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The voluntary intake of hay and silage by lactating cows in response to ruminal infusion of acetate or propionate, or both, with or without distension of the rumen by a balloon

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In order to test the hypothesis that negative feedback signals from abdominal receptors are integrated in an additive manner in the control of voluntary food intake, cows with rumen fistulas were given intraruminal infusions of sodium acetate or sodium propionate, or both, with or without distension of the rumen by balloon. Intakes were monitored during the 3 h experimental period and for 2 h after and samples of rumen fluid were taken for estimation of short-chain fatty acid concentrations and osmolality. Six cows in mid-lactation were fed on hay and concentrates and given, into the rumen, 5·5 mol sodium acetate, 5·2 mol sodium propionate and 7·5 l of distension. Compared with the control (water infusion), neither acetate, propionate nor distension significantly depressed hay intake when given separately. When given in combination, however, the following significantly depressed intake during the 3 h treatment period: propionate + distension, acetate + distension, acetate + propionate + distension. Seven cows in early lactation were fed on silage and concentrates and given, into the rumen, 9·0 mol sodium acetate, 4·0 mol sodium propionate and 10·0 litres of distension. Again, none of the three given alone depressed silage intake to a significant extent during the 3 h treatment period, whereas the following combinations had a significant effect: propionate + distension, acetate + distension, acetate + propionate + distension. Basal rumen osmolalities were similar for the two types of feed but infusion of the sodium salts caused a very much greater increase with silage than with hay. The results are discussed with regard to the implications for our understanding of the control of voluntary intake and the likely role of chemo-, osmo- and mechano-receptors.

Voluntary intake: Intraruminal infusions: Volatile fatty acids: Rumen distension: Dairy cows

During the last 30 years a considerable amount of work has been done to establish the role of metabolic and physical factors in the control of food intake in ruminant animals, and there is now adequate evidence that end-products of carbohydrate fermentation and distension of the rumen are involved in limiting intake. Intraruminal infusion of acetate over periods of several hours depressed hay intake in cattle (Montgomery *et al.* 1963; Bhattacharya & Warner, 1968; Anil *et al.* 1993) and sheep (Baile & McLaughlin, 1970; Bergen, 1972). Infusion of propionate in the rumen depressed feed intake by cattle (Montgomery *et al.* 1963; Anil *et al.* 1993) and sheep (Ternouth, 1967). However, most work has used salts of these acids and there is considerable doubt as to whether their effects are due to the acids themselves or to osmolality. Distension of the rumen depressed forage intake in cattle (Campling & Balch, 1961; Anil *et al.* 1993). However, even at supraphysiological levels, none of these treatments completely prevents feeding and it is unlikely that either rumen volatile fatty acids (VFA) production or distension are entirely responsible for terminating normal feeding (Campling, 1970; Forbes, 1986).

It is likely that the effects of the changes that occur in the rumen during a meal, such as

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production of VFA, changes in pH, osmolality and rumen distension, may be interpreted as a whole by the brain to induce satiety. The effects of feedback signals have been suggested to be additive in sheep (Adams & Forbes, 1981).

These investigations have now been extended to cattle in the present study in which the combined effects of ruminal infusions of acetate or propionate, or both, and rumen distension on the voluntary intake of hay or silage by lactating dairy cows have been examined. Brief accounts of the present work have already been published (Anil *et al.* 1987; Mbanya *et al.* 1989).

MATERIALS AND METHODS

The methods used were generally the same as those described by Anil *et al.* (1993).

Rumen-fistulated Friesian cows were kept in individual stalls and used in $2 \times 2 \times 2$ factorial experiments. Forage was available at all times, including a daily period of group exercise in a small yard, which meant that daily intakes could not be recorded. The treatments imposed involved all combinations of infusions of sodium acetate (Ac), infusions of sodium propionate (Pr) and distension of a balloon (Dist) in the rumen as follows: water (control), Ac, Pr, Dist, Ac+Pr, Ac+Dist, Pr+Dist, Ac+Pr+Dist. Treatments were applied from 11.00 to 14.00 hours and at least 48 h elapsed between successive treatments, which were given in a partial Latin square design. Fresh forage was offered at the start of treatment and refusals were weighed at the end of the 3 h treatment period and again 2 h later. Rumen fluid samples were taken just before the start of treatment and 0·5, 1, 2, 3, 4 and 5 h later. The samples were stored at −20° and later analysed for VFA by gas–liquid chromatography and for osmolality by freezing-point depression after centrifugation to remove all particulate matter. The results were analysed using analysis of variance (Statistical Analysis System, 1982) for the effects of Ac, Pr, Dist, animal and date and for first-order interactions between Ac, Pr and Dist.

Expt 1

Six cows in mid-lactation (mean milk yield, 21 kg/d) and 2 months pregnant were offered ryegrass (*Lolium perenne* L.) hay *ad lib.* and a daily total of 7 kg concentrate ration (fresh weight) given in equal portions at morning (07.00 hours) and afternoon (16.00 hours) milking. The composition of the feeds is given in Table 1. On each of eight experimental days each cow was given a different combination of treatments, with at least 2 d between successive treatments. The experiment was then repeated over a further eight experimental days. The treatments were: Ac, 5·5 mol; Pr, 5·2 mol (both in 4·5 litres water); Dist, 7·5 litres of balloon inflation. These levels were shown in previous work to have small depressing effects on hay intake (Anil *et al.* 1993).

Expt 2

Seven cows in the second and third months of lactation (mean milk yield, 25 kg/d) were offered grass silage *ad lib.* and 3·5 kg concentrate with 2·5 kg sugar-beet pulp (fresh weights) given in two equal portions at morning and afternoon milking. The experiment was carried out over eight experimental days and was not repeated. The treatments were: Ac, 9·0 mol; Pr, 4·0 mol (both in 4·5 litres water); Dist, 10·0 litres of balloon inflation. These levels were shown in previous work to have small depressing effects on silage intake (Anil *et al.* 1993).

RESULTS

Expt 1

Voluntary intake of hay. Mean dry matter (DM) intakes of hay are given in Table 2. There was a significant overall depressing effect of Ac on intake during infusion (2·87 *v.* 2·48; $P = 0{\cdot}01$), whereas the effect of Dist was not quite significant (2·80 *v.* 2·55; $P < 0{\cdot}07$) and

Table 1. *Composition of feeds*

No. of samples (analysed in duplicate)...	Concentrate 5	Sugar-beet pulp 5	Hay 10	Silage 18
Dry matter (DM) (g/kg)	883	909	906	196
Crude protein* (g/kg DM)	167	103	138	151
MAD fibre (g/kg DM)	98	152	386	393
Diethyl ether extract (g/kg DM)	21	11	25	43
Ash (g/kg DM)	43	76	47	87

MAD fibre, modified acid-detergent fibre.
* Nitrogen × 6·25.

Table 2. *Expt 1. Mean hay intake (kg dry matter (DM)) by lactating cows given combinations of acetate (Ac), propionate (Pr) and distension (Dist) in the rumen*†

(Mean values for seven determinations)

Treatment period (h)... Treatment	0–3	3–5	0–5
Water (control)	3·02	0·64	3·66
Ac (5·5 mol)	2·62	0·68	3·31
Pr (5·2 mol)	2·97	0·68	3·66
Dist (7·5 l)	2·89	0·86	3·76
Ac+Pr	2·51	0·28**	2·79**
Pr+Dist	2·44*	0·60	3·05*
Ac+Dist	2·29**	0·66	2·94*
Ac+Pr+Dist	2·42*	0·38*	2·80**
SED	0·26	0·11	0·29

SED, standard error of the difference between treatment means.
Mean values were significantly different from those for water: * $P < 0·05$, ** $P < 0·01$.
† For details of treatments and procedures, see p. 714.

there was no apparent effect of Pr (2·72 *v.* 2·62; $P = 0·46$). None of the first-order interactions was significant ($P > 0·39$). There were significant carry-over effects of Ac ($P < 0·01$) and Pr ($P < 0·001$) during the 2 h after the end of the treatment period, but again there were no significant interactions.

When the eight treatments were considered separately, those 3 h intakes which were significantly ($P < 0·05$) lower than control intakes were: Ac+Dist, Pr+Dist, Ac+Pr+Dist. There were significant ($P < 0·05$) carry-over effects during the 2 h periods after treatment following Ac+Pr and Ac+Pr+Dist.

VFA concentrations in rumen fluid. Mean acetate, propionate and butyrate concentrations in rumen fluid from 0·5 to 3 h and 4 to 5 h are shown in Table 3. The Ac and Ac+Dist treatments caused a significant ($P < 0·05$) increase in rumen fluid acetate concentration during the 3 h treatment period, while with treatments including a combination of Ac+Pr the increase in acetate concentration in rumen fluid just failed to reach significance. During the 2 h post-treatment period, acetate concentration remained slightly higher than the control for the Ac and Ac+Dist treatments although this was not significant.

Administering Pr either separately or in combination with Ac or Dist, or both, increased propionate concentrations ($P < 0·05$) during the three periods and tended to decrease acetate concentration in rumen fluid. Distension of the rumen had no effect on acetate and

Table 3. *Expt 1. Effects of ruminal administration of acetate (Ac), propionate (Pr) and distension (Dist) on volatile fatty acid concentrations and osmolalities in rumen fluid of cows fed on hay*†

(Mean values for no. of observations/cow shown)

	Volatile fatty acids (mmol/l)				Osmolality (mosmol/kg)	
Treatment period (h)...	0·5–3		4–5		0·5–3	4–5
n	18		12			
	Ac	Pr	Ac	Pr		
Water (control)	46·4	15·2	51·5	15·4	263	264
Ac	68·8*	14·1	65·0	12·3	229*	279
Pr	43·5	27·0*	43·8	25·6	292*	274
Dist	49·9	14·1	52·2	15·1	276*	266
Ac+Pr	60·5	27·1*	47·6	22·8	316*	307
Ac+Dist	69·3*	15·3	66·4	14·5	309	279
Pr+Dist	44·7	28·9*	43·9	33·7*	282	283
Ac+Pr+Dist	61·2	29·7*	56·8	28·2*	318*	291
SED	7·3	2·9	8·3	2·9	9·0	5·8

SED, standard error of the difference between treatment means.
Mean values were significantly different from those for water (control): * $P < 0·05$.
† For details of treatments and procedures, see p. 714.

propionate concentrations and butyrate was not affected by any treatment. Acetate concentration in rumen fluid was not correlated with hay intake whereas the latter was correlated with propionate concentration during the 0–3 h (r −0·28, $P < 0·05$) and 0–5 h (r −0·37, $P < 0·05$) periods.

Osmolality of rumen fluid. Mean osmolalities from 0·5 to 3 and 4 to 5 h are shown in Table 3. During the treatment period the main effect of Ac was significant ($P < 0·001$), as was that of Pr ($P < 0·05$). There was no main effect of Dist and no significant interactions between the effects of Ac, Pr and Dist. As expected, osmolality was higher when Ac+Pr was infused, compared with either given alone. There was no significant correlation between rumen osmolality and hay intake even when the results of treatments involving Dist were omitted.

Osmolality returned towards control values after the infusions finished, but the effects of Ac and Pr were still significant ($P < 0·05$).

Expt 2

Voluntary intake of silage. DM intakes of silage are given in Table 4. The depressing effect of Ac on silage intake during the treatment period was significant (1·24 *v.* 0·99; $P < 0·05$), as were the effects of Pr (1·23 *v.* 1·00; $P < 0·05$) and Dist (1·29 *v.* 0·92 kg DM; $P < 0·01$). None of the first-order interactions was significant but with both Ac+Dist and Pr+Dist the depression in intake was approximately twice the sum of the separate effects.

When the data were analysed with the eight combinations as separate treatments those 3 h intakes which were significantly lower ($P < 0·05$) than control intakes were: Ac+Dist, Pr+Dist, Ac+Pr+Dist. During the 2 h after treatment, intakes were still significantly depressed following the Ac, Ac+Pr, Ac+Dist, Pr+Dist, Ac+Pr+Dist treatments.

VFA concentrations in rumen fluid. The mean values for acetate, propionate and butyrate concentrations are given in Table 5. The administration of Ac+Pr+Dist together

Table 4. *Expt 2. Mean silage intake by lactating cows given combinations of acetate (Ac), propionate (Pr) and distension (Dist) in the rumen (kg dry matter (DM))*†

(Mean values for seven determinations)

Treatment period (h)... Treatment	0–3	3–5	0–5
Water (control)	1·40	0·77	2·17
Ac (9·0 mol)	1·20	0·44*	1·64
Pr (4·0 mol)	1·24	0·59	1·83
Dist (10·0 l)	1·25	0·84	2·10
Ac+Pr	1·23	0·41*	1·64
Pr+Dist	0·93*	0·54**	1·47
Ac+Dist	0·93*	0·45*	1·38*
Ac+Pr+Dist	0·51**	0·05**	0·56**
SED	0·20	0·27	0·35

SED, standard error of the difference between treatment means.
Mean values were significantly different from those for water: * $P < 0.05$, ** $P < 0.01$.
† For details of treatments and procedures, see p. 714.

Table 5. *Expt 2. Effects of ruminal administration of acetate (Ac), propionate (Pr) and distension (Dist) on volatile fatty acid concentrations and osmolalities in rumen fluid of cows fed on silage*†

(Mean values for no. of observations/cow shown)

	Volatile fatty acids (mmol/l)				Osmolality (mosmol/kg)	
Treatment period (h)...	0·5–3		4–5		0·5–3	4–5
n	21		14			
	Ac	Pr	Ac	Pr		
Water (control)	54·1	19·5	51·4	19·3	289	278
Ac	90·0	15·3	71·5	13·9	395	348
Pr	52·3	32·2	45·8	26·9	330	306
Dist	64·1	20·3	59·4	17·5	285	284
Ac+Pr	63·9	39·1	79·3	40·8*	452*	359*
Ac+Dist	77·7	21·5	73·1	19·9	444*	312
Pr+Dist	51·9	40·9	48·5	26·7	339	310
Ac+Pr+Dist	129·3**	63·0**	88·6**	38·1*	534*	377*
SED	18·2	10·6	12·8	7·2	50·5	23·8

SED, standard error of the difference between treatment means.
Mean values were significantly different from those for water: * $P < 0.05$, ** $P < 0.01$.
† For details of treatments and procedures, see p. 714.

significantly increased both rumen fluid acetate and propionate concentrations compared with the control during the 0·5–3 h, 3–5 h, and 4–5 h periods. Silage intakes were not significantly correlated with mean acetate or propionate concentrations in the rumen during any of the three periods. The treatments Ac, Ac+Dist and Pr, Pr+Dist caused non-significant increases in acetate and propionate concentrations respectively during the three periods. Butyrate concentrations remained stable and unaffected by treatments.

Osmolality of rumen fluid. Mean osmolalities for 0·5–3 h and 4–5 h are shown in Table 5. The main effect of Ac during the treatment period was significant ($P < 0.001$), while that of Pr did not reach significance ($P < 0.10$). There was no effect of Dist and no significant interactions between the effects of Ac, Pr and Dist. The increase in osmolality during infusion was, as expected, greater when both Ac and Pr were infused than when either was given alone.

The most striking feature of these results is that although the osmolality before the start of infusion was only slightly greater with silage than with hay (Expt 1) the increases during treatment were several-fold greater. Larger amounts of Ac were infused in Expt 2 than in Expt 1 (9·0 *v.* 5·5 mol Ac/3 h) while the amount of Pr given (4·0 mol/3 h) was less than that given in Expt 1 (5·2 mol/3 h). In all treatments which involved Ac, osmolality rose quickly to peak at 1 h, then declined at 2 h to be followed by a second, but smaller, peak at the end of infusion.

There was no significant correlation between individual values of rumen osmolality and corresponding silage intakes, even when results from treatments involving Dist are omitted.

DISCUSSION

The results from the present study confirm previous findings (Adams & Forbes, 1981) that treatments which do not have a significant effect on food intake when given separately in the rumen cause significant depressions in intake when given together.

In previous experiments (Anil *et al.* 1993) it had been shown that levels of treatment (Ac, Pr, Dist) above the physiological range did not cause feeding to cease, so that no single factor could be seriously proposed as the only controller of feeding. We can now speculate that the many small changes occurring in relation to feeding, including intestinal concentrations and abdominal temperature, act together to inform the central nervous system which integrates the incoming signals in an approximately additive manner. If this is true then it has important implications. For example, rather than envisaging a static relationship between the degree of distension of the rumen and food intake, we should think of a dynamic situation in which the degree of distension required to inhibit feeding will depend on the extent to which the other families of abdominal receptors are being stimulated.

Increased rates of utilization of VFA would be expected to lead to reduced concentrations and, therefore, reduced stimulation of VFA-sensitive receptors. A greater degree of rumen distension could, therefore, be accommodated before the total of the signals from abdominal organs became satiating. Tulloh (1968) observed that lactating cows had a greater volume of rumen contents than non-lactating cows and Weston (1984) found that more rapidly growing sheep had greater rumen fill, both these examples being with feeds whose voluntary intake would be expected to be 'physically' limited. Casein infusion into the duodenum of sheep fed on a low-protein forage increased feed intake and rumen fill and led Egan (1970) to state 'that a mechanism of interacting physiological and metabolic factors may be involved throughout the whole range of diets utilized by ruminants, and that there is not simply a switch-over to metabolic regulation at a point where disposal of indigestible bulk is no longer an embarrassment or a limitation to the total digestible energy intake'. This view is entirely consistent with the concept of additivity of feedback signals in the control of feed intake which the currently-reported results support.

The ways in which the sodium salts of VFA affect intake have been discussed by Anil *et al.* (1993) and it is entirely possible that most, if not all, the effects of Ac and Pr observed in these experiments were due to their effects on the osmolality of rumen fluid. There have been brief reports of osmotic effects on receptors in the rumen wall but the results have been very variable (B. F. Leek, personal communication). However, Ternouth & Beattie (1971) and Carter & Grovum (1988) have clearly demonstrated effects of ruminal infusions on feeding in sheep which are related to rumen osmolality rather than to that of abomasal contents or blood plasma. A particularly striking observation in the experiments reported here is the greatly elevated osmolalities when salts of VFA were infused into the rumen of cows fed on silage (Expt 2) compared with hay (Expt 1), despite the lack of such large differences in the ruminal concentration of VFA. This suggests that sodium was being retained in the rumen to a greater extent with silage than with hay, but previous work under the same conditions (Anil *et al.* 1993) showed similar concentrations during infusion for hay and silage; pH was also unaffected by diet or infusion.

A difference in osmolality between hay and silage is not the only reason for differences in intake between the two feeds as silage intakes during water (control) and Dist treatments were considerably lower than for hay despite the relatively small differences in rumen osmolality. Further, even when results from treatments involving Dist were omitted there were no significant correlations between the osmolality of rumen fluid and voluntary intake.

If a major route for effects of sodium salts of VFA on feeding is by elevation of the osmolality of rumen fluid, then it would be expected that Ac would have additive effects with Pr in the experiments reported here; this was not obviously the case in either Expt 1 (Tables 2 and 3) or Expt 2 (Tables 4 and 5) even though osmolality was much higher during the combined infusion of the two salts than with either given alone. We cannot explain why intake was not further depressed with the treatment with Ac and Pr, compared with Ac alone.

Dist had no effect on the concentration of VFA, nor the osmolality of rumen contents, and it can be concluded that the effect of this treatment on food intake was by stimulation of mechano-receptors in the rumen wall.

In conclusion, within physiological conditions a single signal in the rumen is unlikely to be adequate to influence feeding behaviour seriously in the lactating dairy cow. Rather, it appears that satiety can be induced by the additive effects of many negative feedback signals and that daily food intake will depend on the balance between such negative effects and the positive influences of nutrient utilization.

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