et al.* 2000, 2001). An understanding of the factors which influence supplement intake by individual animals in a herd or flock is clearly necessary to improve management strategies to achieve target intakes of supplement by all animals and to optimise cost-effectiveness of supplements. However, to investigate such factors suitable techniques are needed to measure intake of supplements by individual animals in groups while grazing.

The roles and efficacy of markers to measure intake of pasture, pasture components, pasture species and supplements have been reviewed (Kotb and Luckey 1972; Langlands 1975; Holechek *et al.* 1982; Dove and Mayes 1991). Such marker techniques generally depend on the principle of adding a substance to the diet or the digesta, or identifying an unusual component of the diet, a constant proportion of which is excreted by the animal. In particular, marker techniques to measure intake of supplements by grazing ruminants have been recently reviewed by

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McLennan (1999). Tritiated water and deuterium are particularly suitable markers of supplements (Nolan *et al.* 1975; Rocks *et al.* 1982; Dove 1984). However, the use of a radioactive substance such as tritiated water is severely constrained by concerns for human health and the environment, while deuterium is costly for large ruminants or large numbers of animals. Digesta markers that are quantitatively excreted in the faeces, such as chromic oxide (Foot *et al.* 1973; Lobato *et al.* 1980; Ducker *et al.* 1981) and ytterbium (Curtis *et al.* 1994), have been used to measure supplement intake. However, since these markers require either complete collection of faeces for several days (e.g. by collection bags attached to the animals) or important assumptions of forage intake and digestibility, their usefulness is limited. Consequently, the cation of lithium has been investigated as a marker since it has many suitable characteristics. Lithium occurs naturally in the environment, it is harmless to both humans and ruminants at appropriate concentrations, and accurate analysis is simple and economical (Suharyono *et al.* 1991; Suharyono 1992). Although lithium salts have pharmacological and toxic effects and can cause feed aversion (Du Toit *et al.* 1991; Suharyono 1992), the amounts necessary to cause these effects in ruminants are much greater than the amounts generally required as a marker. Lithium salts have been used as a marker of supplement intake for grazing sheep (Suharyono 1992; Kahn 1994; Holst *et al.* 1996) and cattle (Dixon and Petherick 1996; McLennan 1999).

In penned sheep and cattle, plasma lithium concentration has been linearly related to the amount of lithium salt ingested in a supplement (Suharyono 1992; Kahn 1994; McLennan 1999). However, further experimentation is needed to confirm such relationships in cattle and for a variety of dietary and environmental circumstances. Experiments were therefore undertaken to examine lithium concentrations and kinetics in plasma following either ingestion of lithium-labelled supplement or intravenous administration of lithium to evaluate the reliability and accuracy of lithium as a marker of supplement intake for cattle in a tropical environment. Two experiments examined the appearance and disappearance of lithium in plasma following ingestion of various amounts of cottonseed meal (CSM) (experiment 1) or of molasses–urea, 2 types of loose mineral mix (LMM) supplement, or drinking water (experiment 3) labelled with lithium. Experiment 2 examined lithium concentrations in plasma following a single intravenous injection of lithium to measure the kinetics of lithium distribution and excretion. The variability in plasma lithium concentration among cattle offered a hay diet and held under closely controlled pen conditions, and thus of error inherent in the use of lithium as a marker of supplement intake, was determined in experiment 4.

Materials and methods

Experiment 1. Ingestion of various amounts of lithium-labelled CSM supplement

Ten *Bos indicus* × *Bos taurus* crossbred steers [initially 12–14 months of age, liveweight (LW) mean 217 (s.d. 15) kg and body condition score 3–4 on a 9-point scale (NRC 1996)] were housed individually in partially roofed pens (7 by 10 m). During 4 consecutive periods each of 14 days, 8 steers were allocated in a repeated Latin Square experimental design to 1 of 4 levels of supplementation with cottonseed meal (CSM) (0.15, 0.40, 1.00 and 3.00 kg/day). The 2 remaining steers were offered 0.4 kg CSM/day during periods 1 and 2 and were offered 1.0 and 3.0 kg CSM/day during periods 3 and 4. All steers were offered low-quality *Panicum maximum* hay at 20–30% in excess of actual intake, fresh hay being offered and hay refusals removed and weighed 3 times each week. CSM was offered at 0800 hours each day in a separate feeder. DM content of the offered and refused hay and of CSM was determined by drying at 70°C. Water was offered *ad libitum* in drums (570 mm diameter) and water intake was measured daily by change in height of the water in the drums.

On day 8 of each period, the CSM supplement was labelled with lithium chloride (655 mg Li/kg CSM) and offered to the steers at 0800 hours. Thus, steers were offered about 0.5, 1.3, 3.3 or 9.8 mg Li/kg LW. Lithium-labelled CSM was prepared by dissolving the lithium chloride in water (125 g/L) and spraying this solution over CSM while it was being mixed in a horizontal paddle feed mixer. The amounts of lithium-labelled CSM remaining 0.5, 1, 2, 3 and 4 h after the supplement was offered to the steers were measured and any supplement remaining after 4 h was removed. Blood samples were obtained 0.5 h before and 4, 8, 24, 32, 48, 72 and 96 h after the lithium-labelled CSM was offered to the steers. Blood samples were obtained by jugular puncture using vacutainers containing potassium EDTA as an anti-coagulant. Samples were immediately chilled in iced water, centrifuged (3000 *g* for 10 min) to separate plasma and the plasma stored frozen. Plasma proteins were precipitated (1 mL plasma added to 5 mL 2% trichloroacetic acid solution) and lithium concentration in the supernatant resulting from centrifugation was determined using an inductively coupled plasma mass spectrometer (ICPMS) (Model Elan 5000 Perkin Elmer). Steers were weighed at 0730 hours on the day lithium-labelled supplement was offered to the steers.

Net lithium concentrations in plasma were calculated by subtraction of the concentration measured before lithium-labelled CSM was provided (mean 2 μg/L in period 1; mean 63 μg/L in periods 2–4). Plots of net plasma lithium concentration against time indicated that for each of the treatments the concentration increased to a maximum about 24 h after ingestion of the lithium-labelled CSM and thereafter appeared to decline exponentially as observed by Suharyono (1992). Since there were insufficient observations to satisfactorily fit an exponential component between 0 and 24 h, a single exponential equation was calculated to describe the change in net plasma lithium concentration between 24 and 96 h after ingestion of lithium-labelled CSM. This equation was of the form

$$C_{t=} A e^{-kt},$$

where $C_{t=}$ concentration at time (t), A = the Y intercept at time zero, and k = the slope of the exponential component (Shipley and Clarke 1972). The half-time ($T_{1/2}$) was calculated as $0.693/k$.

Data for intake of feed and water, plasma lithium concentration at specific sampling times and the rate constant of disappearance of lithium from plasma were examined by residual maximum likelihood (REML; Patterson and Thompson 1971). Distributional assumptions were assessed by visual inspection of residual and normal probability plots. Paired comparisons between means were made using the protected l.s.d. test adjusted for various sample sizes. In addition, the relationships between lithium concentrations at specific times and the

amount of lithium-labelled CSM ingested were examined by regression procedures.

Experiment 2. Intravenous administration of lithium chloride

Eight of the steers used in experiment 1 continued to be housed in the individual pens, were offered hay and water as described above and were allocated at random to 2 treatments consisting of supplements of either 0.4 or 3.0 kg CSM/day. Mean LW was 235 (s.d. 12) kg. After 11 days, the CSM supplements were offered at 0515 hours, the steers were blood sampled at 0600 hours and at 0630 hours a solution of lithium chloride (3 mg Li/kg LW in 20 mL physiological saline) was injected intravenously into the jugular vein. Blood samples were obtained by venous puncture from the jugular vein not used for the injection of lithium chloride on 16 occasions, after about 0.17, 0.33, 0.67, 1.0, 1.5, 2, 3, 4, 5, 6, 9, 12, 24, 48, 72 and 96 h. Plasma was separated and analysed for lithium as described for experiment 1.

The net concentration of lithium in plasma was calculated by subtraction of background concentration (mean 40 μg/L, corrected during the sampling interval by the rate of disappearance of lithium measured in the respective diets in experiment 1). Concentrations of lithium were then normalised to a common amount of lithium dose (3.0 mg Li/kg LW). From plots of lithium concentration against time it appeared that the decrease in plasma lithium concentrations was best described by 3 exponential components. Thus, an equation of the form

$$C_t = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + A_3 e^{-k_3 t},$$

where C_t = concentration at time (t), A_1, A_2 and A_3 are the Y intercepts at time zero, and k_1, k_2 and k_3 are the slopes of the 3 exponential components, respectively, was examined. This equation was fitted for each animal observation using manual curve-peeling procedures (Shipley and Clarke 1972). The measurements of intake of feed and water and of the rate constants of disappearance of lithium from the various compartments for the 2 dietary treatments were examined by analysis of variance.

Experiment 3. Ingestion of molasses–urea or loose mineral mix supplements, or water labelled with lithium

Sixteen steers, LW mean 265 (s.d. 18) kg, consisting of the 10 steers that had been used in experiment 1 and 6 similar animals were housed and managed as described for experiment 1. Commencing 8 weeks after experiment 2, during each of 2 consecutive 3-week periods the steers were allocated randomly to 4 dietary treatments. All steers were offered chopped low-quality sorghum hay *ad libitum* 3 times weekly and, in a separate feeder, various supplements. Water was freely available. Treatment 1 steers were offered *ad libitum* a supplement of molasses containing 29 g urea/kg (M3U). Treatment 2 steers were offered daily 500 g cottonseed meal. Treatment 3 steers were offered daily 86 g of a loose mineral mix supplement containing (g/kg) 568 chopped lucerne hay, 193 water, 171 urea, 34 molasses and 34 calcium phosphates (LMM-1), while treatment 4 steers were offered 186 g of a loose mineral mix containing (g/kg) 425 chopped lucerne hay, 254 sodium chloride, 144 water, 127 urea, 25 molasses and 25 calcium phosphates (LMM-2). During preparation of the LMM-1 and LMM-2 supplements, the molasses and water were mixed separately before being mixed with the other ingredients. Amounts of hay and supplement offered and refused were weighed and subsamples taken for determination of DM content by oven drying at 70°C.

On day 15 of each period, the M3U (treatment 1), water (treatment 2) or LMM supplements (treatments 3 and 4) offered to the steers between 0800 hours and 1800 hours were labelled with lithium chloride. The lithium chloride was dissolved in water before being mixed with the supplements, the amounts being about 500 mg Li/kg M3U, 40 mg Li/L water, 12 mg Li/g LMM-1 and 6 mg Li/g LMM-2. The amounts of lithium-labelled supplement (treatments 1, 3 and 4) or water (treatment 2) remaining after 1, 2, 4, 6, 8 and 10 h were determined by weight or for water by measurement of change in height in the drum in which it was provided. At 1800 hours, the lithium-labelled supplements were removed and non-labelled M3U (treatment 1) or water (treatment 2) provided. Steers were blood-sampled at 0700 hours before the lithium-labelled supplements were offered to determine background concentrations, and then 8, 24, 32, 48, 56, 72 and 96 h after the lithium-labelled supplements were initially offered. Plasma was centrifuged, stored frozen, protein precipitated and analysed for lithium as described for experiment 1.

The net concentrations of lithium in plasma were calculated by subtraction of background (mean 4 μg Li/L). Concentrations of lithium were then normalised to a common dose of lithium ingested (5.0 mg Li/kg LW). The disappearance of lithium from plasma between 32 and 96 h after the lithium-labelled supplement was initially offered to the steers was calculated as a single exponential equation. Differences between treatments and periods were examined by 2-way analysis of variance. Paired comparisons between means were made using the protected l.s.d. test.

Experiment 4. Ingestion of a meal of lithium-labelled molasses supplement

Twenty-four *Bos indicus* × *Bos taurus* crossbred heifers, 10–14 months of age, LW mean 220 (s.d. 13) kg and body condition score 5–6, were used in the experiment. The heifers were held in individual pens and offered low-quality speargrass (*Heteropogon contortus*) hay and water *ad libitum*. The intake of hay and water was measured as described for experiment 1.

After 13 days, each of the heifers was, at 1100 hours, offered 60 g of lithium-labelled supplement which provided about 2 mg Li/kg LW. This supplement contained (g/kg) 728 molasses, 182 water, 68 lithium sulfate monohydrate and 22 urea, and was offered to the heifers in two 4 g icecream cones manufactured from wheat flour and vegetable oil. Any supplement remaining after 15 min was removed and the actual intake of supplement was determined from the weights of supplement offered and refused. Blood samples were obtained 0.5 h before, and 20, 24 and 28 h after, the supplements were offered and plasma separated following the procedures described in experiment 1. Lithium concentrations in plasma were determined by atomic absorption spectroscopy (Model Z5100, Perkin Elmer). Net lithium concentrations in plasma were calculated by subtraction of background lithium concentrations (mean 8 μg Li/L) and the lithium concentrations were normalised to a common amount of lithium actually ingested (2.0 mg Li/kg LW). Lithium concentrations in plasma at the 3 sampling times were compared by analysis of variance; effects of sampling time were compared within animals.

Results

Experiment 1. Ingestion of various amounts of lithium-labelled CSM supplement

The hay contained 892 g DM/kg as fed, 929 g organic matter and 4.7 g nitrogen per kilogram DM, and had an *in vitro* organic matter digestibility of 256 g/kg DM. CSM contained 924 g DM/kg as fed, and 928 g organic matter and 69 g nitrogen per kilogram DM. The steers were in good health throughout the experiment, had stable intakes of hay and water and always readily consumed their allocations of unlabelled CSM. During periods 1 and 3, all of the steers readily consumed all of the lithium-labelled CSM. During period 2, 2 steers offered 1 kg lithium-labelled CSM consumed only 3 and 60%, respectively, of this allocation during the 4 h when it was offered. In addition, during period 4 one of these steers consumed only 91% of its allocation of

3 kg labelled CSM, while one other steer consumed only 26% of its allocation of 1 kg labelled CSM. These 4 measurements were considered as missing values in the analysis. The remaining steers, with 2 exceptions, consumed their entire allocation of lithium-labelled CSM within 1 h; for these 2 exceptions the lithium-labelled CSM was consumed within 3 h. Other than this incomplete consumption of lithium-labelled CSM by some steers, there was no evidence of feed aversion effects or behavioural changes due to ingestion of the lithium-labelled CSM by the steers. Intakes by individual steers of hay and water were similar during the 3 days before and the 2 days after the lithium-labelled CSM were provided. To address the loss of observations described above when some steers did not consume all the lithium-labelled CSM, measurements were made in 2 additional steers during both periods 3 and 4. By the end of period 4, 8 measurements had been made for the 0.15 and 3.0 kg CSM/day treatments, 9 measurements for 0.4 kg CSM/day treatment and 7 measurements for the 1.0 kg CSM/day treatment.

The intakes of feed and water are shown in Table 1. Hay intake was not changed ($P>0.05$) by CSM supplementation, but total DM intake was increased ($P<0.05$) by provision of 1.0 kg CSM/day and further increased ($P<0.05$) by 3.0 kg CSM/day. Intake of CSM supplement and total DM was linearly related as follows:

$$Y = 17.4 \text{ (s.e. } 0.67) + 0.92 \text{ (s.e. } 0.097)X$$
$$(n = 32;\ r = 0.74;\ \text{r.s.d.} = 5.34;\ P<0.001),$$

where Y (g/kg LW.day) is the intake of total DM and X (g/kg LW.day) is the intake of CSM. Water intake was increased ($P<0.05$) by the provision of 1.0 and 3.0 kg CSM/day, and tended ($r = 0.25$; $P = 0.09$) to be correlated with total DM intake.

The concentrations of lithium in plasma at various intervals after ingestion of the lithium-labelled CSM are shown in Figure 1. After increasing to maxima about 24 h after ingestion of lithium-labelled CSM, the decreases in concentration appeared to be well described as a single exponential relationship. The rate constants for disappearance of lithium from plasma (Table 1) did not differ among the treatments where steers were supplemented with 0.15, 0.4 and 1.0 kg lithium-labelled CSM (0.0117–0.0133/h), but this rate was increased (0.0258/h, $P<0.05$) by provision of 3.0 kg lithium-labelled CSM.

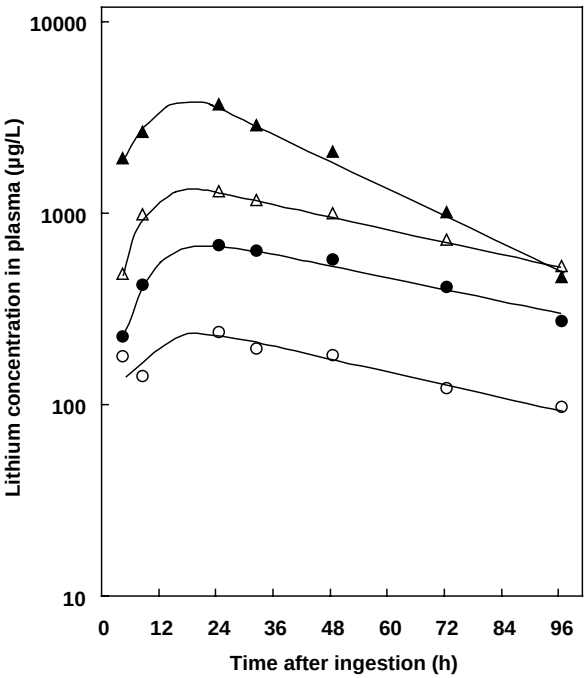

Figure 1. Experiment 1. Mean concentrations of lithium in plasma of steers (n = 7–9) consuming low-quality hay and following ingestion of 0.15 (○), 0.40 (●), 1.0 (△) or 3.0 (▲) kg lithium-labelled cottonseed meal supplement as a single meal.

Table 1. Experiment 1. Intake of hay, cottonseed meal (CSM) and total dry matter (DM), and of water by the steers during the 4 days after ingestion of lithium-labelled cottonseed meal

LW, liveweight; means within each row followed by the same letter are not significantly different at $P = 0.05$

Measurement	Treatment (kg CSM supplement/day)				s.e.d.	Signif.
	0.15	0.4	1.0	3.0		
n	8	9	7	8	—	—
Hay intake (kg DM/day)	3.57	3.63	3.87	3.44	0.178	n.s.
Cottonseed meal intake (kg DM/day)	0.14	0.37	0.92	2.77	—	—
Total dry matter intake (kg/day)	3.71a	4.00a	4.80b	6.22c	0.177	***
Hay intake (g DM/kg LW.day)	16.6	17.0	18.3	16.1	0.80	n.s.
Cottonseed meal intake (g DM/kg LW.day)	0.8	1.9	4.3	13.0	—	—
Total dry matter intake (g/kg LW.day)	17.3a	18.8a	22.7b	29.2c	0.85	***
Water intake (L/day)	19.3a	21.1a	24.1b	30.9c	1.23	***
Water intake (mL/kg LW.day)	91.2a	99.9a	112.5b	144.2c	5.39	***
Rate constant of disappearance of lithium from plasma (per h)	0.0130a	0.0117a	0.0133a	0.0258b	0.00196	***

***$P<0.001$; n.s., not significant.

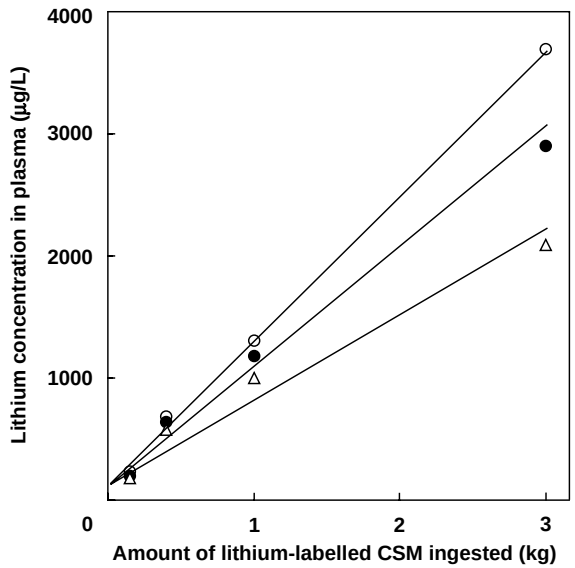

Figure 2. Experiment 1. Relationships between the amount of lithium-labelled cottonseed meal consumed and the concentrations of lithium in plasma after 24 (○), 32 (●) and 48 (△) h.

Because the amount of lithium ingested was confounded with the amounts of CSM, total DM and water ingested (Table 1), it is not possible to conclude whether this difference between diets was due to the amount of lithium ingested or to other differences between the diets. Lithium concentration in plasma after 24, 32 and 48 h was linearly related to the amount of lithium-labelled supplement ingested (P<0.001, n = 32, r = 0.86, 0.87 and 0.82, respectively) (Fig. 2). These relationships after 72 and 96 h were significantly (P<0.001) curvilinear.

Experiment 2. Intravenous administration of lithium chloride

The hay contained 899 g DM/kg as fed, 927 g organic matter and 5.3 g nitrogen per kilogram DM, and had an *in vitro* organic matter digestibility of 295 g/kg DM. CSM contained 933 g DM/kg as fed. Total DM intake was increased (P<0.001) by provision of the higher level of CSM (31.0 and 21.1 g DM/kg LW.day), but hay intake was similar for the 2 treatments (Table 2). Water intake was also increased (P<0.001) by the high level of CSM (155 and 97 mL/kg LW.day). Intakes of hay and water during the 3 days before and the 2 days following administration of lithium chloride were similar.

The concentrations of lithium in plasma following intravenous administration are shown in Figure 3. In general, this relationship appeared to be best described by 3 exponential components, but the results for 1 steer offered 3.0 kg CSM/day where 3 compartments could not be fitted satisfactorily were considered missing values. The rate constants of the 3 compartments are given in Table 2. The rate constant of disappearance of the lithium from compartment 3 was greater (P<0.05) in steers offered the high than the low level of CSM (0.0198 and 0.0105/h, respectively). The Y intercepts of the plasma lithium concentration indicated that the lithium chloride distributed through 18.0% of the liveweight of the steers.

Experiment 3. Ingestion of supplements or water labelled with lithium

The steers were in good health throughout the experiment. The hay contained 917 g DM/kg as fed, 878 g organic matter and 11.2 g nitrogen per kilogram DM and had an *in vitro* organic matter digestibility of 497 g/kg DM. The cottonseed meal, M3U, LMM-1 and LMM-2 supplements

Table 2. Experiment 2. Intake of hay, cottonseed meal (CSM) and total dry matter (DM), and of water by the steers during the 4 days after intravenous injection of lithium chloride

LW, liveweight

Measurement	Treatment (kg CSM supplement/day)		s.e.d.	Signif.
	0.4	3.0		
n	4	4	—	—
Hay intake (kg DM/day)	4.61	4.39	0.29	n.s.
Cottonseed meal intake (kg DM/day)	0.38	2.81	—	—
Total dry matter intake (kg/day)	4.99	7.20	0.29	***
Hay intake (g DM/kg LW.day)	19.5	18.9	0.87	n.s.
Cottonseed meal intake (g DM/kg LW.day)	1.6	12.1	—	—
Total dry matter intake (g/kg LW.day)	21.1	31.0	0.99	***
Water intake (L/day)	22.7	36.1	4.69	*
Water intake (mL/kg LW.day)	97	155	18.9	*
Rate constant of disappearance of lithium from plasma (per h)				
Compartment 1	0.99	1.20	0.334	n.s.
Compartment 2	0.146	0.214	0.0813	n.s.
Compartment 3	0.0105	0.0198	0.00322	*

*P<0.05; ***P<0.001; n.s., not significant.

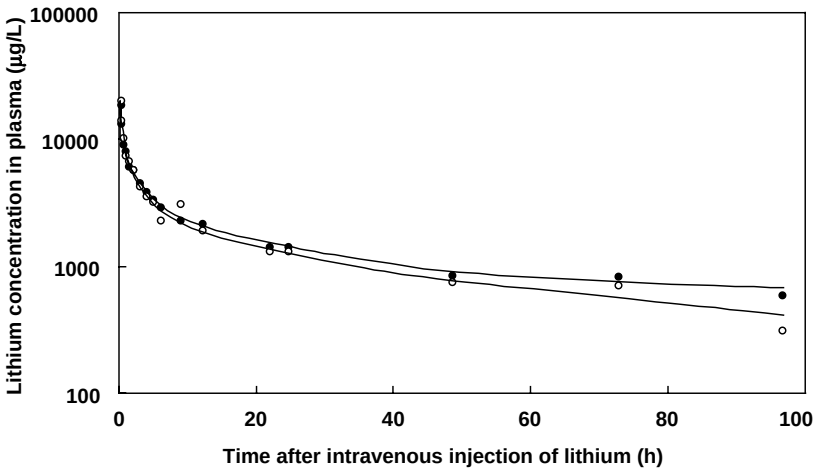

Figure 3. Experiment 2. Mean concentrations of lithium in plasma following intravenous administration of lithium chloride in steers consuming low-quality hay supplemented with 0.4 (○) or 3.0 (●) kg cottonseed meal per day. Plasma lithium concentrations were adjusted to a normalised intake of 3.0 mg lithium/kg liveweight.

contained, on average, 924, 823, 967 and 929 g DM/kg as fed, respectively. Two steers consumed less than 0.9 g DM/day of the unlabelled M3U supplement, although mean intake of the other 6 steers was 2.67 kg DM/day. Also 3 steers consumed less than 28% of the offered unlabelled LMM-2 supplement. Since the amounts of lithium marker ingested were much lower than for the other steers (<0.7 mg Li/kg LW) and the consequent low plasma lithium concentrations led to appreciable analytical error, these 5 observations were discarded.

The mean intakes of hay, supplements and water during the 4 days when lithium-labelled supplements or water were ingested and blood samples obtained, are given in Table 3. Intake of hay did not differ (P>0.05) among the treatments, ranging from 15.5 to 16.8 g DM/kg LW. Voluntary intake of M3U supplement was 10.2 g DM/kg LW and total DM intake was increased (P<0.001) by the provision of this supplement.

Consumption of the lithium-labelled M3U and lithium-labelled water occurred about linearly through the 10-h interval during which they were offered (Fig. 4). However, 78 and 73% of the lithium-labelled LMM-1 and LMM-2 supplements were consumed within the first hour after being provided to the steers. Provision of lithium-labelled supplement did not appear to change hay or water intake. The change in the concentration of lithium in plasma between 32 and 96 h after the lithium-labelled supplements were offered to the steers appeared to be satisfactorily described by a single exponential relationship. The concentration of lithium in plasma appeared to be greatest after 24 h (i.e. 14 h after removal of the lithium-labelled supplements) (Table 3). Neither the concentrations of lithium in plasma at specific times nor the rate constant of disappearance of lithium from plasma were affected by the dietary treatments (Table 3) and were not correlated (P>0.05) with DM or water intake.

Experiment 4. Ingestion of a meal of lithium-labelled molasses supplement

With 1 exception, the heifers consumed all of the lithium-labelled supplement within 15 min; the heifer that

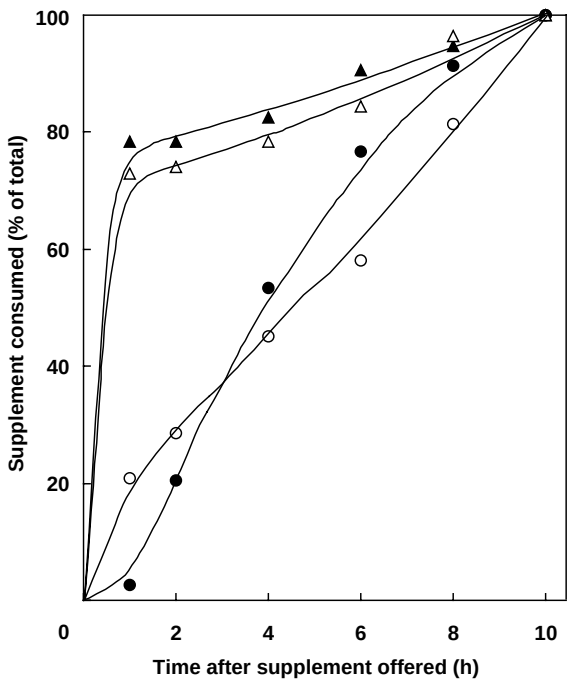

Figure 4. Experiment 3. Intake of 3 lithium-labelled supplements (M3U, ○; LMM-1, △; LMM-2, ▲) or of lithium-labelled water (●) offered at 0800 hours and removed at 1800 hours on one day. On average, the steers consumed 1307 g M3U, 83 g LMM-1, 156 g LMM-2 and 29.7 kg water labelled with lithium.

Table 3. Experiment 3. Intake of hay, supplements and total dry matter (DM), and of water by the steers during the 4 days after ingestion of lithium-labelled supplements

Means within each row followed by the same letter are not significantly different at $P = 0.05$
Plasma lithium concentrations were adjusted to a normalised intake of 5.0 mg Li/kg liveweight (LW)

Measurement	Li-labelled supplement or water				s.e.d.	Signif.
	M3U	Water	LMM-1	LMM-2		
n	6	8	8	5	—	—
Hay intake (kg DM/day)	4.10	4.34	4.40	4.40	0.140	n.s.
Supplement intake (kg DM/day)	2.67	0.46	0.08	0.16	—	—
Total dry matter (kg/day)	6.77[b]	4.80[a]	4.48[a]	4.56[a]	0.246	***
Hay intake (g DM/kg LW.day)	15.5	16.4	16.7	16.8	0.65	n.s.
Supplement intake (g DM/kg LW.day)	10.2	1.8	0.3	0.6	—	—
Total dry matter (g/kg LW.day)	25.7 [b]	18.2[a]	17.0[a]	17.4[a]	1.28	***
Water intake (L/day)	31.6	27.0	27.7	28.3	2.43	n.s.
Water intake (mL/kg LW.day)	120	102	105	108	10.7	n.s.
Amount of Li ingested (mg/kg LW)	2.44	4.28	3.99	3.75	—	—
Plasma Li concentration (μg Li/L)						
24 h	3965	3642	3739	3108	729	n.s.
32 h	3561	3560	2988	2433	658	n.s.
48 h	2475	2629	2256	1854	424	n.s.
Rate constant of disappearance of lithium from plasma (per h)	0.0239	0.0188	0.0156	0.0186	0.00476	n.s.

***$P<0.001$; n.s., not significant.

was the exception consumed only 89% of that offered. The hay contained 922 g DM/kg as fed, 915 g organic matter and 4.5 g nitrogen per kilogram DM, and had an *in vitro* organic matter digestibility of 310 g/kg DM. The molasses supplement contained 590 g DM/kg as fed and 875 g organic matter and 35 g nitrogen per kilogram DM. The heifers thus consumed about 38 g DM and 1.28 g nitrogen in the supplement.

Mean voluntary intake of hay for the 2 days following ingestion of the lithium-labelled supplement was 9.35 (s.d. 2.18) g DM/kg LW, while intake of water was 66.9 (s.d. 12.8) mL/kg LW. Water intake was related to hay intake as follows:

$$Y = 44.0 \text{ (s.e. } 10.9) + 2.44 \text{ (s.e. } 1.14)X,$$
$$(n = 24;\ r = 0.37;\ P<0.05;\ \text{r.s.d.} = 12.8),$$

where Y is the water intake (mL/kg LW.day) and X is the hay intake (g DM/kg LW.day). Intake of hay was not affected by provision of the lithium-labelled supplement.

The net normalised concentration of lithium in plasma 20 h after the Li-labelled supplement was consumed [mean 1090 (s.d. 161) μg Li/L plasma] was lower ($P<0.01$) than the concentrations after 24 and 28 h [mean 1158 (s.d. 190) and 1207 (s.d. 167) μg Li/L plasma, respectively] which were not different from each other ($P>0.05$). Thus, the CV of lithium concentration among heifers ranged from 13.9 to 16.4% for the 3 sampling times. The CV for the means of any 2 of these sampling times was 13.2–14.7% and the CV of the mean concentration at the 3 sampling times was 13.8%. The lithium concentrations measured in individual animals were correlated at each combination of the 3 sampling times ($P<0.001$; r ranged from 0.64 to 0.86). Mean concentration of lithium for the 3 sampling times was related to hay intake as follows:

$$Y = 839 \text{ (s.e. } 132) + 32.7 \text{ (s.e. } 13.8)X$$
$$(n = 24;\ r = 0.41;\ P<0.05;\ \text{r.s.d.} = 158),$$

where Y is the mean concentration of lithium in plasma (μg Li/L) and X is the voluntary intake of hay (g DM/kg LW.day). There was no relationship ($P>0.05$) between mean plasma lithium concentration and water intake.

Discussion

Kinetics of lithium

Following intravenous injection, the change in plasma lithium concentration in experiment 2 appeared to be described most adequately by 3 exponential compartments rather than by 2 exponential compartments as reported by Suharyono (1992). However, in the present experiment the plasma lithium concentrations were measured over 96 h, whereas in the studies of Suharyono (1992), the plasma lithium concentration was measured over only 48 h. The third compartment was only evident because of this extended sampling time (Fig. 3). The third compartment appeared to principally represent renal excretion, since most lithium is excreted from the animal by this pathway (Harrison *et al.* 1963; Ulyatt 1964; Schonewille and Beynen 1999). The first and second compartments observed may have been associated with kinetic compartments of lithium in the extracellular fluid, digesta of the rumen and post-ruminal tract, and other body pools, or with mixing of lithium in

these pools. Lithium injected intravenously would be expected to enter digesta pools since there is extensive transfer of plasma lithium to the rumen via saliva (Ulyatt 1964; Suharyono 1992).

Following ingestion of lithium-labelled supplement, there was an increase in plasma lithium concentration to a maximum followed by an exponential decline. This was a similar pattern to previous observations with cattle (McLennan 1999) but differed from the results of Suharyono (1992) and Kahn (1994) in sheep where there appeared to be a plateau for some time before the decline in plasma lithium concentration. The time to maximum plasma lithium concentration in the present experiments of about 24 h is within the range reported in previous experiments; this maximum has been reported to occur after only about 4–12 h (Kahn 1994; McLennan 1999), 12–24 h (Suharyono 1992), or with fasted sheep 48 h (Ulyatt 1964). These changes in plasma lithium following ingestion of lithium-labelled supplements are consistent with the observation of 3 kinetic pools of lithium, with the disappearance between 24 and 96 h principally reflecting renal excretion. The rates of lithium disappearance in experiment 1 when 0.4 and 3 kg CSM were consumed (0.0117 and 0.0258/h, respectively) were comparable to these rates for compartment 3 measured in the respective diets during experiment 2 (0.0111 and 0.0202/h, respectively). Comparable rate constants were also observed in experiment 3 of the present study. These values compare with a $T_{1/2}$ of 29 h (i.e. a rate constant of 0.024/h) observed by McLennan (1999). The rate of disappearance of lithium from plasma is an established procedure to measure specific aspects of renal function and is influenced by a number of factors including sodium excretion (Thomsen 1984; Boer *et al.* 1988; Koomans *et al.* 1989).

Measurement of supplement intake by individuals in herds

The linear relationship observed in experiment 1 between the amount of Li-labelled supplement consumed and plasma lithium concentration is in agreement with previous reports of this relationship in both cattle (McLennan 1999) and sheep (Suharyono 1992; Kahn 1994). Thus, all of these experiments support the hypothesis that lithium salts can be used to estimate intakes of a supplement by individual animals offered a single meal of lithium-labelled supplement, providing that animals are blood-sampled at about the time of maximal plasma lithium concentrations. However, the present studies also indicate that, because the decline in plasma lithium concentration is rapid and exponential, considerable error can be introduced if plasma is sampled some time after the maximum plasma lithium concentration. This error will be exacerbated when the amount of supplement ingested changes the rate of disappearance of lithium from plasma. The CV of 15% about the estimation of intake of a meal of lithium-labelled supplement by heifers consuming hay *ad libitum* in experiment 4 suggests a minimum error in estimation of supplement intake by individual animals. However, this variation is comparable to that of other marker techniques such as chromic oxide to measure intake of supplements and pasture by grazing ruminants (Langlands 1975; Kendall *et al.* 1980; Parker *et al.* 1990; Williamson *et al.* 2000).

Measurements of lithium-labelled supplement intake by individual animals may be made following a single meal if only restricted amounts of a supplement are being provided to a herd. However, where supplements are provided *ad libitum*, it is clearly desirable that the lithium-labelled supplement should also be provided *ad libitum* and during an interval representative of the intake of supplement by the herd. As with tritiated water as a marker, if lithium-labelled supplement is provided for some hours during a single day then the estimation of supplement intake may be influenced by differences between animals in the time between ingestion of labelled supplement and blood sampling, and also by the rate constant of disappearance of lithium from the animal (Nolan *et al.* 1975). For example if lithium-labelled supplements are offered for 9 h, the rate constant of disappearance of lithium is 0.015/h and the cattle are sampled the following day, calculations indicate that estimated supplement intakes of animals that delayed their entire consumption of lithium-labelled supplement by 3, 6 or 9 h would be 103, 108 and 113% greater, respectively, than for an animal that consumed all of its lithium-labelled supplement when it was initially offered. This potential error has also been shown experimentally; intake of lithium-labelled supplement given in 5 equal meals over 12 h was predicted from 24-h blood samples to be 16% greater than when all of the supplement was consumed as a single meal at the beginning of this interval (McLennan 1999). However, grazing cattle usually spend only part of the day at the water point (Ernst 1973; Squires 1981), so the differences among animals within a herd in the times of ingestion of lithium-labelled supplement offered at the water point are likely to be only several hours. Nevertheless, differences in the time when individual animals consume lithium-labelled supplements offered *ad libitum* may cause appreciable error in the estimation of individual intakes of supplement.

An alternative procedure to use marker-labelled supplement to measure intake of supplements offered *ad libitum* is to provide supplement labelled with tritiated water for a week (Nolan *et al.* 1975) or for many weeks (Wheeler *et al.* 1980) before blood sampling. The error associated with animals consuming labelled supplement on differing days during the interval when it is offered is directly related to the rate constant of disappearance of the marker from the animal and also to the variation between days in supplement intake (Hedges and Rocks 1980; Dove 1984); as the rate constant of disappearance of tritiated water marker from an animal increased from 0.005 to 0.015/h, the error in

estimation of intake of labelled supplement offered for a week increased almost 3-fold. Since lithium has a rate constant of disappearance from cattle in the range 0.010–0.025/h (McLennan 1999; present studies), the errors of this origin in prediction of intake of lithium-labelled supplement consumed for several days will be substantially greater than for tritiated water-labelled supplements. In addition, Dove (1984) showed that as the CV between days in supplement intake increased from 20 to 50%, the error in prediction of supplement intake increased almost 3-fold. Thus, if the experimental procedure involves feeding lithium-labelled supplement for several days, there may be large errors in the measurement of supplement intake by individual animals. Because of these potential errors, we (Dixon and Petherick 1996; Dixon and Smith 2000; Dixon *et al.* 2000, 2001) have used lithium as a marker of supplement offered for 9–10 h on a specific day, and accepted that the measured variability in supplement intake relates solely to a specific day. However, it is well established that there is often large variation between days in voluntary intake of loose mineral mix and block supplements (Rocks *et al.* 1982; Weber *et al.* 1992; Tait and Fisher 1996). A compromise must be made between greater accuracy if lithium-labelled supplement is offered for only 1 day, and making measurements with lesser accuracy but probably more representative of average intake of supplement over several days. Providing lithium-labelled supplement for at least several days will presumably provide more reliable estimates of the proportion of animals in a herd that do not consume any supplement.

In conclusion, lithium salts can be used to measure intake by individuals of a meal of supplement provided on a specific day. Although errors are likely to be greater, lithium salts can also be used to measure individual intakes of supplements provided *ad libitum* with sufficient accuracy to be a valuable field technique.

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