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Preference for flavored wheat straw by lambs conditioned with intraruminal infusions of acetate and propionate

Frederick D Provenza

Preference for Flavored Wheat Straw by Lambs Conditioned with Intraruminal Infusions of Acetate and Propionate[1,2]

Juan J. Villalba and Frederick D. Provenza

Department of Rangeland Resources, Utah State University, Logan 84322-5230

ABSTRACT: We hypothesized that volatile fatty acids in rumen fluid are feedback signals that can condition food preferences or aversions in sheep. Three predictions were tested based on this hypothesis: 1) low doses of sodium propionate or sodium acetate condition preferences, but high doses condition aversions (Exp. 1 and 2); 2) preferences are not caused by osmotic load (Exp. 3 and 4); and 3) low doses of mixtures of acetate:propionate condition preferences (Exp. 4). In Exp. 1, 2, and 4, lambs were divided into four groups (10 lambs/group), and lambs in Exp. 3 were divided into two groups (five lambs/group). In all experiments, alfalfa pellets were the basal diet. On even days, half of the lambs were offered chopped wheat straw containing a distinctive flavor, whereas the other half received straw with a different flavor. During straw ingestion, different groups of lambs received intraruminal infusions of different concentrations (4, 8, or 12% of the daily DE received) of sodium propionate (Exp. 1), sodium acetate (Exp. 2), NaCl at osmotic loads equivalent to those when propionate supplied 4% of the daily DE received (Exp. 3), or different proportions of sodium acetate:sodium propionate (55:45 or 75:25% of the DE of the infusion [4% of the daily DE received]), or equimolar amounts of NaCl (Exp. 4). On odd days, the flavors were switched, and no infusions were administered. After 8 d of conditioning, lambs were offered a choice of wheat straw with the two distinctive flavors. Lambs preferred the flavor paired with the lowest doses of propionate (P = .07) and acetate (P = .08) but avoided the highest doses (P < .001). Excesses of VFA may condition aversions due to increases in rumen fluid osmolality and(or) excessive rates of supply of energy or sodium to the rumen. Lambs also preferred flavored straw associated with combinations of acetate and propionate (P < .001), especially at the highest concentration of propionate (P = .10). Lambs avoided NaCl in Exp. 3 (P < .001) and did not form preferences for NaCl in Exp. 4 (P > .05). Thus, osmolalities were not responsible for flavor preferences. In conclusion, our results support the hypothesis that food preferences and aversions reside along a continuum that depends on the amount of VFA infused.

Key Words: Sodium Propionate, Sodium Acetate, Acetates, Propionates, Osmotic Pressure, Lambs

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Introduction

The energy density of food is important in the regulation of intake by ruminants (Baile and Pfander, 1967; Baile and Forbes, 1974). The major energy sources for ruminants are VFA (i.e., acetic, propionic, butyric), which are the primary end-products of ruminal fermentation. Propionate, a gluconeogenic substrate, may play a primary role in controling food intake (Baile, 1971; Anil and Forbes, 1980). Acetate, the VFA produced and absorbed in greatest quantities, may also control intake (Baile and Pfander, 1966; Baile and Forbes, 1974). Nevertheless, the specificity of VFA in intake regulation is controversial. Some attribute the satiating effects of VFA to a hypertonic load (Grovum, 1995), and others believe propionate and acetate regulate intake through different mechanisms (Baile, 1971; Farningham and Whyte, 1993). Propionate may inhibit feeding by stimulating afferent nerves in the liver (Anil and Forbes, 1980, 1987), and acetate may stimulate neural receptors in the rumen wall (Martin and Baile, 1972; Forbes, 1995). Thus, ruminants may discriminate among foods containing different energy densities by sensing different proportions of VFA produced in the rumen.

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Besides affecting intake, VFA also affect food preferences. Lambs acquire preferences for low-quality foods eaten when sodium propionate is administered intraruminally (Villalba and Provenza, 1996). Low doses of propionate stimulate intake and condition preferences (Villalba and Provenza, 1996), but higher doses depress intake and condition aversions (Ralphs et al., 1995). Thus, food preferences and aversions probably reside along a continuum; either of these states can be conditioned depending on an animal's needs and the nutrient concentrations in the food (Provenza, 1995a).

It is not known to what degree propionate, acetate, and combinations of acetate:propionate condition preferences or aversions in ruminants. Experiments were conducted to determine whether 1) preferences and aversions are influenced by the amount of propionate or acetate delivered into the rumen, 2) preference depends on the combinations of acetate: propionate, and 3) hypertonicity from salt infusion affects food preference.

Materials and Methods

Experiments were conducted at the Green Canyon Ecology Center located at Utah State University in Logan. During each experiment, lambs (Finn-Polypay-Suffolk crossbred lambs of both sexes) were individually penned and had free access to salt blocks and fresh water.

In all experiments, alfalfa pellets were the basal diet and were fed at 1200 daily. Alfalfa pellets plus straw and the amounts of propionate/acetate infused provided approximately 80% of the DE requirement of the lambs (NRC, 1985).

Adaptation to Flavored Straw

Forty lambs in Exp. 1, 2, and 4 (38 kg BW) and 10 lambs in Exp. 3 (39 kg BW) were randomly divided into two groups. From 0800 to 1200 on even days, one group was offered wheat straw (1 to 2 cm particle size) containing a particular flavor (Flavor 1), and the other group was offered straw with a different flavor (Flavor 2). On odd days, straw was again offered to all lambs, but the flavors were switched so lambs that received, for example, Flavor 1 on even days received Flavor 2 on odd days.

After 8 d, lambs were offered straws with each flavor for 15 min, and intake and preference for each flavor were determined. Lambs were then randomly assigned to groups so that the groups were balanced according to flavor preference (10 lambs/group in Exp. 1, 2, and 4; 5 lambs/group in Exp. 3).

Experiments 1, 2, and 4 were conducted in successive order, beginning the day after the preceding experiment was finished. Experiments 2 and 3 were conducted simultaneously.

Experiment 1

The objective of this experiment was to determine whether preference for flavored straw was influenced by the amount of propionate infused into the rumen.

Conditioning. On even days, half of the lambs in each of four groups were offered oregano-flavored straw and the other half were offered onion-flavored straw from 0800 until 1200. Flavors (Pacific Foods, Washington) were mixed with straw at a concentration of 1%. Immediately after beginning to eat straw, lambs in all groups received by gavage intraruminal infusions (200 mL) of water (Control; Group 1) or water solutions of sodium propionate that supplied approximately 4 (414 mmol; Group 2), 8 (828 mmol; Group 3), or 12% (1,242 mmol; Group 4) of the total daily DE received (Treatment 1). On odd days, straw was offered to all lambs, but the flavors were switched so lambs that received, for example, oregano-flavored straw on even days received onion-flavored straw on odd days. During odd days, no water or propionate infusions were administered (Treatment 2); instead, only the tube used for the gavage (on even days) was introduced for a few seconds into each lamb's throat immediately after it started eating. The amount of straw offered on even days was equal to a lamb's intake of straw the previous day, which controlled for exposure to the flavored straw. Lambs consumed 84 g/d (SEM = 2.4) of flavored straw during even days and 86 g/d (SEM = 2.2) during odd days. Alfalfa intake ranged from 1,300 to 1,480 g/d, depending on the dose of VFA administered.

Preference Tests. After 8 d of conditioning (four even and four odd days), lambs received oregano- and onion-flavored straw simultaneously for 15 min, and intake of each food was measured. Preference tests were performed on two consecutive days, and during these days no intraruminal infusions of water or propionate were given.

Experiment 2

This experiment was conducted to determine whether preference for flavored straw by lambs was influenced by the amount of sodium acetate delivered into the rumen. Lambs used in Exp. 1 were used in Exp. 2, but due to their previous experience with onion and oregano, these flavors were replaced with maple and anise (Agrimerica, Inc., IL) at 1% concentration.

Conditioning occurred as in Exp. 1, but on even days lambs received intraruminal infusions (200 mL) of water (Control; Group 1) or of water solutions with sodium acetate, which supplied approximately 4 (725 mmol; Group 2), 8 (1,450 mmol; Group 3), or 12% (2,175 mmol; Group 4) of the total daily DE offered. Due to the high molarity of acetate in the last two doses, we added 100 mL of water to each infusion of 1,450 and 2,175 mmol. Lambs consumed 83 g/d of flavored straw (SEM = 2.5) during even days and 87 g/d (SEM = 2.2) during odd days. After 8 d of conditioning, we tested for preference as in Exp. 1.

Experiment 3

In this experiment, we determined whether osmotic load affected preference for flavored wheat straw. Lambs received NaCl to generate osmotic loads equivalent to the lowest molar concentration delivered with VFA in the previous experiments (414 mmol of sodium propionate; Exp. 1).

After stratification by flavor preference and assignment to two groups, lambs were conditioned as follows. On even days, lambs in Group 1 were offered oregano-flavored straw, and lambs in Group 2 were offered onion-flavored straw from 0800 until 1200. Immediately after beginning to eat the straw, lambs in both groups were given a water solution of NaCl (414 mmol/200 mL; Treatment 1). On odd days, flavors were switched, but no NaCl was given (Treatment 2). Lambs consumed 88 g/d (SEM = 4.4) of flavored straw during even days and 92 g/d (SEM = 4.7) during odd days. After 8 d of conditioning, we tested for preference as in Exp. 1.

Experiment 4

In this experiment, we determined whether lambs preferred flavored straw associated with intraruminal infusions of different molar proportions of acetate and propionate. We also determined whether osmotic loads generated by the infusions affected preference. Lambs used in Exp. 1 and 2 were used in Exp. 4, but due to the previous experience with onion, oregano, maple, and anise, two new flavors (coconut and apple; Agrimerica) were added at 1% concentration to the straw.

The conditioning procedure was as described in Exp. 1, but during even days lambs received intraruminal infusions of water (Control 1; Group 1) or of water solutions with NaCl (647 mmol; Control 2; Group 2), acetate, propionate, and NaCl (399, 187, and 61 mmol, respectively; Group 3), or acetate and propionate (543 and 104 mmol, respectively; Group 4). The amount of DE was the same in Groups 3 and 4 and represented approximately 4% (.152 Mcal) of the total daily DE received, but propionate supplied 45% (Group 3) and 25% (Group 4) of the total DE in the infusion. The NaCl was added to the mixture of VFA containing the highest amount of propionate (Group 3) to control for osmotic effects. Thus, Groups 2, 3, and 4 received intraruminal infusions of the same Na concentration.

Lambs consumed 113 g/d of flavored straw (SEM = 1.5) during even days and 107 g/d (SEM = 1.0) during odd days. After 8 d of conditioning, we tested for preference as in Exp. 1.

Ruminal Determinations

The same diets and conditioning schedules described above were used with an additional group of four lambs. Samples of rumen fluid were obtained through a stomach tube before and .5, 1, and 2 h after infusions of propionate, acetate, mixtures acetate: propionate, water, or NaCl. Ruminal pH was measured and samples (18 mL) of rumen fluid were mixed with 2 mL of 6 *N* HCl, centrifuged, and prepared for VFA determinations (Wiedemeier et al., 1987).

Statistical Analyses

Before Conditioning. After stratification by flavor preference and random assignment to four (Exp. 1, 2, and 4) or two groups (Exp. 3), food intake during the initial preference test was analyzed as a split plot with lambs nested within groups. Group was the between-lamb factor and the treatment to be applied during the following conditioning procedure (1 [metabolite infusions] or 2 [no infusions]) was the within-lamb factor in the split plot.

Preference Tests. Food intake during the preference tests was analyzed as a split-plot design with lambs nested within group. Group was the between-lamb factor, and treatment received during conditioning (1 [metabolite infusions] or 2 [no infusions]) was the within-lamb factor in the split plot. Day was the repeated measure. Means were compared using the LSD test.

To determine whether initial (i.e., before conditioning) flavor preference changed after conditioning, food intake was analyzed as described above, but period (before and after conditioning) was the repeated measure in the split plot. Only one preference test was conducted before conditioning, but two (Exp. 1, 2, and 3) or three consecutive (Exp. 4) tests were conducted after conditioning, so separate analyses were performed to compare responses before conditioning with those obtained for each day of testing after conditioning.

Ruminal Determinations. Values for pH and VFA concentrations were analyzed in a split-plot design with lambs and dose of substance infused as the whole plot and time of rumen fluid extraction as the split plot. When dose × time interactions occurred ($P < .05$), means were compared using the LSD test.

Results

Experiment 1

Lambs did not differ in initial preference for the flavors to be associated with water or with propionate during conditioning: 60 vs 67 (control [water], Group 1); 43 vs 43 (4% DE, Group 2); 61 vs 56 g (8% DE, Group 3); and 58 vs 55 g (12% DE, Group 4), for Treatments 1 and 2, respectively ($P > .05$; SEM = 10). After conditioning, lambs preferred the flavored straw paired with the lowest dose of propionate (4% of the total DE offered; $P = .07$), but they avoided the flavor paired with propionate at 8 ($P = .07$) and 12% ($P < .001$) of the total DE intake (treatment × group

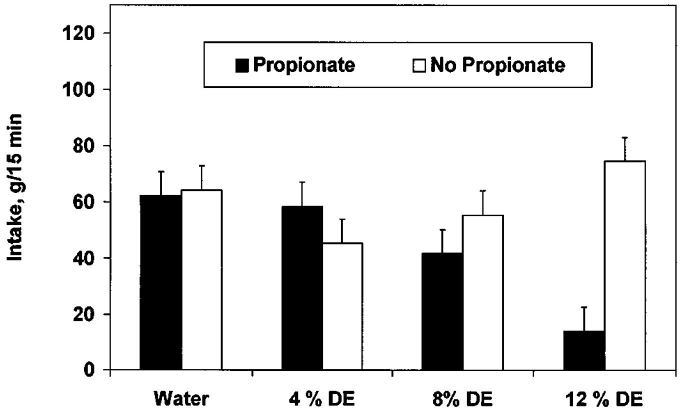

Figure 1. Mean (± SEM) intake of flavored straw by four groups of lambs during a preference test conducted after 8 d of conditioning with (even days, Propionate) or without (odd days, No Propionate) intraruminal infusions of water or sodium propionate. Means differed in groups that received 4 (P = .07), 8 (P = .07), and 12% (P < .001) of the total DE offered daily in sodium propionate.

interaction P < .001; Figure 1). No preference was found for the flavored straw paired with water (control; P > .05; Figure 1).

Experiment 2

Lambs did not differ in initial preference for the flavors to be associated with water or with acetate during conditioning: 67 vs 46 (control [water], Group 1); 59 vs 63 (4% DE, Group 2); 71 vs 57 g (8% DE, Group 3); and 57 vs 62 g (12% DE, Group 4), for Treatments 1 and 2, respectively (P > .05; SEM = 12). After conditioning, lambs preferred the flavored straw paired with the lowest dose of acetate (4% of the total DE offered; P = .08), but they avoided the flavors paired with the highest dose (treatment × group interaction P < .001; Figure 2). Lambs in the control group (Group 1) preferred flavored straw paired with water (P < .05; Figure 2), but their preference before conditioning (67 vs 46 g for Treatments 1 and 2, respectively) did not change after conditioning in either the first (72 vs 43 g) or the second (68 vs 53 g) preference test (P > .05).

Experiment 3

Lambs did not display an initial preference for the flavors to be associated with NaCl during conditioning: 56 vs 48 g for Treatments 1 and 2, respectively (P > .05; SEM = 16). However, lambs avoided flavored straw paired with NaCl after conditioning (19 vs 64 g; P < .001), and there was no treatment × group interaction (P > .05).

Experiment 4

No differences were detected in initial preference for the flavors to be associated with water, NaCl, or VFA mixtures during conditioning: 51 vs 50 (control 1 [water]; Group 1); 66 vs 40 (control 2 [NaCl], Group 2); 56 vs 56 (45% propionate, Group 3); 59 vs 47 (25% propionate, Group 4), for Treatments 1 and 2, respectively (P > .05; SEM = 9). After conditioning, lambs preferred the flavored straw paired with mixtures of VFA (Groups 3 and 4; Figure 3; P < .001). Preference was higher for the acetate:propionate combination in Group 3 (73 vs 39 g) than in Group 4 (74 vs 47 g). Intake was lower for flavored straw not paired with VFA infusions in Group 3 than in Group 4 (P = .10; SEM = 6, Figure 3).

Preference declined on d 2 of testing for flavored straw paired with VFA mixtures. We tested for preference during an additional (third) day to determine whether preference would further decline in the absence of feedback (i.e., during preference tests no VFA infusions were given). Preferences did not differ across days (day effect and treatment × day interaction, P > .05).

Lambs preferred flavored straw paired with infusions of water (P < .05; Figure 3), but they more strongly preferred flavored straw paired with VFA. Intake of flavored straw paired with water (57 g) was lower than intake of flavored straw paired with VFA mixtures (73 g for Group 3 and 74 g for Group 4; P < .001; SEM = 6; Figure 3); intake did not differ for flavors not associated with VFA (P > .05).

When flavored straw was paired with NaCl, intake decreased (P < .05) from 66 g before conditioning to 54 and 46 g in the first and third preference tests after conditioning, respectively; when flavored straw was

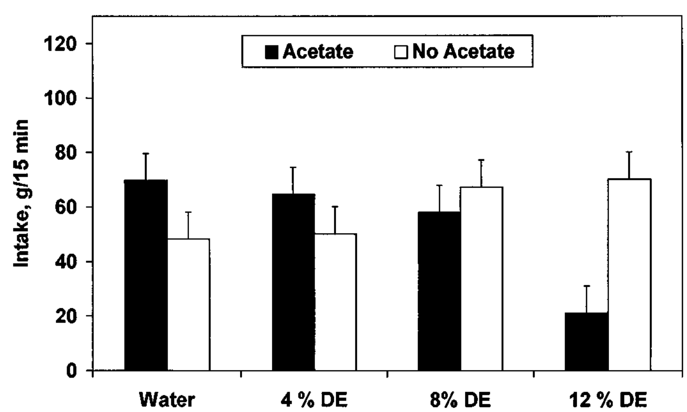

Figure 2. Mean (± SEM) intake of flavored straw by four groups of lambs during a preference test conducted after 8 d of conditioning with (even days, Acetate) or without (odd days, No Acetate) intraruminal infusions of water or sodium acetate. Means differed in groups that received water (P < .05) and 4 (P = .08) and 12% (P < .001) of the total DE offered daily in sodium acetate.

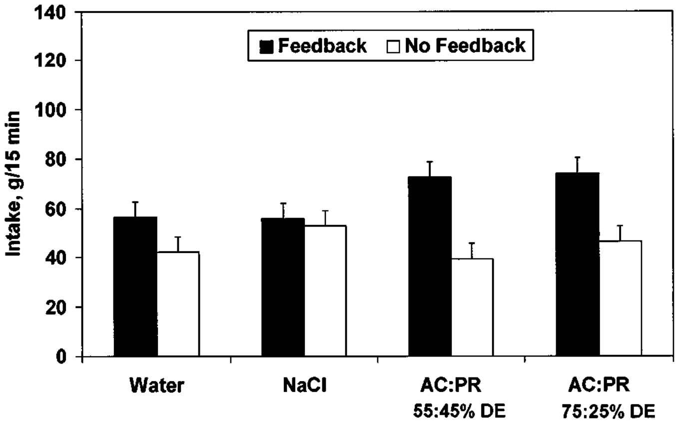

Figure 3. Mean (± SEM) intake of flavored straw by four groups of lambs during a preference test conducted after 8 d of conditioning with (even days, Feedback) or without (odd days, No Feedback) intraruminal infusions of water, sodium chloride, or combinations of sodium acetate:sodium propionate (AC:PR). Means differed in groups that received water ($P < .05$) and AC:PR ($P < .001$) in proportions that represented 55:45 and 75:25% of the DE of the infusions (4% of the total DE offered daily).

not paired with NaCl, intake increased ($P < .05$) from 40 g before conditioning to 55 and 53 g in the first and third preference tests after conditioning, respectively.

Ruminal Determinations

Rumen pH was not affected by the different doses and electrolytes infused, as evidenced by nonsignificant treatment × time interactions ($P > .05$; Table 1). The pH values did not change with time ($P > .05$) and were near neutrality (ranging from 6.7 to 7.4; Table 1), suggesting that acidity associated with VFA infusions did not influence preference or food intake.

Propionate and acetate concentrations in the rumen fluid increased in a dose-dependent manner with the respective VFA infusions, which caused significant treatment × time interactions ($P < .05$; Table 1). Propionate and acetate increased dramatically when they were given at high doses (i.e., 8 and 12% of the daily DE intake) and reached concentrations comparable to or greater than those reported to depress intake in ruminants (Simkins et al., 1965; Engku Azahan and Forbes, 1992; Mbanya et al., 1993). Conversely, administration of NaCl decreased ruminal concentrations of acetate and propionate. Acetate infusions decreased propionate concentrations, probably due to propionate oxidation or propionate conversion to acetate (Seal and Parker, 1994). In general, isobutyrate and isovalerate decreased with VFA administrations, but they increased after the highest propionate dose (Table 1).

Discussion

Continuum Preferences – Aversions

Lambs developed preferences for flavored straw paired with low doses of propionate (414 mmol; Exp. 1) or acetate (725 mmol; Exp. 2). Conversely, when more propionate (i.e., 828 or 1,242 mmol) or acetate (2,175 mmol) was administered, lambs developed strong aversions. Similar responses have been demonstrated with rats using sucrose as the energy source (Warwick and Weingarten, 1996). These findings are consistent with the hypothesis that food preferences and aversions reside along a continuum where either of these states can be conditioned depending on an animal's physiological condition and the nutrient concentrations in the food (Provenza, 1995a).

Preference increases when a feed ameliorates a particular need, and preference decreases when needs are met or exceeded (Provenza, 1995b). In our study, energy deprivation (80% DE required) likely enhanced the preference for flavored straw associated with infusions of low doses of VFA. Energy-restricted lambs prefer straw paired with low doses of propionate (1.9 to 2.8 mmol/kg BW), and the preferences persist even after propionate is no longer administered (Villalba and Provenza, 1996). By contrast, higher doses of VFA, equivalent to or greater than those reported to reduce intake in sheep (Baile and Pfander, 1966; Ternouth and Beattie, 1971; Papas and Hartfield, 1978), caused aversions in our study (Figures 1 and 2). Excesses of VFA may decrease preference by stimulating specific VFA receptors or unspecific osmoreceptors that send negative feedback signals to the central nervous system (Engku Azahan and Forbes, 1992), perhaps by stimulating the emetic system (Provenza et al., 1994).

Inhibition of food intake often requires superphysiological levels of VFA in the rumen (Egan, 1966; Anil et al., 1993; Mbanya et al., 1993), but that does not mean that ruminants cannot sense the positive effects of physiological levels of VFA. We demonstrated that lambs preferred doses of propionate and acetate equivalent to or below those considered ineffective in suppressing feeding (Egan, 1966; Papas and Hartfield, 1978). Thus, low doses of propionate stimulate feeding (Ulyatt, 1965) and enhance preference (Villalba and Provenza, 1996). The linear, dose-dependent relationship between intake depression and VFA infusions (Anil et al., 1993) suggests that responses to VFA are graded.

Our results also suggest that the rate of fermentation affects preference and may be one of the causes for varied diets (Provenza, 1995a). Foods that contain high levels of readily available carbohydrates are likely to be preferred initially but subsequently avoided when ingested in high amounts, because excessive production of organic acids in the rumen may cause a transitory decrease in preference for

Table 1. The pH and VFA in rumen fluid of four lambs before and after intraruminal administrations of water, VFA, and NaCl

Measure and infusion	mmol	Time after infusion, h				SEM
		0	.5	1	2	
		pH				
pH						
Water	0	6.9	6.7	7.2	6.9	
Propionate (PR)	414	7.3	7.4	7.4	7.3	.151
	828	7.2	7.2	7.2	7.3	
	1,242	7.1	6.9	6.8	6.8	
Acetate (AC)	725	7.3	7.3	7.3	7.3	.148
	1,450	7.0	7.0	6.9	7.0	
	2,175	7.1	7.2	7.2	7.1	
NaCl	647	6.9	6.9	6.9	6.8	.154
AC:PR:NaCl	399:187:61	7.3	7.3	7.3	7.2	
AC:PR	543:104	7.2	7.2	7.2	7.2	
		mmol/L				
Acetate						
Water	0	58.12	56.69	52.45	50.72	
PR	414	34.47	31.33	33.98	28.59	3.430
	828	32.77	31.71	31.92	26.39	
	1,242	55.72	58.93	57.15	50.44	
AC	725	30.13[a]	142.64[b]	156.69[b]	98.86[c]	11.978
	1,450	61.82[a]	243.94[b]	232.28[b]	177.03[c]	
	2,175	48.72[a]	281.68[b]	223.68[c]	217.55[c]	
NaCl	647	96.21[a]	73.19[b]	66.84[b]	54.16[c]	6.066
AC:PR:NaCl	399:187:61	49.60[a]	126.15[b]	101.25[c]	71.33[d]	
AC:PR	543:104	47.08[a]	142.68[b]	104.62[c]	83.48[d]	
		mmol/L				
Propionate						
Water	0	12.11	12.27	11.76	11.36	
PR	414	6.32[a]	76.27[b]	64.60[b]	46.99[c]	15.424
	828	6.77[a]	113.94[b]	108.96[b]	106.57[b]	
	1,242	12.28[a]	121.39[b]	147.43[b]	147.46[b]	
AC	725	7.68[a]	5.44[b]	5.13[b]	4.25[c]	.526
	1,450	13.73[a]	12.09[b]	11.91[b]	10.72[c]	
	2,175	11.13[a]	8.69[b]	6.92[c]	5.45[d]	
NaCl	647	20.00[a]	15.74[b]	14.71[b]	11.74[c]	1.597
AC:PR:NaCl	399:187:61	11.00[a]	45.79[b]	35.89[c]	23.57[d]	
AC:PR	543:104	10.03[a]	28.18[b]	19.91[c]	15.97[d]	
		mmol/L				
Butyrate						
Water	0	9.48	9.25	8.92	8.67	
PR	414	4.89	4.36	4.86	3.93	.421
	828	4.67	4.62	4.67	3.78	
	1,242	7.13	7.73	8.95	6.80	
AC	725	3.44	3.24	3.14	2.58	.289
	1,450	6.90	6.45	6.65	5.48	
	2,175	7.04	6.13	5.56	4.49	
NaCl	647	11.16	9.76	8.91	7.02	.471
AC:PR:NaCl	399:187:61	6.75	6.08	5.37	4.27	
AC:PR	543:104	6.77	6.73	5.15	4.36	
		mmol/L				
Isobutyrate						
Water	0	.63	.68	.69	.75	
PR	414	1.00[a]	.90[b]	.97[ab]	.92[ab]	.043
	828	1.17[a]	1.05[b]	1.06[b]	.89[c]	
	1,242	1.05[a]	1.23[b]	1.25[b]	1.20[b]	

(continued)

Table 1 (continued). The pH and VFA in rumen fluid of four lambs before and after intraruminal administrations of water, VFA, and NaCl

Measure and infusion	mmol	Time after infusion, h: 0	.5	1	2	SEM
AC	725	1.16[a]	.90[b]	.97[b]	.69[c]	.040
	1,450	.89[a]	.79[b]	.79[b]	.62[c]	
	2,175	.97[a]	.81[b]	.64[c]	.52[d]	
NaCl	647	.93[a]	.83[b]	.74[c]	.70[c]	.049
AC:PR:NaCl	399:187:61	.99[a]	.95[a]	.88[b]	.71[c]	
AC:PR	543:104	1.00[a]	.97[a]	.83[b]	.71[c]	
		mmol/L				
Isovalerate						
Water	0	.62	.62	.63	.69	
PR	414	1.10[a]	1.02[a]	1.16[b]	1.13[b]	.056
	828	1.33[a]	1.31[a]	1.40[a]	1.27[b]	
	1,242	1.12[a]	1.23[b]	1.51[c]	1.49[c]	
AC	725	1.32[a]	1.16[b]	1.11[b]	.78[c]	.040
	1,450	.91[a]	.90[a]	.94[a]	.72[b]	
	2,175	.96[a]	.89[a]	.80[b]	.75[b]	
NaCl	647	.84	.80	.81	.72	.055
AC:PR:NaCl	399:187:61	1.03	1.02	.99	.84	
AC:PR	543:104	1.11	1.10	.95	.81	
		mmol/L				
Valerate						
Water	0	.86	.82	.80	.78	
PR	414	.45	.47	.57	.57	.066
	828	.47	.58	.65	.65	
	1,242	.92	.95	.74	1.19	
AC	725	.44	.38	.34	.26	.049
	1,450	.97	.84	.87	.80	
	2,175	.91	.74	.64	.49	
NaCl	647	2.53	1.83	1.16	.65	.218
AC:PR:NaCl	399:187:61	.92	.74	.68	.56	
AC:PR	543:104	1.01	1.16	.61	.51	

[a,b,c,d]Means within a row lacking a common superscript letter differ ($P < .001$).

those foods. This phenomenon may explain why lambs often eat a low-energy food after initially preferring one of higher energy density (Provenza et al., 1996). Sheep prefer clover early in the day (e.g., high rates of fermentation and VFA production) after an overnight period without food, but they prefer more slowly digestible grass later (Parsons et al., 1994).

The cyclic pattern of intake typical of rapidly fermentable foods is also consistent with this hypothesis. Gradual increases in consumption of rapidly fermentable foods (e.g., grain) by ruminants may be the result of preferences enhanced by ruminal concentrations of VFA below satiety. Sharp declines in intake after overingestion may be the result of an excessive synthesis of by-products of rumen fermentation (e.g., VFA and other organic acids) with the concomitant acquisition of a food aversion (Provenza, 1996).

Osmolality

Lambs avoided flavors paired with osmotic loads of NaCl equivalent to those that conditioned a preference when the infusate contained sodium propionate or sodium acetate. During all experiments, lambs had free access to mineral blocks. Thus, administrations of sodium propionate, sodium acetate, or sodium chloride likely exceeded sodium needs.

Aversions to NaCl were more evident in Exp. 3 than in Exp. 4, perhaps because of differences in sodium exposure. The effect of NaCl on food intake diminishes with time (Baile and Pfander, 1966), and it is likely that lambs adapt to sodium (Ternouth and Beattie, 1971). In Exp. 1 and 3, lambs were exposed to infusions of sodium for the first time, whereas in Exp. 4 (e.g., the same animals used in Exp. 1 and 2) lambs had received sodium infusions, and some at the highest doses.

Our study suggests that lambs discriminated between infusions of similar osmolalities depending on the chemical composition of the nutrient. Sodium chloride conditioned aversions, whereas sodium propionate, sodium acetate, and mixtures of acetate: propionate conditioned preferences, which suggests lambs formed preferences for flavored straw paired with VFA through a mechanism independent of changes in osmolality.

Rumen fluid osmolality increases with infusions of VFA salts, and some argue that acetate and propionate inhibit feeding through osmotic actions (Grovum, 1995). Food intake is suppressed when excessive amounts of salts are infused into the rumen or when animals have restricted access to water (Ternouth and Beattie, 1971; Grovum and Bignell, 1989). Increases in rumen tonicity may inhibit cell wall fermentation (Bergen, 1972) or induce extracellular dehydration with the concomitant increase in plasma tonicity and appetite inhibition (Ternouth and Beattie; 1971; Silanikove, 1994). Nevertheless, hypertonicity of rumen fluid is not likely to be the satiety signal for an animal's energy requirements, which is probably the reason animals resume eating after drinking water (Barrio et al., 1991; Langhans et al., 1995) or receiving water in the rumen (Baile and Mayer, 1969; Ternouth and Beattie, 1971).

It is likely that satiety results from the additive actions of many feedback signals produced after the ingestion of a meal (Mbanya et al., 1993; Forbes, 1995). If additivity is a result of osmoreceptor stimulation alone, then the summation of osmolalities generated by specific by-products of fermentation (e.g., VFA) should lead to an additive decrease in intake, but such is not the case (Forbes and Barrio, 1992; Mbanya et al., 1993). Portal infusions of sodium propionate cause greater reductions in food intake by sheep than saline infusions of similar osmotic loads (Farningham and Whyte, 1993). Sodium butyrate or sodium bicarbonate infused into the rumen have a less depressing effect on food intake than equimolar amounts of sodium propionate or sodium acetate (Baile and Mayer, 1969). Low doses of propionate or cholecystokinin that alone do not affect intake decrease intake by 44% when administered together (Farningham, 1991; Farningham et al., 1993). Finally, the inhibition of food intake persists longer after infusions of sodium acetate than after infusions of NaCl (Baile and Pfander, 1966; Engku Azahan and Forbes, 1992). All of these results suggest ruminants discriminate between similar osmolalities promoted by different nutrients or by-products of fermentation.

Food ingestion in ruminants may be controlled by the additive actions of different feedback signals acting on particular sensory systems and by the nonspecific actions of rumen fluid tonicity. The former mechanism may be important when an animal has not approached satiety, or when the osmolality of the rumen fluid or by-products of fermentation is within physiological levels, whereas the latter mechanism may acquire particular relevance when VFA and other organic acids increase to superphysiological levels in the rumen, such as when water is withheld or when excessive amounts of concentrates are ingested.

Acetate:Propionate Mixtures

Lambs acquired preferences for flavored straw paired with mixtures acetate:propionate, and they formed stronger preferences for the combination with the highest proportion of sodium propionate (187 mmol). We cannot explain why lambs formed preferences for flavored straw associated with water, although those preferences were of lower magnitude than those formed for VFA infusions. Water infusions have been reported to have no actions on food intake by cattle and sheep when the basal diet is a roughage (Egan, 1966), but water can stimulate intake of a concentrate diet (Baile and Mayer, 1969) or a highly digestible forage (Ternouth and Beattie, 1971), probably by reducing rumen fluid osmolality.

Preferences obtained in this study were of relatively low magnitude compared to the near-total preferences conditioned with macronutrients in studies with nonruminants (Sclafani and Nissenbaum, 1988; Pérez et al., 1996). The experience with flavored straw during adaptation may have allowed the lambs to learn that the two flavors did not differ in their postingestive effects, which in turn could have attenuated preferences for flavors conditioned with VFA.

Lambs acquire preferences for the flavors of foods that provide energy, such as starch (Villalba and Provenza, 1997) or glucose (Burritt and Provenza, 1992; Ralphs et al., 1995). Sheep can discriminate among foods containing different levels of digestible energy and prefer the ones with high energy density (Glimp, 1971; Provenza et al., 1996). Foods with different net energy contents have distinct VFA profiles (Owens and Goetsch, 1988), which likely signal the food's energy density. Molar ratios of acetate:propionate:butyrate are usually near 65:25:10 with roughage diets and 50:40:10 with concentrate diets (Owens and Goetsch, 1988). Consequently, a higher proportion of propionate in the rumen signals a diet with higher levels of readily available energy.

Propionate and acetate may be sensed through different mechanisms, which would allow ruminants to discriminate among different VFA profiles. Some suggest ruminants possess a propionate receptor system in the ruminal vein (Baile, 1971). More recently, it has been proposed that the liver relays information through splanchnic and vagal afferents to the central nervous system to depress intake following the delivery of propionate in the portal vein (Anil and Forbes, 1980, 1987, 1988). In contrast, acetate does not depress intake to the same degree as propionate when infused in the portal vein at equivalent (Farningham and Whyte, 1993) or higher doses (Anil and Forbes, 1980). Consequently, acetate may be sensed through a different system, probably by specific receptors in the rumen wall (Martin and Baile, 1972; Forbes, 1995).

Other evidence also suggests that ruminants can sense proportions of VFA. Mixtures of acetate, propionate, and butyrate infused into the rumen of goats seemed to depress intake to a greater extent than equimolar doses of butyrate alone (Baile and Mayer, 1969). Intraportal infusions of combinations of propionate:acetate had a greater effect on intake depression in sheep when the proportion of propionate in the mixture increased (Farningham and Whyte, 1993).

Ruminants may also sense different VFA profiles through differences in the utilization of the metabolizable energy arising from different proportions of VFA in the rumen. Acetate may be used less efficiently than propionate and butyrate (Armstrong and Blaxter, 1957; Blaxter, 1962), although this hypothesis has been questioned (e.g., Orskov and MacLeod, 1993). Efficiency of VFA use is also influenced by the type of diet. The efficiency of use of ME from acetate increases as the supply of propionate (or protein) increases (Tyrrell et al., 1979; MacRae and Lobley, 1986).

Implications

The acquisition of preferences for flavors paired with low doses of propionate and acetate and the development of aversions at higher doses suggest feedback from the gastrointestinal tract and(or) from organs such as the liver modifies the development of preference for a low-quality food along a continuum that depends on the amount of volatile fatty acids (VFA) present in the rumen fluid. Preferences for combinations of acetate and propionate increased when propionate concentrations were highest, suggesting lambs were able to discriminate between different VFA profiles and acquire stronger preferences for foods associated with proportions of VFA that signal high energy density.

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