Effects of dietary mineral acids on voluntary food intake, digestion, mineral metabolism and acid–base balance of sheep

By J. L. L'ESTRANGE and F. MURPHY

Department of Agricultural Chemistry and Soil Science, Faculty of Agriculture, University College Dublin, Glasnevin, Dublin 9, Republic of Ireland

(*Received* 25 *March* 1971 – *Accepted* 10 *January* 1972)

1. A pelleted grass meal diet alone (control) or supplemented with hydrochloric acid, sulphuric acid or a 1:1 mixture of these acids, at 320 mequiv./kg, was offered *ad lib.* to eight sheep in a double 4 × 4 Latin square design for 20 d.

2. The HCl and HCl–H_2SO_4 treatments each reduced food intake of the sheep by 19% and the H_2SO_4 treatment reduced it by 30%. Average daily intake of acid was 397, 333 and 299 mequiv. on the HCl, HCl–H_2SO_4 and H_2SO_4 treatments respectively.

3. Rumen fluid pH was decreased by each acid treatment by 0·1–0·2 units without any appreciable changes in total or individual volatile fatty-acid concentrations. Dry-matter digestibility was slightly increased by each acid treatment.

4. Blood pH, plasma bicarbonate and blood base excess were decreased by each acid treatment; sulphate concentration in serum was increased by the HCl–H_2SO_4 and H_2SO_4 treatments.

5. In urine the acid treatments decreased the pH from a control value of 8·0 to approximately 6·0; associated with this was a large decrease in urinary bicarbonate and a large increase in urinary ammonia-nitrogen and calcium excretions.

6. The chloride and sulphur contributed by the acid treatments were almost entirely absorbed and excreted in the urine. Each acid treatment caused a negative balance of Ca equal to the increase in urinary Ca. Effects on other minerals were small.

7. The effect of the HCl and the HCl–H_2SO_4 treatments on voluntary food intake is ascribed to metabolic acidosis and of the H_2SO_4 treatment to excess dietary sulphate-S.

Virtanen (1933) formulated a method of fodder preservation as silage by direct acidification with mineral acids which became popularly known as the A.I.V. method. Virtanen (1933) claimed that silage preserved with sulphuric acid alone was much less palatable for cows than silage preserved with a mixture of hydrochloric and sulphuric acids. He suggested that the effect of the sulphuric acid was possibly due to an interference with cellulose digestion by the animals. More recently, ammonium bisulphate and sodium bisulphate have been used as silage additives on the same principle of directly lowering the pH of the grass ensiled. When added at a level of 7·14 g/kg of fresh herbage, ammonium bisulphate gave good silage preservation (McCarrick, 1962, 1964); however, the voluntary intake by cattle of the silage was found to be much lower than that of silage preserved with molasses (e.g. Clifford, 1963; Bryan, 1964; McCarrick, Keane & Tobin, 1965). The last two authors ascribed the reduced silage intake to excess dietary sulphate-sulphur contributed by the salt. McCarrick, Poole & Maguire (1965), on the other hand, ascribed the reduction in silage intake to metabolic acidosis induced by the salt.

In a previous experiment, to clarify this problem, the effects of various sulphate salts and sulphuric acid supplements on the voluntary intake of a pelleted grass-meal

Table 1. *Chemical composition (g/100 g dry matter) of the pelleted grass meal given as the basal diet*

Proximate analysis		Mineral content	
Crude protein	16·8	Potassium	2·50
Ether extract	4·2	Sodium	0·28
Crude fibre	22·0	Calcium	0·73
Ash	9·6	Magnesium	0·20
Nitrogen-free extractives	47·4	Chloride	1·26
		Phosphorus	0·26
		Sulphur	0·45

diet by sheep were compared (L'Estrange, Clarke & McAleese, 1969). In that experiment it was observed that sodium sulphate, added to the diet to provide 1 g S/100 g dry matter (DM), decreased food intake by 22%, whereas ammonium bisulphate, ammonium sulphate or sulphuric acid also added at 1 g S/100 g DM, each decreased food intake by approximately 44%. The effect of sodium sulphate was ascribed to dietary sulphate-S, whereas the increased effect of the other compounds was ascribed to metabolic acidosis, which each induced to the same degree. Because only sulphate compounds were used in that experiment it was not possible to separate completely the effects due to metabolic acidosis and to dietary sulphate.

In this paper these aspects are further examined by comparing the effects of dietary hydrochloric and sulphuric acids, added to a pelleted grass-meal diet, on food intake and metabolism of sheep. Alongside acid–base and mineral metabolism, other factors were studied which might be influenced by the treatments and which have been demonstrated to affect voluntary food intake of ruminants, such as rumen fluid pH (Bhattacharya & Warner, 1967), rumen fluid acetate concentration (Baile, 1968) and DM digestibility (Blaxter & Wilson, 1962).

A preliminary report of the work has already been presented (L'Estrange & Murphy, 1971).

EXPERIMENTAL

Animals

The sheep used were 2-year-old Cheviot wethers (castrated males) and they were housed in metabolism cages throughout the experiment.

Treatments

Pelleted, dried-grass meal, similar in composition (Table 1) to that of good-quality grass for ensilage, was used as the basal diet. The grass-meal pellets were supplemented with either hydrochloric acid or sulphuric acid or a mixture (1:1) of the two acids (HCl–H_2SO_4) at a level of 320 mequiv./kg. This amount of acid reduced the pH of a mixture of grass pellets and distilled water (1:5) to 3·8, and so was chosen as being a desirable level for the proper preservation of silage. Batches of the experimental diets were prepared by pouring 200 ml of acid solution on to 2 kg of the grass pellets in a plastic container which was shaken thoroughly to ensure an even mixture. For the control, the grass-meal pellets were diluted with distilled water to the same extent.

The diets were then stored in plastic bags and each batch was used within 3 d of being prepared.

The four diets, i.e. control, HCl, HCl–H_2SO_4 and H_2SO_4, were given to eight sheep according to two balanced 4×4 Latin squares in a change-over design. In block A the four sheep were non-fistulated and all the blood samples, urine and faeces collections were taken from these animals; in block B the four sheep were fitted with permanent rumen cannulas and all the rumen fluid samples were taken from them.

Feeding

The experimental diets were offered *ad lib.* for 20 d to the sheep in both blocks. The sheep in block A then had an 8 d recovery period on the control diet *ad lib.* before going on to their next treatment. The sheep in block B, after the 20 d *ad lib.* period, continued on their diet at a restricted level of 1 kg/d, offered once daily at 10.00 hours for a further 5 d, which enabled rumen-fluid studies to be made when DM intake was the same on each treatment. This period was followed by a recovery period of 3 d on the control diet *ad lib.* before the sheep went on to their next treatment. During *ad lib.* feeding, each animal was offered approximately 500 g in excess of its intake on the previous day. Food residues were removed every 2nd day and dried. Distilled water was provided throughout and its intake recorded.

Urine and faeces collections

Urine was collected in conical flasks containing a layer of toluene and its volume and pH were recorded daily. Two subsamples of each collection were taken; the one for bicarbonate determination was stored under a layer of toluene and the other, for determination of ammonia-nitrogen and minerals, was acidified with glacial acetic acid and stored under a layer of toluene. Faeces were collected in plastic buckets and their wet weight was recorded every 2 d. A sample of each 2 d collection was dried immediately. Composite samples of both urine and faeces were bulked for days 1–2, 3–4, 5–6, 7–8, 9–20 on the treatments and for days 1–2, 3–4, 5–6 and 7–8 during the recovery period.

Blood and rumen fluid sampling

Blood samples were taken from the jugular vein of the sheep in block A at 11.30 hours on days 0, 2, 4, 6, 10, 17, 21, 23 and 25. One sample for determination of blood pH and plasma total carbon dioxide was collected under a layer of liquid paraffin into a small heparinized McCartney bottle. The pH was recorded immediately and then the sample was centrifuged and the plasma transferred to another bottle containing liquid paraffin before total CO_2 was measured. A second sample was allowed to clot for 24 h and then centrifuged; the serum was withdrawn and stored at $-5°$ for mineral determinations.

Rumen fluid samples were taken from the fistulated sheep in block B, using an Edward's vacuum pump. Samples were taken on days 15 and 17 of the *ad lib.* period, at 08.00, 12.00, 16.00 and 20.00 hours. Samples were also taken on the last 2 d of the 5 d period of restricted intake at 1 h before feeding and at 2, 4 and 6 h after feeding. The pH of whole rumen contents was measured immediately after sampling.

Analytical methods

The DM content of the diets, food residues and faeces were determined by drying to constant weight at 100°. The pH values of urine and rumen fluid were recorded with a Radiometer pH meter (type pH M 26: Radiometer A/S, 72 Emdrupvej, Copenhagen NV, Denmark) using a glass electrode. The pH values of blood were recorded with the same Radiometer pH meter, using a microelectrode unit (type E. 5021) and a water-circulation thermostat (type VTS 13) at 38°. Total CO_2 concentrations in plasma and urine and ammonia-N in urine were determined by the method of Conway (1962). P_{CO_2} and base excess (BE) of blood were calculated from blood pH and plasma CO_2 values according to Davenport (1969). The BE value is the amount of titratable base in blood titrated to pH 7·40 at a P_{CO_2} of 40 mm Hg and at 37°. A zero value for BE in the sheep was chosen as that equivalent to 26 mmol HCO_3^-/l (Billitzer & Jarrett, 1970). In serum, urine, ashed faeces and food samples, calcium and magnesium were determined by atomic absorption spectroscopy, and Na and potassium by flame photometry. Total phosphorus was estimated in ashed urine, faeces and food and in serum by the method of Fiske & Subbarow (1925). Sulphate-S in serum was determined by the method of Dean (1966), total-S in urine, faeces and food by the method of Shaw (1959), Cl in urine and in deproteinized serum by the method of Conway (1962), Cl in faeces and food by the method of the Association of Official Agricultural Chemists (1965), and volatile fatty acids (VFA) in strained rumen fluid by gas–liquid chromatography by the method of Baumgardt (1964).

RESULTS

The eight sheep remained healthy throughout the experiment and increased in mean weight from 41 to 47 kg during the 4-month period of the experiment. The weight gain of the sheep was significantly affected by treatments, being highest on the control and lowest on the H_2SO_4 treatment (Table 2).

DM intake

DM intake (Table 2) was significantly reduced by each of the acid treatments. The effect was greatest with the H_2SO_4 treatment, which reduced intake over the 20 d period to 70% of the control; the HCl and the HCl–H_2SO_4 treatments each reduced intake to 81% of the control. On the control diet, DM intake was constant for the first 6 d at about 1·35 kg/d and then it increased gradually during the last 14 d to about 1·55 kg/d (Fig. 1). The patterns of intake on the HCl and on the HCl–H_2SO_4 treatments were almost identical. Intake on each was slightly below the control on the 1st day and then gradually decreased to its lowest level of about 1 kg on day 4. It then gradually increased over the remaining 16 d, as did intake of the control diet, so that the reduction in intake relative to the control remained at about 0·3 kg/d. Intake on the H_2SO_4 treatment was slightly reduced on the 1st day of treatment and then decreased to its lowest level of 0·72 kg on day 4. It gradually increased over the remaining 16 d at a rate slightly higher than with the other treatments so that the difference between them was somewhat less at the end of the 20 d period.

Table 2. *Live-weight change, dry-matter (DM) intake, water intake, water excretion and DM digestibility for eight sheep given pelleted grass meal alone or supplemented with mineral acids*

	Control (grass meal alone)	Grass meal + HCl	Grass meal + (HCl–H_2SO_4)	Grass meal + H_2SO_4	SE of treatment mean	*F* test†
Live-wt change (g/d)‡ (days 0–20)	168^a	82^{ab}	54^b	22^b	34	*
DM intake (kg/d)‡						
Days 0–20	$1{\cdot}46^a$	$1{\cdot}18^b$	$1{\cdot}18^b$	$1{\cdot}02^c$	0·033	**
8 d recovery period for block A	1·41	1·39	1·26	1·35	0·053	NS
3 d recovery period for block B	1·54	1·48	1·56	1·50	0·040	NS
Water intake (days 0–20)‡						
l/d	$4{\cdot}06^a$	$3{\cdot}46^b$	$3{\cdot}37^b$	$2{\cdot}73^c$	0·11	**
l/kg DM eaten	2·77	2·94	2·83	2·69	0·07	NS
Urine volume (days 0–20)§						
l/d	1·39	1·80	1·48	1·16	0·14	NS
l/kg DM eaten	$1{\cdot}05^c$	$1{\cdot}64^a$	$1{\cdot}45^{ab}$	$1{\cdot}26^{bc}$	0·07	**
% H_2O in faeces (days 0–20)§	69·4	64·4	64·0	68·1	1·84	NS
DM digestibility§						
Days 0–8	54·5	56·0	55·6	53·3	0·81	NS
Days 8–20	$54{\cdot}5^a$	$56{\cdot}0^b$	$56{\cdot}0^b$	$58{\cdot}2^c$	0·41	**

† Where the *F* test shows a significant treatment effect the means are significantly different from each other ($P < 0{\cdot}05$) if they do not have a letter in common. Significance levels: NS, $P > 0{\cdot}05$; *$0{\cdot}05 > P > 0{\cdot}01$; **$0{\cdot}01 > P > 0{\cdot}001$.

‡ Mean for eight sheep/treatment.

§ Mean for four sheep/treatment.

DM intake during the recovery period showed no significant carry-over treatment effects (Table 2 and Fig. 1).

Water intake and excretion

Daily water intake (Table 2) was significantly lower on each of the acid treatments than on the control. Water intake per unit of food eaten was not significantly affected by treatment, though it was higher on the HCl and the HCl–H_2SO_4 treatments and lower on the H_2SO_4 treatment than on the control. There was no significant treatment effect on the volume of urine excreted or on the moisture content of the faeces. The volume of urine excreted per kg food eaten was, however, significantly higher on both the HCl and the HCl–H_2SO_4 treatments than on the control.

DM digestibility

The apparent digestibility of DM was significantly higher on each of the acid treatments for the second period (days 8–20) but not for the first period (Table 2). This higher digestibility, which showed that the acid supplements did not adversely affect digestion of the grass meal, was probably due to the reduction in DM intake during the acid treatments.

pH and VFA in rumen fluid

During *ad lib.* feeding the mean pH of rumen fluid during the day was slightly lower on each acid treatment than on the control (Table 3) though only the value on the HCl treatment was significantly so ($P < 0{\cdot}05$). On each treatment the highest pH values

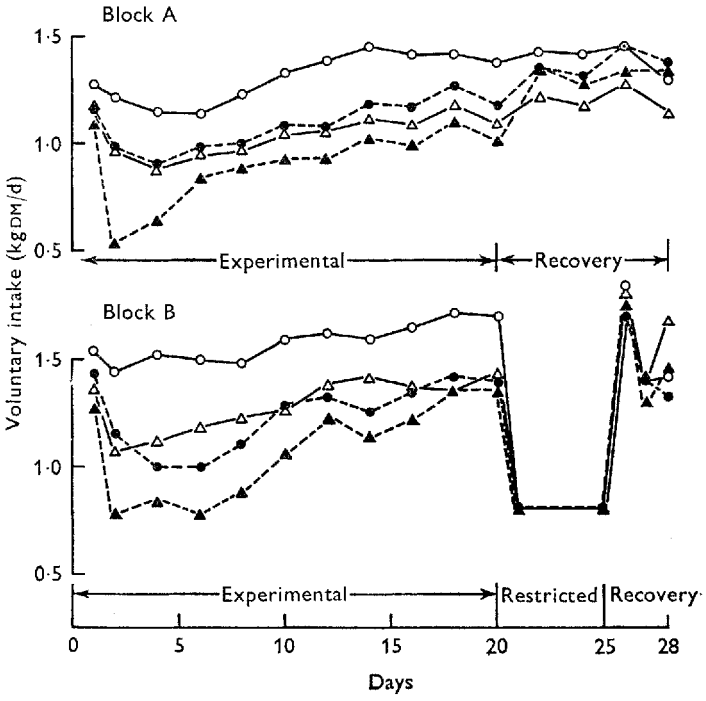

Fig. 1. Dry-matter (DM) intake by the sheep given grass-meal pellets alone (○), or supplemented (320 mequiv./kg food) with HCl (●), HCl–H_2SO_4 (△) or H_2SO_4 (▲). In block A the diets were given *ad lib.* for 20 d followed by an 8 d recovery period on the grass-meal pellets alone. In block B the diets were given *ad lib.* for 20 d followed by a 5 d restricted period and by a 3 d recovery period on the grass-meal pellets alone. Values in each block are the means for four sheep/treatment.

were at 08.00 hours and the lowest at 16.00 hours (Fig. 2). The difference between the values for the acid treatments and the control was greatest at 12.00 hours, though the time of sampling by treatment interaction was not significant. This pattern was clearly related to the fact that the food was changed daily at 10.00 hours and some was immediately consumed.

During restricted feeding the mean pH of rumen fluid for the four sampling times during the day was significantly lower on each acid treatment than on the control ($P < 0{\cdot}05$), though the difference was less than 0·2 units (Table 3). On each treatment values were highest 1 h before feeding and lowest 2 h after feeding (Fig. 2). The food was always eaten within 2 h of being offered. The difference between the values on the acid treatment and on the control was greatest at 2 and 4 h after feeding though the time of sampling by treatment interaction was not significant ($P \geqslant 0{\cdot}05$).

There was no significant treatment effect on total VFA concentration in rumen fluid during either *ad lib.* or restricted feeding. During *ad lib.* feeding total VFA concentration on each treatment was lowest at 08.00 hours and increased during the day to a peak at 20.00 hours (Fig. 2). During restricted feeding the values were lowest 1 h before feeding, and highest at 2–4 h after feeding. The time of sampling by treatment interaction was not significant ($P \geqslant 0{\cdot}05$).

Table 3. *Effects of the treatments on the pH and volatile fatty acid (VFA) contents in rumen fluid of four sheep given grass meal, alone or supplemented with mineral acids*

	Control (grass meal alone)	Grass meal + HCl	Grass meal + ($HCl–H_2SO_4$)	Grass meal + H_2SO_4	SE of treatment mean	*F* test†
Ad lib. feeding period‡						
pH of rumen fluid	$6·51^{a}$	$6·36^{b}$	$6·44^{ab}$	$6·43^{ab}$	0·04	*
Total VFA (mmol/l)	109	95	99	104	4·37	NS
Molar % of individual VFA:						
Acetic	66·8	67·4	67·1	66·1	0·84	NS
Propionic	17·5	17·5	16·6	16·9	0·55	NS
Isobutyric	2·2	2·2	2·1	2·7	0·28	NS
Butyric	12·3	12·1	12·5	13·4	0·66	NS
Isovaleric	1·47	1·39	1·46	1·66	0·15	NS
Valeric	1·10	1·10	1·00	1·00	0·08	NS
Restricted intake period‡						
pH of rumen fluid	$6·73^{a}$	$6·56^{b}$	$6·59^{b}$	$6·62^{b}$	0·022	***
Total VFA (mmol/l)	73	68	68	72	3·30	NS
Molar % of individual VFA:						
Acetic	64·2	64·6	61·5	64·4	1·06	NS
Propionic	$17·9^{bc}$	$19·7^{ab}$	$20·0^{a}$	$16·8^{c}$	0·66	**
Isobutyric	3·2	3·3	3·5	3·3	0·26	NS
Butyric	11·9	11·3	13·4	13·6	0·76	NS
Isovaleric	2·1	2·1	2·0	2·3	0·10	NS
Valeric	$1·2^{a}$	$1·0^{b}$	$1·1^{ab}$	$0·8^{c}$	0·04	***

† Where the *F* test shows a significant treatment effect the means are significantly different from each other ($P < 0·05$) if they do not have a letter in common. Significance levels: NS, $P > 0·05$; *$0·05 > P > 0·01$; **$0·01 > P > 0·001$; ***$P < 0·001$.

‡ Values for the *ad lib.* feeding period are the means for samples taken at 08.00, 12.00, 16.00 and 20.00 hours on days 15 and 17 from four sheep/treatment. Values for the restricted intake period are the means for samples taken at 1 h before feeding and at 2, 4 and 6 h after feeding on the last 2 days from four sheep/treatment.

During *ad lib.* feeding there was no significant treatment effect on the molar percentage of the individual VFA (Table 3). During restricted feeding there was a significant treatment effect ($P < 0·01$) on the molar percentage of propionic acid, the value being highest on the HCl and $HCl–H_2SO_4$ treatments and lowest on the H_2SO_4 treatment. Although significant, the extent of the differences was small and was not associated with a significant treatment effect on the molar percentages of the other VFA except for valeric acid. The molar percentage of the individual VFA was little affected by time of sampling during either *ad lib.* or restricted feeding and the time of sampling by treatment interactions on individual VFA values were also not significant.

Blood acid–base status

Each acid treatment caused a degree of metabolic acidosis in the sheep as indicated by blood pH, blood BE and plasma CO_2 values (Fig. 3). The P_{CO_2} values were not affected by treatment, mean values for days 2–17 on the control, HCl, $HCl–H_2SO_4$ and H_2SO_4 treatments being 37, 38, 37 and 37 mm Hg respectively.

The decrease in blood pH, which was never greater than 0·15 units, was gradual up to day 4. Plasma CO_2 on the HCl and $HCl–H_2SO_4$ treatments decreased within 2 d to 22 and 21 mmol/l respectively, remained at around these levels for some days and

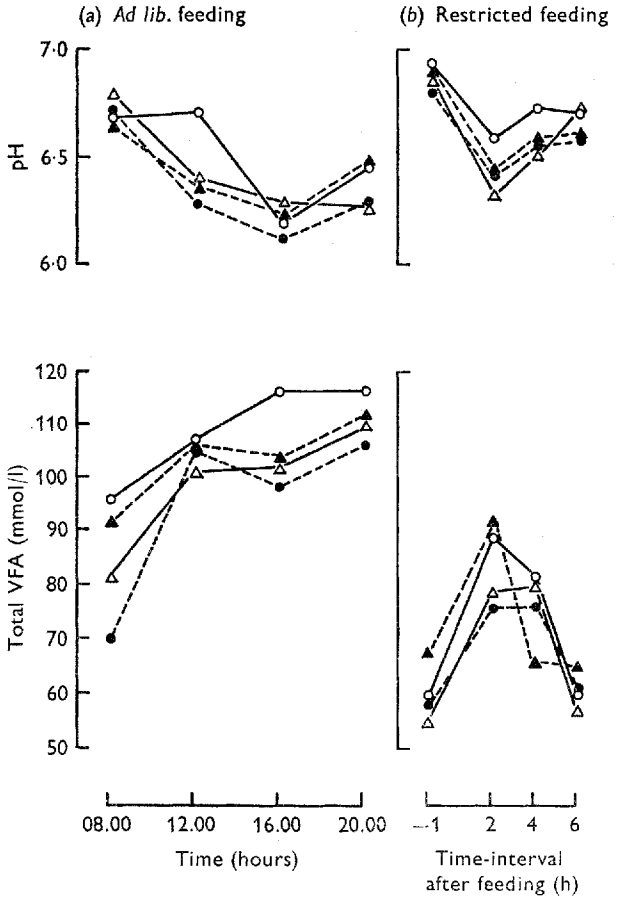

Fig. 2. pH and total volatile fatty acids (VFA) concentration of rumen liquor samples from sheep on control (○), HCl (●), HCl–H_2SO_4 (△) and H_2SO_4 (▲) treatments. The values are the means for samples from the four sheep in block B; (*a*) for days 15 and 17 during *ad lib.* feeding, (*b*) for days 24 and 25 during restricted feeding. Other details as in Fig. 1.

increased slightly towards the end of the 20 d period. Plasma CO_2 on the H_2SO_4 treatment decreased more gradually up to day 10. The values for blood BE showed a trend similar to that of plasma CO_2, the lowest value being −6 mmol/l. The degree of metabolic acidosis on the HCl and HCl–H_2SO_4 treatments was clearly greater for the first 6 d of treatment than subsequently. On the H_2SO_4 treatment the acidosis developed more towards the end of the 20 d period.

During the recovery period the blood pH increased to the levels on the control diet within 1 d of withdrawal of the supplements; plasma CO_2 and blood BE both increased to slightly higher values than the values on the control diet.

Minerals in serum

The mean concentration of sulphate-S in the serum of the sheep was significantly higher on the H_2SO_4 treatment than on the HCl–H_2SO_4 treatment, which in turn was significantly higher than on the HCl or control treatment (Table 4). Serum sulphate-S

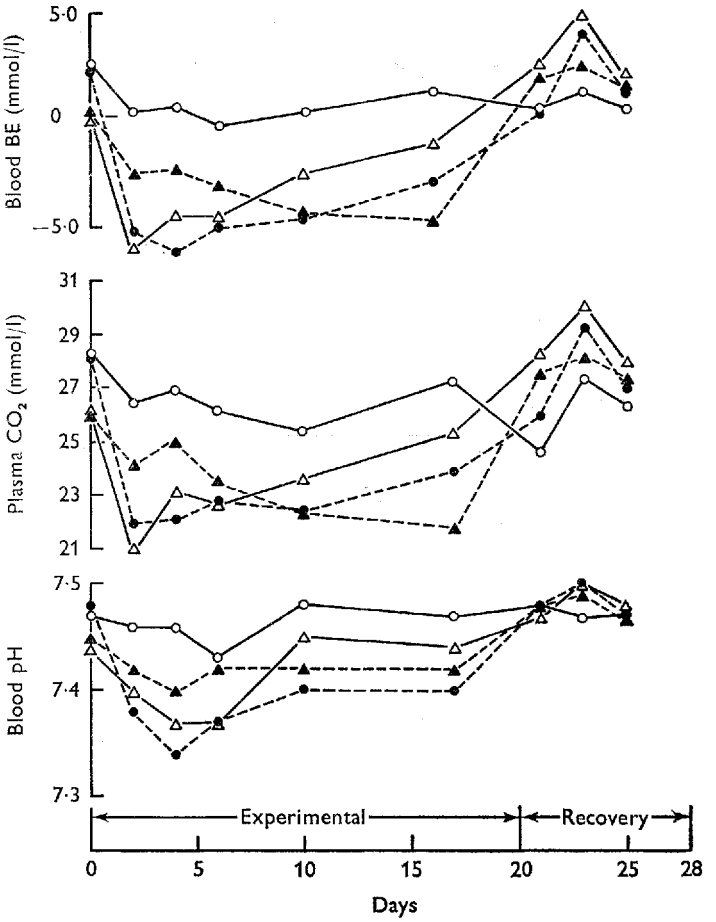

Fig. 3. Mean blood pH, plasma total CO_2 and blood base excess (BE) values for the four sheep in block A throughout the experiment on the treatments: control (○), HCl (●), HCl–H_2SO_4 (△) and H_2SO_4 (▲). Other details as in Fig. 1.

Table 4. *Effects of the treatments on the concentration of minerals (mg/100 ml) in serum of sheep given grass meal alone or supplemented with mineral acids*

(Means for five samples taken from day 2 to day 17 from four sheep/treatment)

	Control (grass meal alone)	Grass meal + HCl	Grass meal + (HCl–H_2SO_4)	Grass meal + H_2SO_4	SE of treatment mean	*F* test†
Sodium	330·0	324·2	318·8	333·4	7·59	NS
Potassium	22·6	22·7	21·6	22·3	0·50	NS
Calcium	7·80	7·65	7·75	7·77	0·70	NS
Magnesium	1·97	1·85	2·06	1·82	0·11	NS
Inorganic phosphate	6·82	6·75	6·49	6·61	0·61	NS
Chloride	372	388	394	386	5·0	NS
Sulphate-S	6·52[a]	6·84[a]	7·58[b]	8·22[c]	0·21	**

† Where the *F* test shows a significant treatment effect the means are significantly different from each other ($P < 0·05$) if they do not have a letter in common. NS, $P > 0·05$; **$0·017 > P > 0·001$.

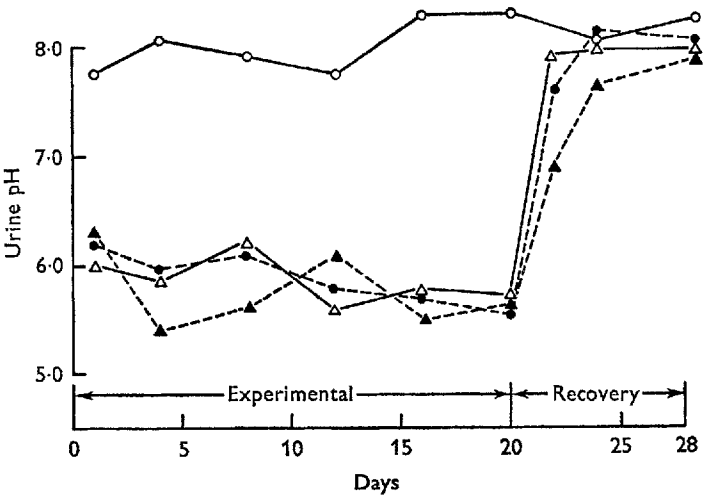

Fig. 4. Mean urine pH for the four sheep in block A on the treatments: control (○), HCl (●), HCl–H_2SO_4 (△) and H_2SO_4 (▲). Other details as in Fig. 1.

on the H_2SO_4 and HCl–H_2SO_4 treatments increased by about 2·0 and 1·0 mg/100 ml respectively from day 2 of treatment, and then remained constant relative to the control values before decreasing to the original values within 1 d of the beginning of the recovery period. The concentrations of Cl, Ca, Mg, K, Na and inorganic P in the serum of the sheep were not significantly affected by treatment (Table 4).

Urinary pH, bicarbonate and ammonia-N excretion

The pH of the urine (Fig. 4) of the sheep on the control diet remained constant throughout the experiment at around 8·0. The values on each of the acid treatments decreased to 6·0 within 24 h and they remained at this or slightly below it throughout the 20 d. When the acid supplements were withdrawn the values returned to normal within 3 d, the increase being more gradual after the H_2SO_4 treatment than after the other acid treatments.

On the control diet the excretion of bicarbonate in urine (Fig. 5) was around 130 mmol/d throughout the experiment. On each acid treatment bicarbonate excretion in urine decreased within 2 d to about 30 mmol/d and remained at this value except for days 4–8 on the HCl and HCl–H_2SO_4 treatments. During the recovery period bicarbonate excretion increased to the control level within 4 d except after the H_2SO_4 treatment, when the increase was more gradual.

Excretion of ammonia-N in urine (Fig. 5) was constant for the sheep on the control diet at about 25 mmol/d. On each of the acid treatments ammonia-N excretion increased gradually up to day 6 and then remained relatively constant at 100–150 mmol/d. The rate of increase and the amount excreted on the H_2SO_4 treatment was lower than on the HCl or HCl–H_2SO_4 treatment. During the recovery period after each acid treatment ammonia-N excretion decreased to the control value within about 4 d.

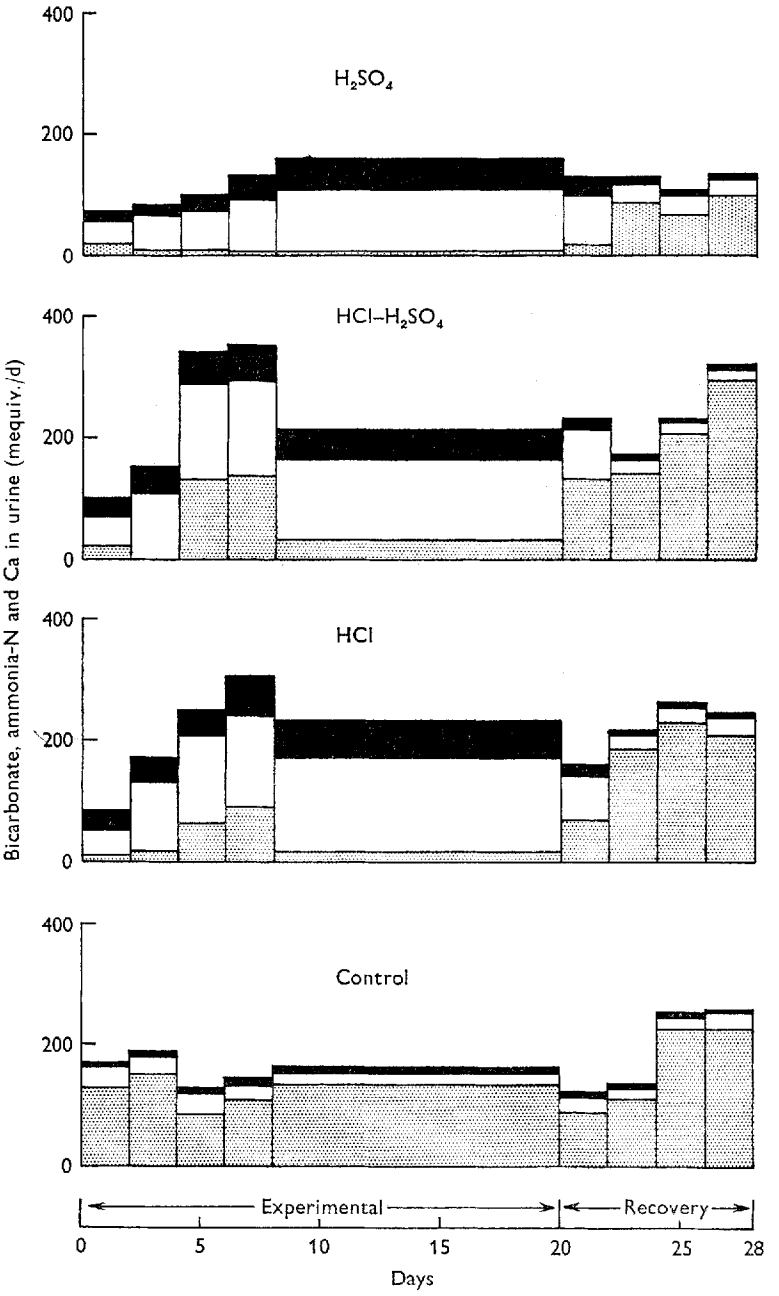

Fig. 5. Mean urinary excretion of bicarbonate (▦), ammonia-N (□) and calcium (■) by the four sheep in block A. Other details as in Fig. 1.

Intake, excretion and balance of S and Cl

During the 8–20 d period, intake of S was increased from the control level of 6·5 g/d to 8·04 and 9·89 g/d on the $HCl–H_2SO_4$ and H_2SO_4 treatments respectively (Table 5). Faecal S excretion expressed in g/d was lower, though not significantly so, on each acid

Table 5. *Intake, faecal and urinary excretion and balance of sulphur and chloride for days 8 to 20 by sheep given grass meal alone or supplemented with mineral acids*

(Means for four sheep/treatment)

	Control (grass meal alone)	Grass meal + HCl	Grass meal + (HCl–H_2SO_4)	Grass meal + H_2SO_4	SE of treatment mean	F test†
Sulphur:						
Intake (g/d)	6·50	5·38	8·04	9·89	—	—
Faecal S (g/d)	3·50	2·94	2·88	2·65	0·29	NS
Faecal S (g/100 g DM eaten)	0·25	0·26	0·26	0·26	0·01	NS
Faecal S as % of intake	54^a	55^a	36^b	27^c	1·36	***
Urinary S (g/d)	$2·81^a$	$2·54^a$	$4·54^b$	$5·94^b$	0·41	**
Urinary S as % of intake	43^a	48^a	56^b	61^b	1·98	**
Balance (g/d)	0·16	0·08	0·64	1·32	0·31	NS
Chloride:						
Intake (g/d)	17·8	29·2	20·7	12·8	—	—
Faecal Cl (g/d)	1·1	0·8	0·7	1·1	0·20	NS
Faecal Cl (g/100 g DM eaten)	0·077	0·064	0·064	0·073	0·018	NS
Faecal Cl as % of intake	$6·4^{ab}$	$2·5^a$	$3·4^a$	$9·2^b$	1·28	*
Urinary Cl (g/d)	$13·8^{ac}$	$24·2^b$	$16·2^c$	$9·6^a$	1·67	**
Urinary Cl as % of intake	78	83	80	75	3·94	NS
Balance (g/d)	2·9	4·2	3·6	2·2	0·36	NS

† Where the F test shows a significant treatment effect the means are significantly different from each other ($P < 0·05$) if they do not have a letter in common. NS, $P > 0·05$; *$0·05 > P > 0·01$; **$0·01 > P > 0·001$; ***$P < 0·001$.

treatment than on the control treatment; expressed in g/100 g DM eaten, it was the same on all four treatments. Faecal S expressed as a percentage of S intake was significantly lower on the HCl–H_2SO_4 and H_2SO_4 treatments than on the control and HCl treatments. Urinary S excretion expressed in g/d and as a percentage of intake was significantly higher ($P < 0·01$) on the HCl–H_2SO_4 and the H_2SO_4 treatments. There was no significant treatment effect on the balance of S though it was highest on the HCl–H_2SO_4 and the H_2SO_4 treatments.

The excretion of S in faeces on the H_2SO_4 treatments was highest for the first 2 d of treatment and then decreased to a constant level (Fig. 6*a*), indicating that the absorption of the supplementary sulphate was lowest immediately after the introduction of the treatment. Urinary S excretion on the H_2SO_4 treatment was as high for the first 2 d of treatment as subsequently and it quickly decreased during the recovery period, indicating that the supplementary sulphate was rapidly absorbed and excreted in the urine. This pattern of S excretion was not so clear on the HCl–H_2SO_4 treatment, where of course the increase in S intake was not so great.

Cl intake increased from the control level of 17·8 to 29·2 and 20·7 g/d on the HCl and HCl–H_2SO_4 treatments respectively for days 8–20 (Table 5). Faecal excretion of Cl, expressed in g/d or in g/100 g DM eaten, was not affected by treatment but was significantly decreased by the HCl treatment when expressed as a percentage of Cl intake (Fig. 6*b*). The HCl and the HCl–H_2SO_4 treatments significantly increased urinary Cl excretion when expressed in g/d, but not when expressed as a percentage of Cl intake. There was no significant treatment effect on the balance of Cl. On the HCl and HCl–H_2SO_4 treatments urinary Cl excretion was almost as high on the first 2 d

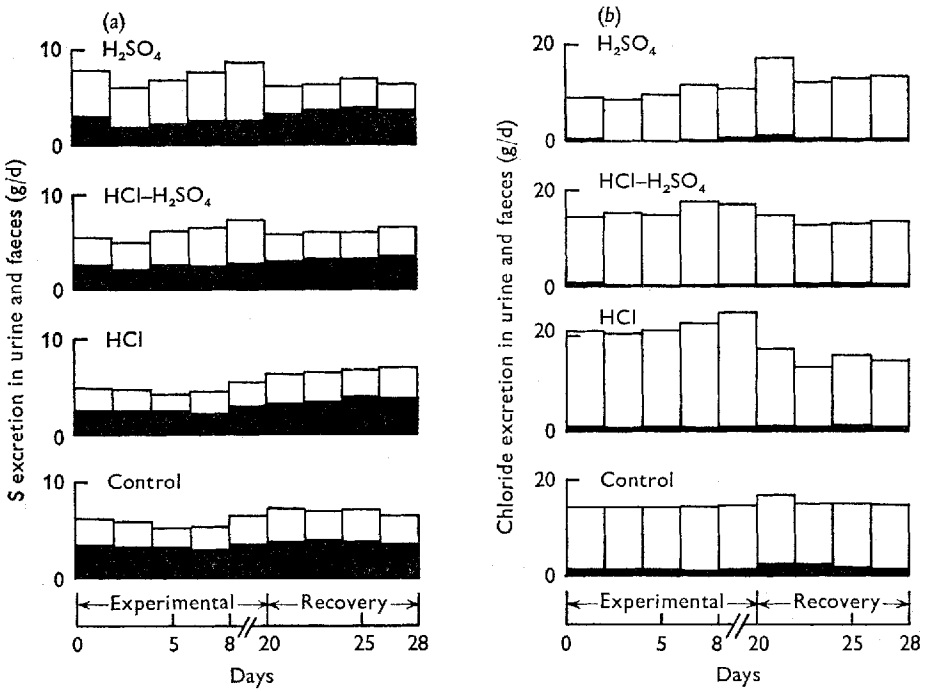

Fig. 6. Pattern of excretion throughout the experiment of (*a*) sulphur and (*b*) chloride in faeces (■) and in urine (□) by the four sheep/treatment in block A. Other details as in Fig. 1.

of treatment as subsequently and the values decreased to the control levels within 2 d of the recovery period. Faecal Cl showed no increase after introduction of the acid treatments.

Intake, excretion and balance of Ca, P, Mg, K and Na

Urinary Ca excretion was increased to ten times its value by each acid treatment (Table 6), the output increasing gradually during the first 8 d after introduction of the acids (Fig. 5). During the recovery period urinary Ca excretion decreased rapidly to the control values within 4 d. Faecal Ca excretion was not affected by treatment and consequently the balance of Ca was negative on each acid treatment at about 0·5 g/d compared with a positive balance of 0·6 g/d on the control (Table 6).

The percentage of dietary P excreted in faeces was significantly increased by each acid treatment compared with the control (Table 6). Urinary P excretion was not affected by treatment. The balances of P on each acid treatment were the same at approximately 0·05 g/d, which was significantly lower than the balance on the control at 0·49 g/d.

Mg excretion in urine expressed as a percentage of intake was significantly higher on each acid treatment than on the control but faecal excretion and balance of Mg were not affected by treatment (Table 6). There was no significant treatment effect on urinary or faecal excretion or balance of K (Table 6).

The percentage of dietary Na excreted in faeces was lower on each acid treatment than on the control treatment, though the difference was not significant (Table 6).

Table 6. *Intake, faecal and urinary excretion and balance of calcium, phosphorus, magnesium, potassium and sodium for days 8–20 by sheep given grass meal alone or supplemented with mineral acids*

(Means for four sheep/treatment)

	Control (grass meal alone)	Grass meal + HCl	Grass meal + ($HCl–H_2SO_4$)	Grass meal + H_2SO_4	SE of treatment mean	*F* test†
Calcium						
Intake (g/d)	10·33	8·60	8·05	7·37	—	—
Faecal Ca as % of intake	94	93	95	92	2·00	NS
Urinary Ca as % of intake	$0·93^a$	$13·4^b$	$11·7^b$	$13·0^b$	0·70	***
Balance (g/d)	$0·60^a$	$-0·65^b$	$-0·44^b$	$-0·34^b$	0·22	**
Phosphorus						
Intake (g/d)	3·71	3·07	2·90	2·64	—	—
Faecal P as % of intake	86^a	96^b	96^b	96^b	1·76	*
Urinary P as % of intake	1·31	3·44	1·65	2·20	0·64	NS
Balance (g/d)	$0·49^a$	$0·02^b$	$0·07^b$	$0·06^b$	0·06	**
Magnesium						
Intake (g/d)	2·82	2·33	2·22	2·01	—	—
Faecal Mg as % of intake	86	87	86	88	2·5	NS
Urinary Mg as % of intake	$14·2^a$	$17·6^b$	$17·2^b$	$18·4^b$	0·79	*
Balance (g/d)	−0·02	−0·10	−0·03	−0·14	0·06	NS
Potassium						
Intake (g/d)	35·3	29·3	27·4	25·2	—	—
Faecal K as % of intake	7·4	7·9	7·4	9·1	1·39	NS
Urinary K as % of intake	83	86	86	83	2·38	NS
Balance (g/d)	3·6	1·7	2·2	1·9	0·45	NS
Sodium						
Intake (g/d)	3·80	3·12	2·98	2·68	—	—
Faecal Na as % of intake	49	21	25	40	6·1	NS
Urinary Na as % of intake	37^a	75^c	59^{bc}	51^b	6·2	*
Balance (g/d)	0·45	0·31	0·33	0·24	0·14	NS

† Where the *F* test shows a significant treatment effect the means are significantly different from each other ($P < 0·05$) if they do not have a letter in common. NS, $P > 0·05$; *$0·05 > P > 0·01$; **$0·01 > P > 0·001$; ***$P < 0·001$.

However, the percentage of dietary Na excreted in urine was significantly higher on two acid treatments than on the control ($P < 0·05$). There was no significant treatment effect on the balance of Na. Thus, the acid treatments changed the excretory pattern of Na by decreasing faecal output and increasing urinary output. The increase in urinary Na excretion was greatest during the first 4 d of treatment, the decrease in faecal output was greatest from days 2 to 8, but both values returned to the control values within 2 d of the beginning of the recovery period.

Intake of acid and excretion of electrolytes in urine

The intakes of mineral acid calculated from the amount added and from the Cl and S contents of the food residue were 397, 333 and 299 mequiv./d on the HCl, $HCl–H_2SO_4$ and H_2SO_4 treatments respectively, for days 0–20. Although DM intakes were the same on the HCl and $HCl–H_2SO_4$ treatments, the amount of acid left in the food residue, as indicated by Cl and S contents, was greater on the $HCl–H_2SO_4$ than on the HCl treatment. It should be noted that the food residues were dried before

Table 7. *Intake of acid and the excretion of electrolytes in urine for days 8–20 by sheep given grass meal alone or supplemented with mineral acids*

(Means for four sheep/treatment)

		Control (grass meal alone)	Grass meal + HCl	Grass meal + (HCl–H_2SO_4)	Grass meal + H_2SO_4
Acid intake (mequiv./d)		0	414	352	319
Electrolytes in urine (mequiv./d)	Ca^{2+}	5	59	49	48
	Mg^{2+}	33	34	33	31
	Na^{+}	63	94	79	62
	K^{+}	745	645	592	538
	NH_4^{+}	20	159	127	105
	HPO_4^{2-}	3·0	1·2	0·4	0·6
	$H_2PO_4^{-}$	0·1	2·9	1·3	1·6
	Cl^{-}	384	682	457	274
	SO_4^{2-}	192	158	283	370
	HCO_3^{-}	137	17	39	6

analysis, which may have resulted in some loss of free HCl by volatilization. The treatment effects on electrolyte excretion in urine have already been presented, but the values for each electrolyte expressed in mequiv./d are compared with acid intake for days 8–20 in Table 7. It is clear that, apart from Cl and sulphate excretion, the major changes caused by the acid treatments were on Ca, ammonia-N and bicarbonate excretion. The effect on K, which was the predominant electrolyte in urine, was due to decreased food and consequently decreased K intake.

DISCUSSION

The 19% reduction in voluntary intake of the pelleted grass meal caused by the HCl treatment can be ascribed to excess acid rather than Cl intake, since much higher levels of dietary Cl as NaCl had little effect on voluntary food intake of sheep (Meyer & Weir, 1954; Pierce, 1957; Upton, 1970). The cause of this reduced intake could be related to several factors known to affect voluntary food intake of ruminants, such as rumen pH, rumen acetate concentration, DM digestibility, prolonged food retention, palatability and metabolic disturbance.

Bhattacharya & Warner (1967) observed that direct infusion of lactic, citric or phosphoric acid into the rumen of steers to maintain the pH of rumen fluid at 6·0 or 0·6 units below normal control values caused a 50–70% reduction in food intake, which they ascribed to the lower rumen fluid pH. In our experiment the HCl treatment reduced rumen fluid pH of the sheep by less than 0·2 units which would seem to rule out this factor as the main cause of the reduction in food intake. Furthermore, the HCl treatment had little or no effect on total or individual VFA concentrations in rumen fluid, which rules out high acetate concentration, shown by Baile (1968) to affect food intake, as a possible cause. Likewise DM digestibility is ruled out, as it was, in fact, slightly increased by the HCl treatment. It is possible that the increase in DM digestibility resulted from prolonged food retention in the rumen due to an effect of the HCl treatment on rumen motility. It is more likely, however, that the increase in DM

digestibility of the grass pellets was caused by the lower DM intake as shown by Wainman, Smith & Blaxter (1971).

Reduced palatability due to the low pH (3·8) of the diet could be a factor, particularly in view of the recent report of McLeod, Williams & Raymond (1970) that increasing the pH of silage (made without mineral acids) with $NaHCO_3$ increased the intake of the silage by sheep and cattle. In our experiment DM intake was reduced only slightly on the 1st day of treatment (Fig. 1), which indicates that palatability was not a major factor involved.

Consequently, it appears that the effect of the HCl was a metabolic one and was closely related to its effect on the acid–base status of the animals. The HCl treatment induced a chronic degree of metabolic acidosis which developed rapidly, persisted throughout the 20 d feeding period and was quickly rectified during the recovery period. The effects of acid intake on metabolic acidosis noted here are comparable with observations on acid loading in cattle (Lebeda, Bouda & Kučera, 1970) and less severe than those observed in goats (Fencl, Miller & Pappenheimer, 1966). As the acidosis developed, food intake decreased, likewise when it was rectified during the recovery period food intake returned to normal (Figs 1, 4).

The extra reduction in food intake caused by the H_2SO_4 treatment is ascribed to the high content of dietary sulphate-S (0·56 g/100 g DM) contributed by this treatment, whereas the content contributed by the HCl–H_2SO_4 treatment (0·28 g/100 g DM) was too low to have any effect. This extra effect cannot be ascribed to changes in rumen fluid pH or VFA concentrations or DM digestibility as these were the same as on the HCl treatment. Palatability also appears to be ruled out as intake was reduced only slightly on the 1st day of treatment (Fig. 1). The degree of metabolic acidosis induced by the H_2SO_4 treatment was somewhat less than that induced by the other acid treatments and accordingly can be discounted as contributing to the extra reduction in food intake. Excess dietary sulphate-S as sodium sulphate has previously been shown to affect food intake of sheep adversely (L'Estrange *et al.* 1969; Upton, 1970). Weeth & Hunter (1971) have shown recently that high levels of sulphate in drinking-water reduced voluntary food intake of cattle.

The reduction in food intake on the H_2SO_4 treatment was less than would be expected from the report of Virtanen (1933), who observed with cows that silage preserved with H_2SO_4 alone was associated with extremely low intakes compared with silage preserved with a 1:1 mixture of HCl and H_2SO_4. The results here are, however, similar to those of a previous experiment on sheep (L'Estrange *et al.* 1969) in which ammonium bisulphate, ammonium sulphate or sulphuric acid, added to a grass-meal diet to provide 1 % S in the food DM, each caused a reduction of approximately 44 % in DM intake. They also agree with the effect of adding ammonium bisulphate to silage on voluntary intake by cattle (McCarrick, Maguire, Poole & Spillane, 1965).

The effects of the mineral acids on mineral metabolism were closely related. The major effect was an increase in urinary Ca excretion. There was also an increase in urinary Na and Mg excretion, a decrease in faecal Na excretion and an increase in faecal P excretion. The mineral acids adversely affected the balance of Ca and P but were without effect on the excretion or balance of K. These results are similar to those

previously reported for sheep given ammonium bisulphate, ammonium sulphate and sulphuric acid (L'Estrange, 1970).

The metabolisms of the supplementary S and of the supplementary Cl were similar in many respects, both elements being almost entirely absorbed and excreted in the urine. The only major difference noted between them was that the supplementary S increased serum sulphate-S concentration, whereas the supplementary Cl had no effect on serum Cl concentration, possibly because Cl has a much greater volume of distribution in the body than sulphate (Wolf & McDowell, 1954). The different distribution within body fluids may partly explain why voluntary food intake is more sensitive to sulphate than to chloride in the diet.

We are indebted to Professor D. M. McAleese for helpful advice and criticism and to Dr D. Kellegher for advice on statistical analysis. We thank Mr S. Dillon, Miss K. McCormack and Miss A. McGuigan for their skilled technical assistance.

REFERENCES

Association of Official Agricultural Chemists (1965). *Official Methods of Analysis* 10th ed. Washington DC: Association of Official Agricultural Chemists.
Baile, C. A. (1968). *Fedn Proc. Fedn Am. Socs exp. Biol.* **27**, 1361.
Baumgardt, B. R. (1964). *Bull. Wis. Univ. Dep. Dairy Sci.* no. 1.
Bhattacharya, A. N. & Warner, R. G. (1967). *J. Dairy Sci.* **50**, 1116.
Billitzer, A. W. & Jarrett, I. G. (1970). *Aust. J. biol. Sci.* **23**, 915.
Blaxter, K. L. & Wilson, R. S. (1962). *Anim. Prod.* **4**, 351.
Bryan, W. B. (1964). Consumption and physiological effects in the dairy cow of grass silage with different additives. MAgrSc Thesis, National University of Ireland.
Clifford, A. J. (1963). Comparative studies on the physiological effects of feeding grass silage with different additives on dairy cows. MAgrSc Thesis, National University of Ireland.
Conway, E. J. (1962). *Microdiffusion Analysis and Volumetric Error* 5th revised ed. London: Crosby Lockwood.
Davenport, H. W. (1969). *The ABC of Acid-Base Chemistry* 5th ed. Chicago and London: University of Chicago Press.
Dean, G. A. (1966). *Analyst, Lond.* **91**, 530.
Fencl, V., Miller, T. B. & Pappenheimer, J. R. (1966). *Am. J. Physiol.* **210**, 459.
Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
Lebeda, M., Bouda, J. & Kučera, A. (1970). *Acta vet. Brno.* **39**, 415.
L'Estrange, J. L. (1970). *Ir. J. agric. Res.* **9**, 161.
L'Estrange, J. L., Clarke, J. J. & McAleese, D. M. (1969). *Ir. J. agric. Res.* **8**, 133.
L'Estrange, J. L. & Murphy, F. (1971). *Proc. Nutr. Soc.* **30**, 21A.
McCarrick, R. B. (1962). *Ir. J. agric. Res.* **1**, 267.
McCarrick, R. B. (1964). *Ir. J. agric. Res.* **3**, 99.
McCarrick, R. B., Keane, E. & Tobin, J. (1965). *Ir. J. agric. Res.* **4**, 115.
McCarrick, R. B., Maguire, M. F., Poole, D. B. R. & Spillane, T. (1965). *Ir. J. agric. Res.* **4**, 135.
McCarrick, R. B., Poole, D. B. R. & Maguire, M. F. (1965). *Ir. J. agric. Res.* **4**, 125.
McLeod, D. S., Williams, R. J. & Raymond, W. F. (1970). *J. agric. Sci., Camb.* **75**, 311.
Meyer, J. H. & Weir, W. C. (1954). *J. Anim. Sci.* **13**, 443.
Pierce, A. W. (1957). *Aust. J. agric. Res.* **8**, 711.
Shaw, W. M. (1959). *J. agric. Fd Chem.* **7**, 843.
Upton, P. K. (1970). Some biological and chemical aspects of sulphur metabolism in the ruminant. PhD Thesis, National University of Ireland.
Virtanen, A. J. (1933). *Emp. J. exp. Agric.* **1**, 143.
Wainman, F. W., Smith, J. S. & Blaxter, K. L. (1971). *Proc. Nutr. Soc.* **30**, 23A.
Weeth, H. J. & Hunter, J. E. (1971). *J. Anim. Sci.* **32**, 277.
Wolf, A. V. & McDowell, M. E. (1954). *Am. J. Physiol.* **176**, 207.

Printed in Great Britain