Acid–base balance in ruminating calves given sodium hydroxide-treated straw

BY SØREN WAMBERG[1] AND KNUD ENGEL[2]

Departments of [1]Physiology and [2]Clinical Chemistry, Odense University Medical School, DK-5230 Odense M, Denmark

AND PETER STIGSEN*

Department of Animal Physiology and Chemistry, National Institute of Animal Science, DK-1958 Copenhagen, Denmark

(*Received 29 November 1984 – Accepted 4 June 1985*)

1. Studies of whole-body balances of non-metabolizable base (NB) and several minerals, and of relevant acid–base quantities in blood and urine, were carried out in two 6-month-old ruminating Holstein × Friesian bull calves fed on fixed rations containing 500 g barley straw/kg diet (group A) to examine the quantitatively important components of the balance of NB and determine the rates of mineral and NB retention associated with normal body growth.

2. Parallel balance studies were conducted in six other bull calves given fixed rations containing 500 g alkali-treated barley straw/kg diet to evaluate the effects of long-term alkali-straw feeding on the rates of body growth and skeletal mineral and NB deposition and the renal control of extracellular electrolyte and acid–base status. The straw component was treated either with 50 g sodium hydroxide/kg dry matter (DM) (group B; two calves), or with 50 g or 100 g NaOH/kg DM and subsequently neutralized with hydrochloric acid (groups C and D; two calves per group). In all groups the animals were given free access to tap water.

3. Throughout the total 105 d experiment, all animals remained healthy and gained weight. Normal body growth (group A) was associated with a positive balance of NB (1–2 mmol/kg live weight (LW) per d) due to continuing deposition of dietary NB in 'new tissue', largely in the developing skeleton.

4. During 105 d alkali-straw feeding, the animals showed a remarkable ability to cope with dietary loads of NaOH or sodium chloride, up to about 30 mmol/kg LW per d, without any significant disturbance of extracellular acid–base and electrolyte status or body growth rate. The surplus mineral and NB loads were absorbed and subsequently excreted in an increased volume of urine. Rates of mineral and NB retention were not significantly different from the reference values of group A and remained within the range of values reported from similar studies. In all groups, maintenance of normal whole blood and plasma acid–base and electrolyte status was accounted for by efficient renal control of the composition of the extracellular fluid compartment.

In the past two decades the development of new techniques for industrial production of alkali-treated roughages has encouraged a number of animal nutritionists to study the effects of alkali-straw feeding on dietary intake, body growth, rumen microbial activity, in vivo digestibility, etc. (Saxena *et al.* 1971; Oloade & Mowat, 1975; Jackson, 1977; Friis Kristensen *et al.* 1978; Coombe *et al.* 1979; Sriskandarajah & Kellaway, 1984). Apparently, however, only limited information on the effects of alkali-straw feeding on acid–base metabolism and mineral turnover in the ruminant is available (Voigt & Piatkowski, 1974; Friis Kristensen *et al.* 1978).

In the present study, investigations of mineral and acid–base metabolism were carried out in two 6-month-old ruminating Holstein × Friesian bull calves, fed on a basic ration containing 500 g barley straw/kg diet, in order to determine quantitatively the components of the whole-body balance of (titratable) non-metabolizable base (NB), and to determine the rates of mineral and NB retention associated with normal body growth. Parallel series of balance studies were conducted in two bull calves subjected to long-term feeding on alkali-straw rations and in four calves given alkali-straw neutralized with hydrochloric acid

* Present address: Flexplan Agro Projects A/S, DK-2970 Hørsholm, Denmark.

Table 1. *Age, live weight, and rates of weight gain and dietary and water intake*
(Mean values and standard errors (4 df) for two calves per group studied during periods 1–6)*

Group	Ration†	Age on arrival (d)	Live wt				Dietary intake					
			Initial (kg)	Final (kg)	Gain (kg/d)		Dry matter (kg/d)		Gross energy (MJ/d)		Water intake (l/d)	
					Mean	SE	Mean	SE	Mean	SE	Mean	SE
A	US	155	214·0	269·0	0·71	0·09	5·52	0·30	102·1	5·4	21·8	2·27
B	AS	149	216·5	278·0	0·80		5·92		106·9		36·7	
C	NS50	146	206·0	268·0	0·82		5·68		101·9		26·6	
D	NS100	141	205·5	258·0	0·68		5·52		97·4		26·2	

US, untreated barley straw; AS, alkali-treated barley straw; NS50, NS100, neutralized barley straw.
* For details, see Fig. 1 (p. 661).
† For details, see Tables 2 and 3 (p. 657).

in order to examine the effects of excessive dietary loading with sodium hydroxide (i.e. NB) or sodium chloride on (1) rates of body growth and mineral and NB retention, (2) several acid–base quantities in blood and urine, and (3) renal control of extracellular acid–base and electrolyte status. The results were compared with the reference values obtained in the two animals given untreated barley straw.

EXPERIMENTAL

Animals and management

Eight Holstein × Friesian bull calves were raised on milk-substitute, concentrates including vitamin and mineral supplements, and hay until approximately 6 months of age. On arrival at the National Institute of Animal Science, the animals were treated against parasites with Thibenzole vet. (MSD, Rahway, NJ, USA; 100 mg/g) and, following premedication with Rompun vet. (Bayer Chemie, Leverkusen, FRG; 20 mg/ml) and application of anaesthetic by local injection of lidocaine (DAK, Copenhagen; 10 mg/ml), the calves were castrated and the right carotid artery exteriorized to a cervical skin loop for repetitive sampling of arterial blood. On recovery, the animals were randomly allocated to four groups (A–D) of two calves (see Table 1) and confined to individual metabolism crates fitted with a rubber-coated floor with a receptacle below and a box behind for quantitative collection of urine and faeces. Throughout the study each group was fed on a composite ration as described later and, following 3 weeks of adaptation, the animals were studied for six consecutive balance periods, each consisting of a 7 d conditioning period followed by a 7 d collection period (see Fig. 1, p. 661). Mean live weight (LW) and rates of weight gain were obtained by weighing the animals immediately before and after each collection period (Table 1). For further details of the techniques employed, see Thorbek (1980) and Wamberg *et al.* (1976*a*, *b*).

Dietary preparations

Throughout the study the calves were given weighed portions of the experimental rations specified in Table 2, daily at 07.00 and 15.00 hours. The total amount of feed offered in each collection period was equal to the *ad lib.* intake of the control animals of group A during the preceding 7-d conditioning period. All animals were given free access to tap water.

Table 2. *Composition of experimental rations (g/kg)*

Feed component	Ration			
	US	AS	NS50	NS100
Untreated barley straw	500	—	—	—
Treated barley straw (50 g sodium hydroxide/kg DM)	—	500	—	—
Treated straw (50 g NaOH/kg DM), neutralized with hydrochloric acid	—	—	500	—
Treated straw (100 g NaOH/kg DM), neutralized with hydrochloric acid	—	—	—	500
Barley, rolled	120	120	120	120
Wheat bran	60	60	60	60
Molasses	70	70	70	70
Soya-bean meal	170	170	170	170
Linseed cake	40	40	40	40
Linseed	20	20	20	20
Mineral–vitamin mixture*	20	20	20	20

DM, dry matter.
* Minerals (g/kg): $CaHPO_4 . 2H_2O$ 388·5, $CaCO_3$ 400, NaCl 180, $FeSO_4 . 7H_2O$ 10, MnO, 5, $CuSO_4 . 5H_2O$ 9, $CoSO_4 . 7H_2O$ 0·4, ZnO 7; $Ca(IO_3)_2$ 0·1. Vitamins (μg/g): retinol 120, ergocalciferol 3, α-tocopherol 100.

Table 3. *Average chemical composition of experimental rations*

Ration...	US	AS	NS50	NS100
Dry matter (DM) (g/kg)	894	877	870	836
Composition of DM (g/kg)				
Organic matter (OM)	928	901	901	884
Crude protein (nitrogen × 6·25)	156	160	157	158
Diethyl ether extracts	28	28	28	30
Crude fibre	250	236	239	227
Nitrogen-free extracts	495	477	477	468
Neutral-detergent fibre	537	454	449	418
Acid-detergent fibre	309	297	271	280
Detergent lignin	43	42	36	40
Gross energy				
MJ/kg DM	18·5	18·1	17·9	17·7
MJ/kg OM	19·9	20·0	19·9	20·0
Mineral composition (mmol/kg DM)				
Sodium	134·0	618·0	604·0	919·5
Potassium	468·0	496·0	478·0	415·0
Calcium	199·0	192·5	188·0	192·0
Magnesium	85·0	86·0	81·5	83·0
Chloride	136·0	175·0	464·0	813·0
Phosphorus	125·5	124·0	120·0	133·5
NB*	808·1	1272·8	941·0	831·2

NB, non-metabolizable base.
* Calculated according to eqn (2) (p. 659).

Details of the chemical composition of the ration for each experimental group are given in Table 3. All feed mixtures contained 500 g barley straw/kg diet and were pressed into 14-mm cobs. The chopped barley straw was either untreated (ration US) or spray-treated with 8 M-NaOH corresponding to approximately 50 g NaOH/kg dry matter (DM) (rations AS and NS50) or 100 g NaOH/kg DM (ration NS100). Before the final processing step, residual amounts of NaOH in rations NS50 and NS100 were neutralized with HCl (Table 3). For further details of the alkali-spray technique, developed at the Biotechnical Institute, Kolding, Denmark, see Rexen & Vestergaard Thomsen (1976) and Friis Kristensen *et al.* (1978).

Sampling

Diets. Dietary intake was measured by weighing out the daily rations and correcting for any food residue. For each balance period, portions were taken for chemical analysis.

Water. The daily water intake of each animal was measured by means of a calibrated water meter.

Faeces and urine. Each day faeces and urine were collected quantitatively at 08.00, 12.00 and 16.00 hours and thoroughly mixed. Samples of approximately 10% of the faeces and 5% of the urine (with a few crystals of mercuric iodide) were frozen for subsequent analysis.

Blood. During each balance period, arterial (carotid) and venous (jugular) blood samples were drawn anaerobically in heparinized glass syringes. However, due to anxiety and excitement of the animals, this sampling procedure failed to give reliable blood acid–base values, and during the final collection period (period 6) blood was obtained by means of indwelling carotid catheters inserted under local anaesthesia on day 92 (see Table 6, p. 664).

Sample preparation

Diets. Samples of the composite feed cobs were ground, dried to constant weight at 100° and boiled in 0·7 M-nitric acid for 1 h under reflux. After cooling, samples of the filtrate were frozen for subsequent analysis.

Faeces. Homogenized samples of faeces were freeze-dried and weighed portions were boiled in 0·7 M-HNO_3, filtered and stored at −20° until analysed.

Urine. Thawed, filtered specimens of urine were analysed within 6 h or refrozen.

Blood. The acid–base status of arterial blood was determined within 2 h of sampling. Residual blood was centrifuged and the plasma frozen for mineral analyses.

Analytical methods

Dietary DM composition and gross energy were determined as previously described (Thorbek, 1980). Concentrations of sodium, potassium, calcium, magnesium, chloride and total phosphorus in tap water and in the diet, faeces, urine and plasma, as well as concentrations of sulphate in urine, were measured by methods described previously (Wamberg *et al.* 1976*a*, *b*). Arterial blood acid–base status was determined by the equilibration technique (Siggaard-Andersen, 1974) and urinary (titratable) non-carbonic acid (NCA) and ammonia were measured by potentiometric titration at 37° and a carbon dioxide pressure of 0 mmHg as described by Jørgensen (1957). All measurements were performed in duplicate; estimates of analytical accuracy were obtained by analysis of an aqueous standard solution with each batch of samples (Wamberg *et al.* 1976*a*) showing a coefficient of variation of less than 2·4% for any component.

Statistical analysis of results

All values are presented as means with their pooled standard errors.

In all cases no significant interaction between balance periods and dietary groups was

found ($P > 0{\cdot}05$). The standard error for comparison of dietary groups was based on the variation between calves within groups (4 df) and that for balance-period comparisons was based on the interaction between periods and calves within dietary groups (20 df). In the case of blood acid–base values (Table 6, p. 664) the same analysis was applied, except that balance periods were replaced by time of blood sampling (3 df). The computations were performed by means of the GLIM system developed by Baker & Nelder (1978).

Calculations

In traditional acid–base physiology, investigations are usually limited to measurements of carbonic acid (CA) and NCA in blood, plasma, urine, etc. (Hills, 1973; Dobson, 1980). However, in studies of nutritional and system-physiological aspects of acid–base metabolism it is necessary to distinguish between two kinds of NCA (or base), i.e. metabolizable organic acid (MA) subject to metabolic control, and non-metabolizable acid (NA) or base (NB) subject to renal control and elimination (Kildeberg & Winters, 1978; Kildeberg, 1981). Thus, for any system the concentration (c) of NCA is

$$c\mathrm{NCA} = c\mathrm{MA} + c\mathrm{NA} = c\mathrm{MA} - c\mathrm{NB}. \quad (1)$$

In the organism, metabolic processes invariably result in positive concentrations of CA and MA in cell water and plasma and, in order to maintain (normal) extracellular pH values slightly above the chemical point of neutrality (pH 7·00 at 25°), the organism is dependent on an external source of NB. In the diet, the stoichiometric content of NB is to some extent neutralized ('titrated') by MA. However, by oxidative degradation of absorbed MA absorbed NB is reconstituted and thus available for processes of growth and homeostasis.

In the present study, concentrations of NB in the diet, faeces, urine and plasma were calculated from measured concentrations of non-metabolizable ions multiplied by their average net charge at pH 7·40 (Wamberg *et al.* 1976*b*; Kildeberg, 1981, 1983),

$$c\mathrm{NB} = c\mathrm{Na}^+ + c\mathrm{K}^+ + 2c\mathrm{Ca}^{2+} + 2c\mathrm{Mg}^{2+} - c\mathrm{Cl}^- - 1{\cdot}8c\mathrm{tP} - 2c\mathrm{SO_4}^{2-}, \quad (2)$$

where tP is total P. In the case of the diet, faeces and plasma the SO_4^{2-} was ignored (see Table 4, p. 662).

It follows that cNB represents the sum of titratable values of stoichiometrical concentrations of non-metabolizable Brønsted bases (NaOH, KOH, $Ca(OH)_2$, $Mg(OH)_2$) minus that of non-metabolizable Brønsted acids (HCl, H_3PO_4, H_2SO_4) in the sample.

The concentration of NB may also be obtained by direct titration, and in the case of urine the principle of electroneutrality requires that

$$c\mathrm{NB(U)} = c\mathrm{MA_g(U)} - [c\mathrm{NCA(U)} + c\mathrm{NH_4}^+\mathrm{(U)}], \quad (3)$$

where $c\mathrm{MA_g(U)}$ is the concentration in urine of MA lost by the process of glomerular filtration (Engel & Kildeberg, 1977; Kildeberg, 1981). For plasma, the following applies

$$c\mathrm{NB(P)} = c\mathrm{MA(P)} - c\mathrm{NCA(P)} = c\mathrm{MA(P)} + c\mathrm{BE(P)} + K, \quad (4)$$

where cBE(P) is the 'base excess' of plasma (Siggaard-Andersen, 1974) and K is a constant, approximately 24 mmol/l. Thus, by measuring cNB values according to eqn (2), $c\mathrm{NCA(U)} + c\mathrm{NH_4}^+\mathrm{(U)}$ according to Jørgensen (1957), and cBE(P) according to Siggaard-Andersen (1974), values of $c\mathrm{MA_g(U)}$ and cMA(P) can be obtained by subtraction.

Balance of NB

In animal physiology, the balance technique is widely used in studies of changes in whole-body content of various substances. By definition, the whole-body balance of a given substance is the difference between the mean rate of gain by all sources (input) and the mean rate

of loss by all routes (output). The distinction between input and output is mathematically arbitrary, depending on a sign convention, and may therefore be based on considerations of physiological control mechanisms. In the case of acid–base metabolism, constancy of the concentration of NB in extracellular fluid is achieved by renal mechanisms (Kildeberg, 1981; Wamberg *et al.* 1983). Accordingly, in studies of the balance of NB the output is by definition the mean rate of renal NB excretion, the input being the mean rate of gain of NB by all extra-renal processes such as gastrointestinal absorption, endogenous production, and distribution between body compartments (see Fig. 2, p. 665). Because the mean rate of endogenous production of H_2SO_4 cannot be assessed independently of the rate of renal excretion, the assumption of a zero sulphate balance is requisite to the calculation of the balance of NB (Kildeberg & Winters, 1978; Kildeberg, 1981). In deriving meaningful balance values it is of course essential that the same object of measurement be applied to the various sources of gain and loss. In the case of the balance (b) of NB (bNB), gain of acid corresponds to loss of base and vice versa:

$$b\text{NB} = \dot{n}\text{NB(i)} - \dot{n}\text{NB(o)} = \dot{n}\text{NB(d)} - \dot{n}\text{NB(f)} + \dot{n}\text{NB(e)} - \dot{n}\text{NB(u)}, \quad (5)$$

where $\dot{n}$NB denotes mean substance rate of NB (mmol/kg LW per d) and the process specifications in parentheses denote input (i), output (o), dietary intake (d), faecal loss (f), endogenous production (e) and renal excretion (u). As explained previously, the mean rate of endogenous NA production ($-\dot{n}$NB(e)) is taken to be equal to twice the mean rate of urinary sulphate excretion ($\dot{n}SO_4^{2-}$(u)) (see Table 4, p. 662 and Fig. 2, p. 665).

RESULTS

Body growth

In all groups the animals remained healthy throughout the study and, except for the final balance period (period 6), rates of weight gain were not significantly different. According to the values in Table 1, mean rates of dietary DM intake and feed conversion ratios (MJ/kg weight gain) were rather similar in all groups and comparable to the results obtained by Thorbek (1980).

Water metabolism

In group B, dietary loading with extra Na and NB resulted in a highly significant increase (from 21·8 to 36·7 l/d) in the mean rate of water intake and a similar increase (from 6·2 to 19·7 l/d) in mean daily urinary volume. By contrast, in response to dietary NaCl loading, the observed increase in daily water intake by the animals of groups C and D (4·8 and 4·4 l/d respectively) was distinctly lower. At the same time the mean daily volume of urine rose by 5·2 and 10·3 l/d in groups C and D respectively, apparently due in part to a shift in water excretion from faeces to urine (see p. 664).

Mineral metabolism

Table 4 shows mean rates of dietary intake and faecal and urinary excretion as well as mean rates of absorption and retention of the six minerals studied. Means of intra-individual whole-body balances of NB, calculated according to eqns (2) and (5), are presented in Table 4 and Fig. 2 (pp. 662 and 665); and pertinent urinary acid–base and electrolyte values are given in Table 5. The results show that in groups B and C the surplus loads of dietary Na and NB were almost quantitatively absorbed from the gastrointestinal tract along with K, Cl and additional water, and subsequently excreted in the urine. In group D, a similar response to dietary NaCl loading was observed. Furthermore, in all groups the rates of turnover of Ca, Mg and total-P followed the same pattern and remained not significantly different

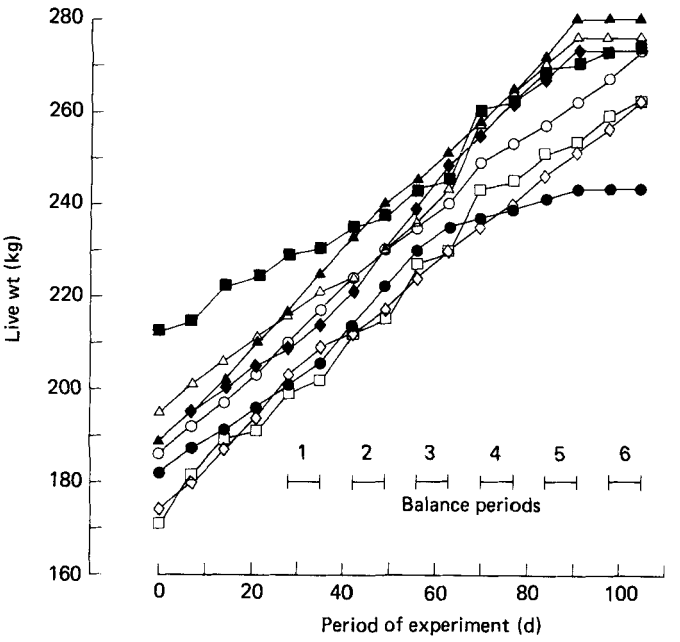

Fig. 1. Individual live-weight gains of ruminating calves in relation to balance periods 1–6. Group A (untreated barley straw) (■ □), group B (alkali-treated barley straw) (▲ △), group C (neutralized barley straw, ration NS50) (◆ ◇), group D (neutralized barley straw, ration NS100) (● ○). For details of rations, see Tables 2 and 3.

($P > 0{\cdot}05$). However, an unexpected but statistically significant difference between balance periods for K, Ca, P and NB was found, apparently due to dietary batch variations.

Acid-base metabolism

During alkali-straw feeding the animals of group B ingested and absorbed considerable amounts of dietary Na and NB, while faecal NB excretion remained not significantly different ($P > 0{\cdot}05$) from the control value of group A (Table 4). At the same time the estimated average rate of endogenous H_2SO_4 production rose slightly and, as a result, the animals of groups B and C faced a net extra-renal load of NB (input) averaging 20·1 and 12·5 mmol/kg LW per d respectively (Fig. 2). In both groups, however, the increase in NB input was closely matched by a corresponding rise in the mean rate of renal NB excretion (11·2 and 4·1 mmol/kg LW per d respectively), accounted for by increased volumes of urine with moderately elevated concentrations of sodium bicarbonate (negative values of *c*NCA(U)) but low concentrations of (filtered) MA (Table 5). In all groups, irrespective of the magnitude of dietary NB loading, normal body growth was associated with a positive balance of NB of about 1–2 mmol/kg per d (Table 4 and Fig. 2).

Blood acid–base and electrolyte status

In Table 6, mean arterial blood and plasma acid–base values obtained during days 4 and 5 of the final collection period are presented. It appears that in spite of the magnitude of alkali loading, arterial blood acid–base status of the animals of groups B, C and D remained within the normal range (Dobson, 1980). Similarly, the plasma concentrations of BE, NB and MA obtained in these groups were not significantly different from those of group A

Table 4. *Whole-body mineral and NB* balance values (mmol/kg live weight per d) of ruminating calves given 500 g untreated (US), sodium hydroxide-treated (AS), or neutralized (NS50 and NS100) barley straw/kg diet*†

(Mean values with their standard errors (4 df) for twelve observations during balance periods 1–6)‡

		Sodium		Potassium		Calcium		Magnesium		Chloride		Phosphorus		Sulphate		NB	
Group	Ration	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Intake																	
A	US	3·1	0·46	10·6	0·54	4·5	0·22	1·9	0·09	3·1	0·31	2·9	0·14	—		18·4	1·08
B	AS	14·6		11·7		4·6		2·0		4·6		2·9		—		30·1	
C	NS50	14·4		11·4		4·5		2·0		11·1		2·9		—		22·5	
D	NS100	21·6		9·7		4·5		1·9		19·1		3·1		—		19·5	
Faeces																	
A	US	1·3	0·31	0·9	0·10	3·6	0·21	1·5	0·13	0·7	0·12	1·8	0·20	—		8·5	0·68
B	AS	1·6		0·7		3·8		1·7		0·6		2·1		—		8·8	
C	NS50	1·2		0·8		3·8		1·7		0·9		2·1		—		8·6	
D	NS100	1·4		0·7		3·5		1·5		0·6		1·7		—		8·5	
Absorption																	
A	US	1·7	0·53	9·7	0·47	0·9	0·10	0·4	0·04	2·4	0·30	1·0	0·20	—		9·9	0·47
B	AS	13·1		11·0		0·8		0·3		3·6		0·8		—		21·4	
C	NS50	13·2		10·6		0·7		0·2		10·3		0·8		—		14·0	
D	NS100	20·2		9·1		1·0		0·4		18·5		1·4		—		11·0	
Endogenous NA production§																	
A	US	—		—		—		—		—		—		—		−1·0	0·10
B	AS	—		—		—		—		—		—		—		−1·2	
C	NS50	—		—		—		—		—		—		—		−1·4	
D	NS100	—		—		—		—		—		—		—		−2·0	
Urine																	
A	US	1·4	0·86	9·1	0·64	0·02	0·003	0·2	0·11	2·8	0·60	0·0	0·06	0·5	0·05	7·1	0·74
B	AS	12·6		10·3		0·04		0·3		4·0		0·0		0·6		18·3	
C	NS50	13·4		10·5		0·03		0·2		11·9		0·0		0·7		11·0	
D	NS100	19·8		8·6		0·03		0·2		19·4		0·1		1·0		7·3	
Balance																	
A	US	0·3	0·35	0·7	0·27	0·9	0·10	0·2	0·11	−0·4	0·40	1·0	0·15	—		1·8	0·49
B	AS	0·5		0·7		0·8		0·0		−0·5		0·8		—		1·9	
C	NS50	−0·2		0·2		0·7		0·0		−1·4		0·8		—		1·5	
D	NS100	0·4		0·5		1·0		0·2		−0·9		1·2		—		1·7	

NB, non-metabolizable base.

* Calculated according to eqn (2) (p. 659).

† For details, see Tables 1–3.

‡ For details, see Fig 1.

§ For details, see p. 660.

Table 5. *Urinary volumes (l/d), pH, and concentrations of acid–base quantities and electrolytes (mmol/l) of ruminating calves given 500 g untreated (US), sodium hydroxide-treated (AS), or neutralized (NS50 and NS100) barley straw/kg diet**

(Mean values with their standard errors (4 df) for twelve observations during balance periods 1–6)†

		Volume		pH		*c*NCA(U)+ *c*NH_4^+(U)		*c*MA_g(U)		*c*NB(U)		*c*Na^+(U)		*c*K^+(U)		*c*Cl^-(U)	
Group	Ration	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
A	US	6·2		8·6		−166·1		114·0		280·3		57·2		367·5		113·0	
B	AS	19·7	1·32	8·5	0·1	−206·0	21·0	39·9	4·3	245·9	24·5	168·3	17·5	138·5	16·3	53·6	10·3
C	NS50	11·4		8·4		−176·9		54·4		231·3		281·8		219·8		250·0	
D	NS100	16·5		8·3		−80·1		27·2		107·4		285·3		124·3		279·7	

*c*NCA(U)+*c*NH_4^+(U), concentrations of titratable non-carbonic acid and ammonium in urine, determined according to Jørgensen (1957) (see p. 659); *c*MA_g(U), concentration in urine of filtered metabolizable organic acid (see p. 659); *c*NB(U), concentration in urine of non-metabolizable base, calculated according to eqn (2) (p. 659); *c*Na^+(U), *c*K^+(U) and *c*Cl^-(U), concentrations of sodium, potassium and chloride in urine.

* For details, see Tables 1–3.

† For details, see Fig. 1.

(Table 6). Moreover, in all groups plasma concentrations of Na (137·1–143·7 mmol/l), K (4·0–4·6 mmol/l) and Cl (97·2–102·3 mmol/l) remained within the normal range published by McSherry & Grinyer (1954), irrespective of the time of blood sampling.

DISCUSSION

The main result of the present study was a demonstration of the ability of the ruminating calf to tolerate long-term alkali-straw feeding, given free access to fresh water. Thus, in all groups a normal plasma acid–base and electrolyte status was maintained and adverse effects on health or body growth were not observed. This is in agreement with previous observations in ruminating cattle given various amounts of alkali-treated roughage (Stigsen, 1975; Jackson, 1977) or dietary supplements of alkali-salt mixtures (Brouwer, 1935; Bhattacharya & Warner, 1968).

Effects of alkali-loading

It appears from the present findings (Table 4 and Fig. 2) that the basic ration of group A provided a dietary intake of NB of about 15–18 mmol/kg LW per d, approximately 50% of which was lost by faecal excretion. Apparently then, during normal body growth, the net rate of gastrointestinal NB absorption exceeds the sum of rates of endogenous H_2SO_4 production and NB retention, leaving considerable amounts of dietary NB to be excreted (mainly as potassium bicarbonate) in an alkaline urine with a pH value above 8 (Table 5). The positive balance of NB (1–2 mmol/kg LW per d) reflects the deposition of absorbed NB along with dietary mineral constituents in 'new tissue', largely the skeleton (Forbes, 1909; Shohl & Sato, 1923; Wamberg *et al.* 1976*b*). Considering the processes of bone formation it appears that 0·92 mmol NB is required for every 1 mmol Ca deposited as crystalline hydroxyapatite $(Ca_3(PO_4)_2)_3 . Ca(OH)_2$ (Kildeberg, 1981). In the present study NB and Ca were retained at a molar ratio of about 2 and, therefore, additional amounts of NB may have been deposited as phosphates and carbonates along with Ca, Mg and Na in amorphous bone mineral. Thus, as indicated by the present findings, the growing calf is in a state of 'relative hypermineralization' due to the rapid development of the skeleton.

Table 6. *Arterial blood and plasma acid–base values of ruminating calves given 500 g untreated (US), sodium hydroxide-treated (AS) or neutralized (NS50 and NS100) barley straw/kg diet**

(Mean values with their standard errors (4 df) for four samples per group obtained by permanent catheterization during days 4 and 5 of period 6)†

Time of day of blood sampling (hours)...			08·00		10.00		12.30		15.00	
Variable	Group	Ration	Mean	SE	Mean	SE	Mean	SE	Mean	SE
pH	A	US	7·45	0·04	7·46	0·03	7·44	0·02	7·44	0·05
	B	AS	7·46		7·47		7·46		7·48	
	C	NS50	7·44		7·44		7·43		7·45	
	D	NS100	7·46		7·46		7·45		7·47	
Carbon dioxide potential (mmHg)	A	US	35·0	2·1	35·2	3·6	35·8	3·9	35·1	4·9
	B	AS	31·8		33·3		32·8		31·2	
	C	NS50	34·4		34·2		34·7		35·4	
	D	NS100	33·5		33·1		33·1		31·7	
*c*BE(P) (mmol/l)	A	US	0·7	1·0	1·5	0·7	0·7	1·1	−0·1	1·2
	B	AS	−0·6		−0·3		−0·2		−0·1	
	C	NS50	−0·3		−0·6		−0·8		0·4	
	D	NS100	0·1		0·1		−0·7		0·1	
*c*NB(P) (mmol/l)	A	US	43·1	4·8	47·2	3·5	47·2	3·0	45·1	4·2
	B	AS	45·9		42·9		46·3		44·6	
	C	NS50	44·3		42·9		42·7		41·3	
	D	NS100	41·6		45·4		40·1		42·0	
*c*MA(P) (mmol/l)	A	US	18·4	5·9	21·6	3·3	22·5	3·3	21·1	4·8
	B	AS	22·0		18·2		22·5		20·7	
	C	NS50	20·6		18·3		19·5		16·9	
	D	NS100	17·3		21·4		16·6		17·9	

*c*BE(P), concentration of plasma 'base excess' (Siggaard-Andersen, 1974); *c*NB(P) and *c*MA(P), concentrations in plasma of non-metabolizable base and metabolizable organic acid, calculated according to eqns (2) and (4) respectively (see p. 659).

In all cases the difference observed between dietary groups or time of blood sampling were not statistically significant ($P > 0{\cdot}05$) (see p. 658).

* For details, see Tables 1–3.

† For details, see p. 658.

In the case of alkali-straw feeding it appears from the present findings that the amount of dietary NB ingested by the ruminating animal may be increased to about 30 mmol/kg LW per d without significant changes in faecal NB excretion (Table 4) or in blood and plasma acid–base status (Table 6), the amount of absorbed dietary NB being quantitatively excreted by the kidneys. In group B, urinary NB concentration was slightly lower than the apparent maximum value of about 300 mmol/l (Brouwer, 1935) and the excess load of NB was excreted by means of a threefold increase in urinary volume, accounted for by a corresponding increase in water intake (see p. 660). In comparison, the animals of group D absorbed an extra load of about 18 mmol NaCl/kg LW per d and excreted it in a relatively less increased volume of urine (Table 5). In these animals, however, the concurrent rise in water intake (Table 1) accounted for only 50% of the increase in urinary volume. Since the animals behaved normally, gained weight and did not develop acidosis, it seems unlikely that dehydration or decreased perspiratory or respiratory water loss could have contributed to the increase in urinary volume. Possibly, therefore, additional water was provided by a shift in water excretion from faeces to urine (see p. 660). Finally, in spite of 'alkalinization' or Na and Cl loading of the diet, or both, the mean rates of mineral

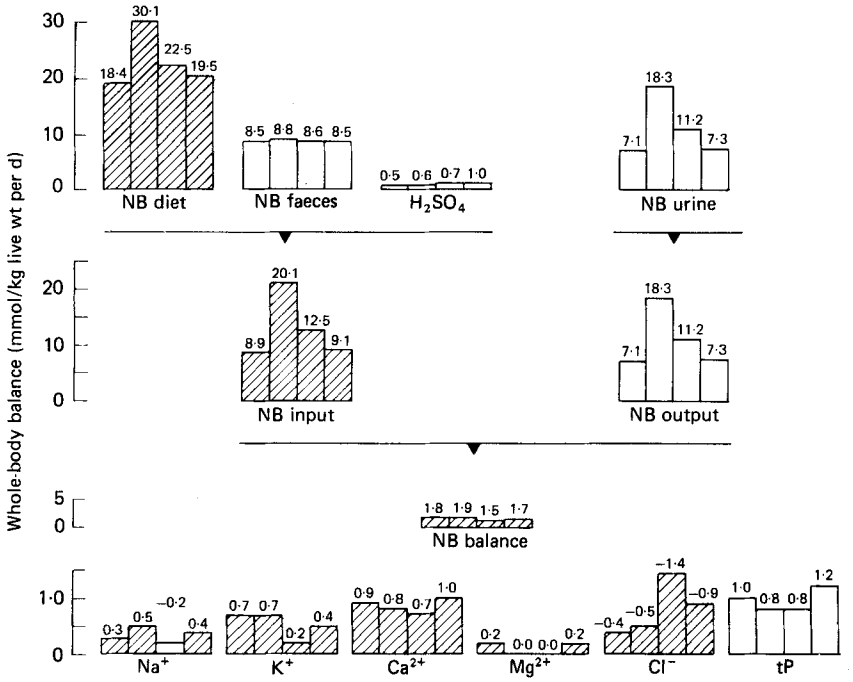

Fig. 2. Histograms representing the components of whole-body balance of non-metabolizable base (NB) in four groups (A–D) of ruminating calves given untreated (US), alkali-treated (AS) or neutralized (NS50 and NS100) barley straw rations (for details of rations, see Tables 2 and 3). The groups of four columns, from left to right, represent the values obtained in groups A, B, C and D respectively. Gains of NB (▨), gains of non-metabolizable acid (NA) (□) (see p. 660). tP, Total P.

retention obtained in the present study were almost identical in the four groups, and within the range of the values reported in the literature (Ellenberger *et al.* 1950; Duncan, 1958; Singh & Jackson, 1971).

The present findings are in accordance with the observation by Kellaway *et al.* (1977) that supplementation of the ration with $NaHCO_3$ up to about 700 mmol/kg DM is well tolerated by young non-ruminating calves, whereas dietary loading above this level produces frank non-carbonic alkalosis. In a similar study in young growing rats given dietary loads of $NaHCO_3$ or sodium citrate (about 50 mmol/kg LW per d), we observed a remarkable increase in Na and NB retention (Wamberg *et al.* 1978), indicating a relative hypermineralization of the skeleton, without any apparent effects on blood acid–base status or rate of body growth. Moreover, according to Brouwer (1935), milk cows may be given dietary alkali loads up to about 30 mmol/kg per d for 54 d without unfavourable consequences.

In principle, renal control of the acid–base status of the extracellular fluid compartment is maintained by continuous removal of NB by glomerular filtration and controlled replacement by tubular 'reabsorption' of NaOH. In any dynamic steady-state (including constant extracellular volume) the concentration of NB in extracellular water (E) is given by:

$$cNB(E) = \frac{\dot{n}NB(t) + \dot{n}NB(i)}{GFR}, \qquad (6)$$

where $\dot{n}NB(t)$ is the rate of tubular regeneration ('reabsorption') of filtered NB, equal to the rate of tubular secretion of hydrogen ion; $\dot{n}NB(i)$ is the net rate of gain of NB by the

extracellular compartment by all non-renal processes; GFR is the rate of glomerular volume filtration (Kildeberg, 1981). In all groups of the present study the mean concentration of NB in plasma was approximately 44 mmol/l (Table 6), similar to the value that can be calculated from the values of McSherry & Grinyer (1954). In the alkali-loaded animals of group B, the mean rate of renal NB excretion was 18·3 mmol/kg LW per d and taking the average rate of glomerular filtration to be about 3·7 l/kg LW per d (Anderson & Mixner, 1960), it appears that the fractional excretion of filtered NB in this group may have been about 12%. This value, which is almost identical to the value of 13% observed by Voigt & Piatkowski (1974) in alkali-straw-fed cows and similar to the value of 15% obtained by us in $NaHCO_3$-loaded growing rats (Wamberg *et al.* 1978), illustrates the capacity of the mammalian kidney to maintain a normal extracellular electrolyte and acid–base status during Na and NB loading.

Effects of neutralization

Considering the effects of neutralization of the rations with HCl it appears that the findings of the present study agree well with several reports of the ability of mature ruminants to excrete massive dietary loads of Na and Cl, provided adequate water is available. Thus, Meyer *et al.* (1955) observed a 50% increase in water intake by steers given 33 mmol NaCl/kg LW per d for 84 d without any detrimental effects. Similarly, in steers given up to 18 mmol NaCl/kg LW per d, Nelson *et al.* (1955) found a threefold increase in daily urinary volume and a small but significant increase in whole-body balances of Na and Cl, whereas the mean rate of N retention remained unchanged. Water supplies containing 10 g NaCl/l (about 36 mmol NaCl/kg LW per d) are well tolerated by young heifers (Weeth & Haverland, 1961) whereas larger loads depress feed consumption and growth rate. In heifers given 15 g NaCl/l drinking water, Weeth & Lesperance (1965) observed pronounced hypernatraemia, increased plasma osmolality and a tenfold increase in daily urinary Na excretion without significant changes in urinary osmolality.

Even in simple-stomached animals it appears to be difficult to induce salt poisoning by dietary NaCl loading, due partly to impalatability (Medway & Kare, 1959; Gyrd-Hansen, 1972) but mainly to the renal capacity for excretion of dietary alkali-salts by the process of glomerular filtration (Potter, 1961, 1963; Mason & Scott, 1974; Kildeberg, 1981).

Dr Grete Thorbek and Dr Lise Neergaard are gratefully acknowledged for continual encouragement and support. The authors are grateful to Professor P. Kildeberg for critical reading of the manuscript and to Bent Jørgensen for statistical guidance and help. We also wish to thank H. B. Kelmann Hansen, Anette Linde, Anne-Grethe Lorenzen, Vibeke Nielsen, Inge Roth and Inge Staffelt for excellent technical assistance. The study was supported by grant no. 513–5133 from the Danish Agricultural and Veterinary Research Council.

REFERENCES

Anderson, R. R. & Mixner, J. P. (1960). *Journal of Dairy Science* **43**, 1476–1479.
Baker, R. J. & Nelder, J. A. (1978). *The GLIM System: Generalised Linear Interactive Modelling, Release 3.* London: Royal Statistical Society.
Bhattacharya, A. N. & Warner, R. G. (1968). *Journal of Animal Science* **27**, 1418–1425.
Brouwer, E. (1935). *Tierernährung* **7**, 463–495.
Coombe, J. B., Dinius, D. A. & Wheeler, W. E. (1979). *Journal of Animal Science* **49**, 169–176.
Dobson, A. (1980). In *Scientific Foundations of Veterinary Medicine*, pp. 112–125. [A. T. Phillipson, L. W. Hall and W. R. Pritchard, editors]. London: Heinemann Medical Books Ltd.
Duncan, D. L. (1958). *Nutrition Abstracts and Reviews* **28**, 695–715.
Ellenberger, H. B., Newlander, J. A. & Jones, C. H. (1950). *Vermont Agricultural Experiment Station Bulletin* no. 558, pp. 1–66.

Engel, K. & Kildeberg, P. (1977). *Scandinavian Journal of Clinical & Laboratory Investigation* **37**, Suppl. 146, 21–26.
Forbes, E. B. (1909). *Ohio Agricultural Experiment Station Bulletin* no. 207, pp. 23–52.
Friis Kristensen, V., Andersen, P. E., Stigsen, P., Vestergaard Thomsen, K., Andersen, H. R., Sørensen, M., Ali, C. S., Mason, V. C., Rexen, F., Israelsen, M. & Wolstrup, J. (1978). *National Institute of Animal Science Report* no. 464, pp. 1–218.
Gyrd-Hansen, N. (1972). Studies on salt poisoning in pigs. PhD Thesis, The Royal Veterinary and Agricultural University, Copenhagen.
Hills, A. G. (1973). *Acid–Base Balance. Chemistry, Physiology, Pathophysiology*. Baltimore: Williams & Wilkins Co.
Jackson, M. G. (1977). *Animal Feed Science and Technology* **2**, 105–130.
Jørgensen, K. (1957). *Scandinavian Journal of Clinical & Laboratory Investigation* **9**, 287–291.
Kellaway, R. C., Thomson, D. J., Beever, D. E. & Osbourn, D. F. (1977). *Journal of Agricultural Science* **88**, 1–9.
Kildeberg, P. (1981). *Quantitative Acid–Base Physiology. System Physiology and Pathophysiology of Renal, Gastrointestinal, and Skeletal Acid–Base Metabolism*. Odense: Odense University Press. New York and Tokyo: Igaku-Shoin Medical Publishers.
Kildeberg, P. (1983). *Scandinavian Journal of Clinical & Laboratory Investigation* **43**, 103–109.
Kildeberg, P. & Winters, R. W. (1978). In *Advances in Pediatrics* vol. 25, pp. 349–381. [L. A. Barnes, editor]. New York: Year Book Medical Publishers Inc.
McSherry, B. J. & Grinyer, I. (1954). *American Journal of Veterinary Research* **15**, 509–510.
Mason, G. D. & Scott, D. (1974). *Quarterly Journal of Experimental Physiology* **59**, 103–112.
Medway, W. & Kare, M. R. (1959). *Cornell Veterinarian* **49**, 241–251.
Meyer, J. H., Weir, W. C., Ittner, N. R. & Smith, J. D. (1955). *Journal of Animal Science* **14**, 412–418.
Nelson, A. B., MacVicar, R. W., Archer, Wm. Jr & Meiske, J. C.(1955). *Journal of Animal Science* **14**, 825–830.
Oloade, B. G. & Mowat, D. N. (1975). *Journal of Animal Science* **40**, 351–357.
Potter, B. J. (1961). *Australian Journal of Agricultural Research* **12**, 440–445.
Potter, B. J. (1963). *Australian Journal of Agricultural Research* **14**, 518–528.
Rexen, F. & Vestergaard Thomsen, K. (1976). *Animal Feed Science and Technology* **1**, 73–83.
Saxena, S. K., Otterby, D. E., Donker, J. D. & Good, A. L. (1971). *Journal of Animal Science* **33**, 485–490.
Shohl, A. T. & Sato, A. (1923). *Journal of Biological Chemistry* **58**, 235–255.
Siggaard-Andersen, O. (1974). *The Acid–Base Status of the Blood*, 4th revised ed. Copenhagen: Munksgaard. Baltimore: Williams & Wilkins Co.
Singh, M. & Jackson, M. G. (1971). *Journal of Agricultural Science, Cambridge* **77**, 5–10.
Sriskandarajah, N. & Kellaway, R. C. (1984). *British Journal of Nutrition* **51**, 289–296.
Stigsen, P. (1975). Kulhydratkildens og neutralisationens betydning for udnyttelse af natriumhydroxydbehandlet halm hos malkekøer. PhD Thesis, The Royal Veterinary and Agricultural University, Copenhagen.
Thorbek, G. (1980). *National Institute of Animal Science Report* no. 498 (Denmark), pp. 1–104 (in English).
Voigt, J. & Piatkowski, B. (1974). *Archiv für Tierernährung* **24**, 589–600.
Wamberg, S., Engel, K. & Kildeberg, P. (1983). *Scandinavian Journal of Clinical & Laboratory Investigation* **43**, 73–83.
Wamberg, S., Hansen, A. C., Engel, K. & Kildeberg P. (1978). *Biology of the Neonate* **34**, 24–31.
Wamberg, S., Kildeberg, P. & Engel, K. (1976*a*). *Biology of the Neonate* **28**, 160–170.
Wamberg, S., Kildeberg, P. & Engel, K. (1976*b*). *Biology of the Neonate* **28**, 171–190.
Weeth, H. J. & Haverland, L. H. (1961). *Journal of Animal Science* **20**, 518–521.
Weeth, H. J. & Lesperance, A. L. (1965). *Journal of Animal Science* **24**, 441–447.

Printed in Great Britain