The rumen buffering system of sheep fed pelleted roughage–concentrate rations

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1. Three wethers fitted with permanent rumen cannulas were given a pelleted roughage–concentrate ration containing 45% ground barley straw as roughage. The animals were given 1 kg of the diet at 08.00 h and 500 g at 17.00 h.

2. The measurements made to determine the buffering components of the rumen fluid were: rumen pH, buffering capacity value (β), total volatile fatty acids (VFA), CO_2 and inorganic phosphorus concentrations. β was expressed as the quantity of titrant (m-equiv./l.) required to bring about 2 unit changes in rumen fluid pH. The pH ranges in which the β values were measured were 4–6, 5–7 and 6–8. All measurements were made at hourly intervals over a 9 h period commencing 1 h before feeding.

3. Rumen pH values were high before feeding, decreased to a minimum 2 h after feeding and then increased to approximately the original values. The concentrations of total VFA followed an inverse pattern. There was diurnal variation in the concentrations of CO_2, but inorganic P levels varied very little throughout the day.

4. Significant correlations were obtained between VFA, inorganic P and CO_2 levels and the β values. It would appear that the bicarbonate and phosphate buffers were not effective in regulating the rumen pH when sheep were given a roughage–concentrate ration.

A number of factors affect the buffering system of the rumen contents. The most important of these are the amount and composition of parotid saliva secreted (Kay, 1960; Bailey, 1961), the concentration of the end-products of fermentation in the rumen, particularly of volatile fatty acids (VFA) and carbon dioxide as well as the rate of absorption of these compounds through the rumen epithelium (Turner & Hodgetts, 1955*a*). The rate of passage of the food through the alimentary tract and the level of dietary buffers also affect the buffering system (Nicholson, Cunningham & Friend, 1962). The feeding of finely ground or pelleted concentrate and roughage–concentrate rations to ruminants is now widely accepted practice. Associated with the feeding of these rations is a marked reduction in salivary secretion and the lowering of the rumen pH (Balch, 1958; Lawlor, Giesecke & Walser-Kärst, 1966). Such changes may result in a suboptimum environment in terms of rumen buffering capacity, thereby reducing the efficiency of utilization of the ration, particularly the roughage part.

The object of the present work was to examine the buffering system in the rumen of sheep given roughage–concentrate rations. The following were measured: rumen fluid pH and buffering capacity (β), total VFA, CO_2 and inorganic P concentrations in the rumen.

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EXPERIMENTAL

Animals and diet: Three mature Suffolk crossbred wethers fitted with permanent rumen cannulas were used. The sheep were housed in individual pens with slatted wooden floors. The components of the roughage–concentrate ration are given in Table 1. The animals had been given this diet for a period of 1 year before the experiment began. They were fed twice daily as follows: 1 kg at 08.00 h and 500 g at 17.00 h. The sheep normally consumed the food within 30 min. They had free access to drinking water.

Table 1. *Components* (%) *of the pelleted roughage–concentrate diet*

Ground barley straw	45·0
Ground barley	10·0
Ground oats	18·5
Maize	10·0
Soya-bean meal	15·0
Dicalcium phosphate	1·0
Mineral + vitamin mixture*	0·5

* Providing (per kg diet): iodized NaCl 5·0 g, $CaCO_3$ 13·4 mg, vitamin A 3000 i.u., vitamin D 500 i.u., vitamin E 33·0 mg.

Sampling. Samples of rumen contents were obtained through the cannulas by means of a polyvinyl chloride tube which was inserted approximately halfway into the rumen contents. This tube was connected to a 250 ml glass centrifuge tube which in turn was connected to a vacuum pump (0·125 hp). Rumen samples were obtained immediately before feeding and at hourly intervals for 9 h after feeding.

A major difficulty associated with the collection of rumen samples when studying the buffering characteristics is the loss of CO_2 and contamination with atmospheric CO_2. In order to overcome these problems the samples were withdrawn directly into 10 ml of a 4:1 (v/v) mixture of liquid paraffin and light petroleum. The inclusion of light petroleum avoided the problems of high viscosity which are encountered when liquid paraffin only is used. The centrifuge tubes with contents were placed in a deep-freeze immediately after sampling. Subsequently the samples were defrosted and centrifuged and the supernatant fluid was stored in a cold room at 4° under the liquid paraffin–light petroleum mixture.

Measurement of rumen pH. The pH values of the rumen fluid were determined on samples of whole rumen contents obtained separately at sampling time. A glass electrode pH meter equipped with a scale expander was used to determine pH.

Determination of buffering capacity of rumen fluid. Samples (2 ml) of the rumen fluid were used for the titration analyses which were carried out under a layer of liquid paraffin. For each titration two portions were used: (1) titrated against 0·5 N-HCl to pH 4 and (2) titrated against 0·5 N-NaOH to pH 8. An automatic titrating unit (Radiometer, Copenhagen) equipped with an autoburette of 2·5 ml capacity and a titrigraph was used to obtain the titration curves. The buffering capacity (β) was expressed as the quantity of titrant (m-equiv./l. rumen fluid) required to bring about 2 unit changes in pH.

Total rumen VFA concentrations. Samples (3 ml) of prepared rumen fluid were treated with equal amounts of N-H_2SO_4 saturated with $MgSO_4$ and centrifuged. A Markham distillation unit was used to distil 1 ml quantities of the supernatant fraction and the distillate was titrated against CO_2-free 0·01 N-KOH in an autotitrator.

CO_2 and inorganic P content of rumen fluid. The microdiffusion technique of Conway (1950) was used to measure the total CO_2 liberated by treating 1 ml samples of the rumen fluid with 1 ml of N-H_2SO_4. The method of Fiske & Subbarow (1925) was used to measure the inorganic P. The proteins were precipitated by treating 1 ml of the rumen fluid with 4 ml of trichloroacetic acid. Duplicate measurements were made on all samples.

Table 2. *The pH and concentration (m-equiv./l.) of volatile fatty acids (VFA), inorganic phosphorus and CO_2 in the rumen contents of sheep 1 h before and at hourly periods after feeding*

(Each value is a mean of five determinations)

	Sheep	−1 h	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	9 h
pH	D	6·45	6·00	5·80	5·97	5·89	6·15	6·21	6·34	6·35	6·49
	E	6·45	5·45	5·25	5·35	5·40	5·55	5·80	6·00	6·10	6·35
	F	6·45	5·70	5·55	5·50	5·50	5·70	5·75	5·80	6·00	6·05
	Mean	6·45	5·72	5·53	5·61	5·60	5·80	5·92	6·05	6·15	6·30
Total VFA	D	77·4	140·1	141·4	140·9	137·7	130·4	128·2	113·3	116·3	106·9
	E	73·7	140·3	136·6	133·7	121·2	108·8	96·5	94·2	78·4	73·5
	F	78·1	150·3	150·9	151·5	150·7	139·5	140·6	132·6	121·0	100·6
	Mean	76·4	143·6	143·0	142·0	136·5	126·2	121·8	113·4	105·2	93·7
Inorganic P	D	45·4	43·9	42·7	47·5	47·5	44·7	45·7	47·0	47·5	49·0
	E	47·7	46·8	43·1	42·4	39·7	39·5	39·2	39·1	38·3	37·6
	F	49·9	47·4	44·4	45·1	44·7	44·8	47·2	44·9	44·0	44·2
	Mean	47·7	46·0	43·4	45·0	44·0	43·0	44·0	43·7	43·3	43·6
CO_2	D	41·4	39·6	40·6	36·6	41·2	52·4	52·2	33·0	58·8	58·0
	E	55·8	50·6	49·6	46·0	48·4	61·4	60·8	59·0	65·0	58·5
	F	44·8	33·0	23·2	23·4	28·8	30·0	39·0	23·0	38·2	50·2
	Mean	47·3	41·4	37·8	35·3	39·5	47·9	50·7	38·3	54·0	55·6

RESULTS

The pH of the rumen fluid and the concentrations of VFA, inorganic P and total CO_2 are given in Table 2. The pH values were highest before feeding and declined sharply thereafter, reaching a minimum (5·25–5·80) 2 h after feeding. This was followed by a gradual increase in pH to approximately the original values at the end of the 9 h sampling period. The pH pattern was the same for all three sheep. The VFA concentrations in the rumen were low before feeding, increased to peak levels between 1 and 3 h and then decreased to the original values. The rumen inorganic P concentrations were fairly uniform for all three sheep and did not show any consistent pattern with time after feeding. Rumen CO_2 concentrations varied considerably both between animals and with time after feeding; they tended to decrease 2–3 h after feeding but increased again at the end of the 9 h sampling period. The β values are shown graphically in Fig. 1. The values obtained for the various pH ranges are shown separately. The β values in the pH range 4–6, where the VFA are most effective as buffers, were

low (75·62–80·0) before feeding, reached a maximum (123·12–133·75) 1 h after feeding and then gradually decreased to the original values. There was little variation throughout the sampling period in the β values over the pH range 6–8, where the phosphate buffers predominate; the pattern in the pH range 5–7 was similar, though less marked, to that in the pH range 4–6.

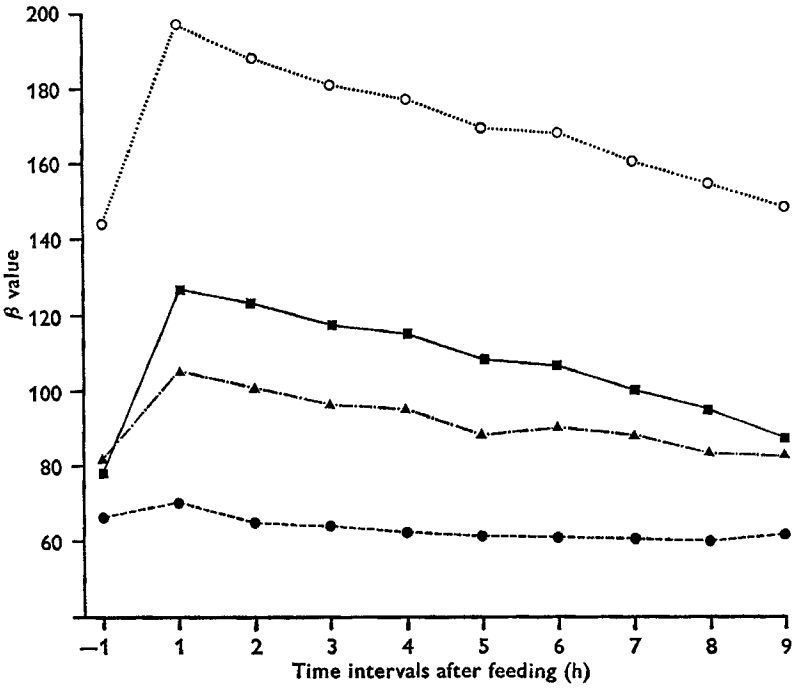

Fig. 1. Mean values for three sheep for the buffering capacity (β) of the rumen fluid in the pH ranges 6–8 (●- - -●), 5–7 (▲- · - · -▲), 4–6 (■——■) and 4–8 (○- - -○).

The relationships between the rumen pH, VFA, inorganic P, CO_2 concentrations and β values are shown in the histograms presented in Fig. 2. The VFA concentrations were negatively correlated (r = 0·978) with rumen fluid pH and positively correlated (r = 0·986) with rumen fluid β values. There was a positive correlation (r = 0·71) between inorganic P concentrations and β values in the pH range 6–8.

DISCUSSION

Despite the extensive and wide-ranging studies which have been conducted on the fermentation of mixtures of roughages and concentrate feeds in the rumen, the buffering system in the rumen of sheep fed a pelleted roughage–concentrate diet does not appear to have been investigated previously. In the present studies the rumen pH values followed the usual trend, being high just before feeding and decreasing to minimum values some 2–3 h after feeding. pH values were closely related to rumen VFA concentrations which increased after feeding thus depressing the pH. The

minimum pH values (5·25–5·80) obtained in these studies are similar to the value of 5·50 reported by Cullison (1961) for a pelleted ration. On the other hand Raun, Burroughs & Woods (1962) reported pH values of from 6·3 to 6·6 for animals fed a 50% concentrate–50% roughage ration. However, the concentrations of rumen VFA observed by the latter workers were considerably lower than the peak levels recorded in the present studies. In general, rumen pH decreases with increasing levels of total VFA (Balch & Rowland, 1957; Van Adrichem, 1962). The concentra-

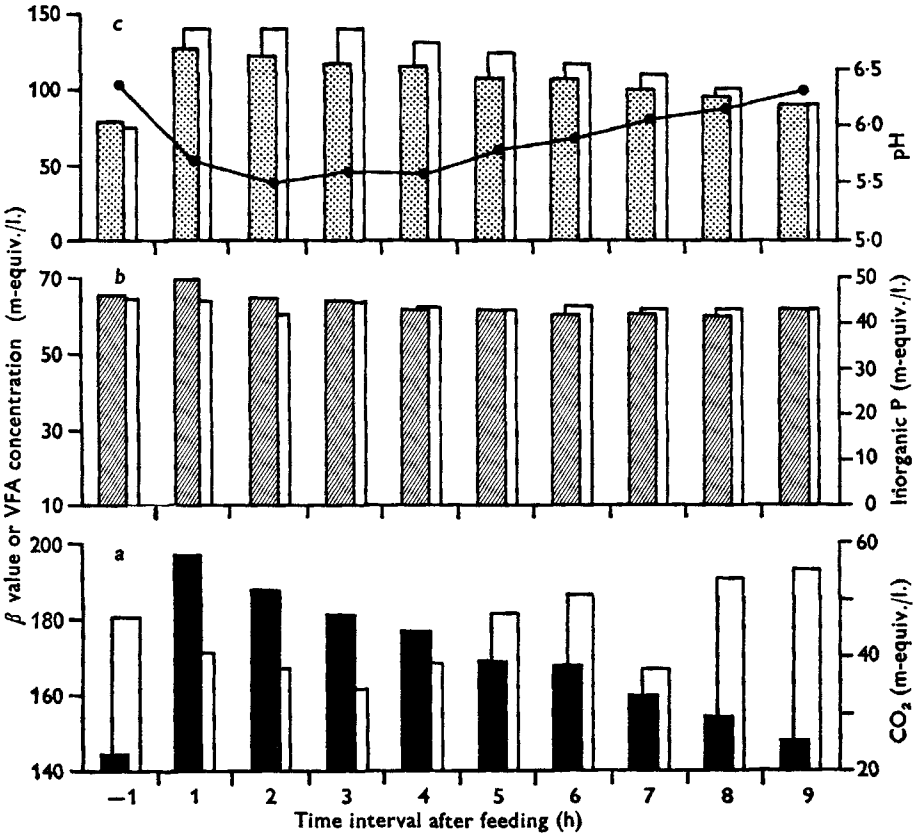

Fig. 2. Relationships at 1 h before and hourly intervals after feeding between (*a*) concentration of CO_2 in rumen fluid of sheep (□) and the β values (■) in the pH range 4–8; (*b*) the inorganic phosphorus concentration (□) and the β values (▨) in the pH range 6–8 and (*c*) rumen pH (●——●), volatile fatty acid (VFA) concentration (□) and the β values (▦) in the pH range 4–6.

tions of inorganic P in the rumen remained fairly constant for each animal throughout the sampling period. A difference of 10 m-equiv./l. between maximum and minimum concentrations is almost identical with that obtained by Bailey (1961). These observations contrast with those of Tomas, Moir & Somers (1967), who found considerably lower inorganic P levels in the rumen fluid 6 h after feeding than obtained immediately before feeding. They indicate that this was possibly due to the rapid consumption of the diet. We disagree with this suggestion, as in the present studies the sheep

consumed the 1 kg of feed within 30 min. Tomas *et al.* (1967) were primarily interested in P turnover in sheep and their results indicated that salivary P was the major source of P to the rumen and was the principal determinant of rumen fluid inorganic P levels. Thus it is likely that the high levels of inorganic P which occurred in the rumen immediately before feeding were attributable to an increase in parotid secretion at that time. The patterns of parotid secretion were subject to marked changes in flow rates throughout the day (Lawlor *et al.* 1966) and in this connexion it is relevant that Tomas *et al.* (1967) presented the ration to the animals only once daily.

Very little information is available in the literature on the concentrations of CO_2 in rumen fluid. The CO_2 concentrations are subject to great variation depending on the quantity and type of diet fed, pH of the rumen fluid, and the intraruminal pCO_2 in addition to the method of sampling of the rumen contents. Turner & Hodgetts (1955*a*) have stressed the high pCO_2 of rumen fluid which may be more than ten times that of arterial blood. These authors emphasize the importance of avoiding CO_2 loss when sampling, and in the present studies every precaution was taken to minimize such loss. In general, there was a reduction in the CO_2 concentrations in the rumen fluid 2–3 h after feeding; with the exception of those for sheep D and F 7 h after feeding, the values increased again at the end of the sampling period. This pattern follows that observed for the pH values, and the concentrations are within the range of theoretical values given by Cole, Huffman, Kleiber, Olson & Schalk (1945).They estimate the total CO_2 in aqueous solutions, when in contact with gas at 1 atmosphere with 70% CO_2, to lie between 21·2 and 59·5 m-moles/l. for a pH range of 5·5–6·5 respectively. Cole *et al.* (1945) emphasize the contribution which parotid saliva makes to rumen CO_2 content when the rumen pH is below 6·9. It may be that the variations in rumen CO_2 concentrations observed in the present study, and especially the unexplained decrease in rumen CO_2 in sheep D and F 7 h after feeding, reflect fluctuations in the volume of parotid saliva secreted. The CO_2 concentrations obtained by Turner & Hodgetts (1955*a*) are considerably lower than those obtained here, which probably reflect differences in the types of diet fed. Measurements of the buffering capacity of rumen fluid in animals receiving a variety of different feeds have been made by several workers (Turner & Hodgetts, 1955*b*; Cabezas, Hentges & Ammerman, 1964; Raun *et al.* 1962; Nicholson, Cunningham & Friend, 1963). The results of these various observations however are difficult to compare since a variety of methods was used to measure and express the buffering capacity. In some instances precautions were not taken to avoid loss of CO_2 and the buffering capacity was expressed either as the quantity of 0·1 N-KOH required to titrate acidified rumen fluid from pH 3·5 to pH 7·0 (Raun *et al.* 1962) or the quantity of HCl required to reduce the pH of 100 ml of strained rumen fluid to pH 4·5 (Nicholson *et al.* 1963). The method employed by Turner & Hodgetts (1955*a*) measured the β value of the rumen fluid at the pH value at which it was withdrawn from the rumen. In the present studies the main objective was to correlate and quantify, under the conditions resulting in the rumen from the diet being studied, the importance of VFA, CO_2 and inorganic P as rumen buffers. For this reason the β values were measured for the pH ranges 4–6, 5–7 and 6–8.

The β values obtained in the pH 4–6 range were significantly higher ($P < 0.001$) than those obtained in the 6–8 and 5–7 ranges. Similarly the β values in the pH 5–7 range were significantly higher ($P < 0.001$) than those in the 6–8 range. The high β values in the pH 5–7 region reflect a contribution from the VFA which are effective as buffers between pH 5 and 6.

A very close correlation ($r = 0.96$) was found between rumen VFA concentrations and β values in the pH range 4–6. This correlation was even more marked than that ($r = 0.81$) which Raun *et al.* (1962) obtained. The VFA are produced in the rumen in their acid form and when partly neutralized are effective buffers. These results emphasize the very important contribution of VFA to the buffering capacity in the rumen when a pelleted roughage–concentrate ration is fed, especially for the period 2–3 h after feeding when the rumen pH is low. The relationship between rumen fluid inorganic P concentrations and β values in the pH range 6–8 was also quite marked. The β values did not vary with time after feeding, as did the inorganic P levels. This would indicate that the quantities of phosphate buffers in the rumen fluid with the dietary regime used by us were inadequate in effectively regulating rumen pH in the 6–8 region. Observations on the buffering capacity of the rumen fluid in the pH 5–7 region were less conclusive. In view of the high CO_2 levels obtained it may be assumed that H_2CO_3, which has a pK value of 6·37, was the principal buffer but, as indicated, it is also likely that a substantial contribution to the buffering values over the pH 5–7 range came from the VFA. It was not possible to assess accurately the relative contribution of each.

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REFERENCES

Bailey, C. B. (1961). *Br. J. Nutr.* **15**, 489.
Balch, C. C. (1958). *Br. J. Nutr.* **12**, 330.
Balch, D. A. & Rowland, S. J. (1957). *Br. J. Nutr.* **11**, 288.
Cabezas, M. T., Hentges, J. F. Jr & Ammerman, C. B. (1964). *J. Anim. Sci.* **23**, 303.
Cole, H. H., Huffman, C. F., Kleiber, M., Olson, T. M. & Schalk, A. F. (1945). *J. Anim. Sci.* **4**, 183.
Conway, E. J. (1950). *Microdiffusion Analysis and Volumetric Error*, 3rd ed. p. 208.
Cullison, A. E. (1961). *J. Anim. Sci.* **20**, 478.
Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
Kay, R. N. B. (1960). *J. Physiol., Lond.* **150**, 515.
Lawlor, M. J., Giesecke, D. & Walser-Kärst K. (1966). *Br. J. Nutr.* **20**, 373.
Nicholson, J. W. G., Cunningham, H. M. & Friend, D. W. (1962). *Can. J. Anim. Sci.* **42**, 75.
Nicholson, J. W. G., Cunningham, H. M. & Friend, D. W. (1963). *Can. J. Anim. Sci.* **43**, 309.
Raun, N. S., Burroughs, W. & Woods, W. (1962). *J. Anim. Sci.* **21**, 838.
Tomas, F. M., Moir, R. J. & Somers, M. (1967). *Aust. J. agric. Res.* **18**, 635.
Turner, A. W. & Hodgetts, V. E. (1955*a*). *Aust. J. agric. Res.* **6**, 115.
Turner, A. W. & Hodgetts, V. E. (1955*b*). *Aust. J. agric. Res.* **6**, 124.
Van Adrichem, P. W. M., (1962). *De Invloed Van Het Voeder Op Enige Fermentatierprodukten in de Pens Van Normale Runderen en Van Acetonaemiepatienten*, p. 33. Drukkerij Stempel, Hoorn.

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