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Reticular groove contraction in yearling cattle detected by breath test

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ABSTRACT

A novel method was used to detect the incidence of reticular groove contraction in yearling cattle. Following its oral administration, ^{13}C octanoic acid is absorbed from the intestine, metabolised by the liver and the ^{13}C label excreted in the CO_2 of breath. Contraction of the reticular groove should result in a quicker and greater peak appearance of ^{13}C in breath, than when swallowed material is delivered into the reticulo-rumen. Mean breath ^{13}C enrichment after drenching with NaCl was greater ($P<0.001$) than with water. Mean increases in enrichment after sucking molasses and label were greater than drenching with water ($P<0.001$) and NaCl ($P<0.05$). These results are consistent with sodium and sucking stimulating reticular groove contraction and support the use of this method as a simple, repeatable, non-radioactive and non-invasive means of detecting the route taken of orally administered therapeutic agents or nutrients. The test, for example, would identify those animals at risk by rumen bypass of anti-bloat agents.

Keywords: reticular groove; breath test; rumen bypass.

INTRODUCTION

In ruminants reticular groove contraction, a reflex activated by stimulation of receptors in the mouth, results in swallowed fluids by-passing the reticulo-rumen (Titchen 1968). This mechanism is responsible for the passage of milk in the suckling animal passing directly into the abomasum for hydrolytic digestion. The patency of this reflex is usually lost with increasing age of the animals, but in some adults it is retained and may be easily elicited on drenching. It is possible this may be responsible for the death of some animals by bloat, despite being drenched with anti-bloat agents (Carruthers *et al*. 1994). Following investigations of the incidence of rumen by-pass on drenching in adult cattle using a dye indicator and slaughter technique (Carruthers *et al*. 1994), we developed a simple breath test to detect the fate of swallowed fluids in cattle (McLeay *et al*. 1997). This test was based on the administration of ^{13}C octanoic acid which is absorbed by the intestine, metabolised by the liver and the C label excreted in the CO_2 of expired breath. Placement of the octanoic acid through the reticulo-omasal orifice of rumen-fistulated cows resulted in a more rapid and greater peak appearance of the label in breath, than when the material was placed in the reticulo-rumen. We now report detailed observations on testing this technique in yearling cattle, known to have a high incidence of reticular groove contraction in response to sodium salts (Reik 1954). In addition some of these animals sucked from a bottle, which is one of the most potent stimulants of reticular groove contraction (Orskov *et al*. 1970).

METHODS

Cattle- Sixteen 12- month-old (yearling) Holstein Friesian bulls or steers (castrated at 6 months of age), with liveweight between 235 and 296 kg, were maintained on pasture and 1 kg of a mixed grain supplement containing molasses. The yearlings were removed from pasture at 0700h and brought indoors to head stalls at 0830h or 1130h without having access to food or water. One experiment was undertaken on each animal each day. The experiments were approved by the Animal Ethics Committees of the University of Waikato and Ruakura Agricultural Research Centre.

Breath collection and analysis- Exhaled breath was collected and analysed for carbon isotope ratios of CO_2 by use of gas chromotography-mass spectrometry as described previously (McLeay *et al*. 1997).

Experiments- Animals were drenched with a drench gun which included an attached syringe containing the octanoic acid (Octanoic acid-1-^{13}C, 99 atom % ^{13}C, Isotec Inc. Miamisburg and Cambridge Isotope Labs., Andover, Mass.USA). The 0.2 ml volume of octanoic acid required to administer the 200 mg dose was solubilised in 15 ml 40% ethanol. Each animal received two treatments on days 1 and 3 or 2 and 4 in a cross-over design over 4 days with breath collected before and at 10 and 20 minutes after drenching. Treatments were: 70 ml water and 15 ml 40% ethanol containing 200 mg ^{13}C octanoic acid; and 70 ml NaCl (37 g in 100 ml water) and 15 ml 40% ethanol containing 200 mg 13C octanoic acid.

When drenching with NaCl, the animal's mouth was washed out with 20 ml of this solution, 60-120 seconds before the drench. These cattle were unaccustomed to drenching. Results for one animal were discarded because of water and NaCl drench spillage, and for two other animals because of water drench spillage.

Four of the 16 yearlings showed interest in sucking, via a calf rearing teat, a solution of molasses from a bottle. The animals were not trained to suck, but eagerly sought the molasses solution. Two of the animals were particularly

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excited at the prospect of sucking from the bottle. Molasses was diluted in warm tap water to give a 25% solution. The animals sucked the molasses solution from the bottle, whilst 200 mg ^{13}C octanoic acid in 15 ml 40% ethanol was introduced over 20-30 sec into the teat. Each animal continuously sucked molasses solution for about 60 sec before and after the administration of the ethanol and label, consuming about 120 ml. Breath was collected before and at 10 and 20 minutes after administration of the label.

Statistical Analyses- Statistical significance of differences were tested using predicted treatment means obtained from residual maximum likelihood (REML) analyses with animal and date as random effects and treatment as a fixed effect. Because of total confounding of sucking treatment with day in the 4 yearlings, date was not included as a random effect and raw means are presented. The variation in background values for these 4 cattle over the 3 treatment days was within 0.8 ‰.

RESULTS

Mean values of breath ^{13}C enrichment at 10 and 20 minutes after drenching yearlings with NaCl were significantly ($P<0.001$) greater than those after drenching with water (Table 1) . On an individual basis, 12 of 14 cattle had higher enrichment on drenching with NaCl than with water, with the increase at 10 minutes 1.6 to 6 times greater for NaCl than for water. The 2 exceptions (#1 and #10 in Table 1) had high enrichment when drenched with both water and NaCl. Only 3 of 14 yearlings had values >2.1 ‰ at 10 minutes and >3.5 ‰ at 20 minutes after drenching with water (#1, #4 and #10, Table 1).

TABLE 1: Enrichment of ^{13}C (‰) in breath of yearlings at 10 and 20 minutes following the administration of ^{13}C octanoic acid by drenching with H_2O or NaCl .

Yearling	‰ at 10 min		‰ at 20 min	
	H_2O	NaCl	H_2O	NaCl
1	5.4	5.5	7.6	7.8
2		5.1		7.0
3	1.0	2.9#	2.5	4.3#
4	3.6	6.1	5.2	7.9
5	1.1	7.5	2.4	9.2
6	1.7	3.2	3.0	5.5
7	1.8	3.0#	3.3	5.4#
8	1.7	2.8	2.3	5.2
9	0.3	2.5	1.9	4.2
10	4.5	3.3	6.6	5.8
12		5.2		8.8
13	1.0	6.0	3.0	9.8
14	0.6	3.0	1.6	5.3
15	1.3	4.7	3.0	7.7
16	0.7	3.9	1.4	6.2
18	2.1	6.1	3.5	8.0
mean[a]	2.0	4.6	3.5	7.0
sed	0.51		0.59	

\# indicates a small loss of isotopic label on drenching of NaCl and these figures are not included in the means. Where loss occurred with water, no figures are presented for those animals.

[a] Predicted mean from REML analysis with animals random and treatment fixed, sed = the standard error of the difference between treatments at 10 minutes and 20 minutes.

Mean breath ^{13}C enrichment values at 10 minutes in yearlings that sucked molasses and label, were greater ($P<0.05$) than those when drenched with water, but were not different from those after drenching with NaCl (Table 2). The mean increases in enrichment at 20 minutes after sucking were more pronounced, especially compared with values after water ($P<0.001$) and NaCl ($P<0.05$) drenchings (Table 2). The highest values of enrichment of 11.9 and 10.4 ‰ obtained in any of the experiments, occurred after sucking by the two cattle most eager to suck (#1 and #18, respectively, in Table 2).

TABLE 2: A comparison of the enrichment of ^{13}C (‰) in breath of yearlings at 10 and 20 minutes, following the administration of ^{13}C octanoic acid by drenching with H_2O or NaCl or by sucking.

	‰ at 10 min			‰ at 20 min		
	H_2O	NaCl	Suck	H_2O	NaCl	Suck
Yearling						
1	5.4	5.5	6.1	7.6	7.8	11.9
8	1.7	2.8	4.9	2.3	5.2	9.1
16	0.7	3.9	3.7	1.4	6.2	7
18	2.1	6.1	4.2	3.5	8	10.4
mean	2.5	4.6	4.7	3.7	6.8	9.6
sed		0.78			0.83	

sed = standard error of the difference between treatments at 10 minutes and 20 minutes.

DISCUSSION

The highly significant increase in mean enrichment with the sodium drench compared with water and the high values in all individuals drenched with sodium, provides strong evidence for the use of the breath test as an indicator of groove contraction, given the known effect of sodium on reticular groove contraction in young cattle (Reik 1954). The high values of enrichment also achieved in three animals on drenching water, indicated the groove contracted on water alone, which has been reported in a minority of animals previously (Reik 1954). Additional support for the breath test as an indictor of reticular groove contraction was obtained by the high levels of enrichment following the sucking of molasses and in fact this procedure resulted in the highest levels of enrichment observed (see Table 2). Sucking is recognised as a most potent stimulant of groove contraction (Orskov *et al*. 1970). The greater differences in enrichment between drenching water or NaCl and sucking at 20 min, compared with sucking at 10 min, may be explained by the act of sucking causing an inhibition of gastric motility (Kay and Ruckebusch 1971), with a resultant delay in the entry of label into the small intestine from where it is absorbed.

These results are consistent with sodium and sucking stimulating reticular groove contraction and supports the use of this method as a means of detecting partial or complete contraction of the reticular groove. Irrespective of reticular groove contraction, the method reveals, by say, a 10 min value >2.5 ‰, the rapid delivery of swallowed material to the intestines. This could occur by either groove contraction and/or rapid exit from the reticulo-rumen (McLeay *et al*. 1997) . Thus the method may be used to detect the route taken of orally administered therapeutic agents or nutrients, and for example, would identify those animals at risk by rumen bypass of anti-bloat agents.

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