Chapter 4 The Buffering Capacity of Caecal Digesta Exceeds that of Rumen Digesta from Sheep Fed Pasture or Roughage Diets

4.1 Introduction

When ruminants ingest large amounts of starch or feed rich in readily fermentable carbohydrates, VFAs and lactic acid can accumulate in the rumen and hindgut causing the pH of the gut contents to fall. If the production of lactic acid and corresponding fall in pH are of sufficient magnitude, the clinical condition of acidosis can occur. Acidosis arising from rapid fermentation of carbohydrate is wide-spread in ruminant production systems and is a condition with severe consequences for the animal. An understanding of how acidosis affects the animal is a fundamental requirement in developing effective methods of prevention.

Previous studies on the pH of rumen contents in animals with acidosis indicate that pH is often below 5.5 (Dunlop 1972; Murray *et al.* 1992; Godfrey *et al.* 1992; Andrighetto and Bailoni 1994). Since lactic acid is poorly absorbed from the rumen (Williams and Mackenzie 1965), it accumulates in the digestive tract and makes a major contribution towards low rumen fluid pH and the development of acidosis. These acidic conditions are partly ameliorated by *in situ* buffer systems. Turner and Hodgetts (1955) established that the most important buffering components within the usual pH range of the rumen digesta are bicarbonate and phosphate, and that bicarbonate is more important than phosphate in providing buffering against acid conditions in the rumen of fasted sheep. Subsequent studies have demonstrated that bicarbonate and VFAs are the main components of the buffering system in the rumen fluid of dairy cattle under a range of feeding conditions (Counotte *et al.* 1979), and that saliva phosphates are of little importance as a buffer, despite neutralising a limited quantity of the acid produced in the rumen.

Dietary supplements may also be used to manipulate rumen fluid pH. Included in the diet of steers and sheep consuming corn silage, alfalfa hay, orchard-grass hay, and concentrate mixture of corn and soybean, sodium bicarbonate ($NaHCO_3$) can increase rumen fluid pH (Rogers *et al.* 1979; Rogers and Davis 1982a, b; Kovacik *et al.* 1986). Furthermore, administration of sodium carbonate (Na_2CO_3) into the rumen prevents the anticipated drop in rumen digesta pH in sheep fed rations containing high concentrations of wheaten starch (Reid *et al.* 1957).

Recent studies indicate that acidosis can also occur in the hindgut as a result of the rapid fermentation of carbohydrates (Godfrey *et al.* 1992, 1994b). However, there is little information on the buffering capacity of the ruminant hindgut. The expansion of feedlots in Australia and the increasing use of carbohydrate-rich diets in ruminant production systems demands that the potential capacity of animals to effectively neutralise the acid productions of fermentation within the whole digestive tract be established and understood. Therefore, the aim of these studies was to investigate the relative buffering capacities of the caecal and rumen digesta of sheep under a range of dietary conditions, and to determine the main chemical factors that affect the buffering capacity of the rumen digesta *in vitro*.

4.2 Materials and methods

4.2.1 Experimental design

Four separate groups of Merino sheep, weighing 30 to 45 kg and aged approximately 2 years, were used in the experiments.

(i) Group 1

Six wethers were individually penned and fed 900 g/day of oaten chaff containing 1% urea for 4 weeks before the experiment. The feed was given hourly in equal amounts.

(ii) Group 2

Three wethers were fed 900 g/day of oaten chaff containing 1% urea for 2 weeks, at hourly intervals, before and while receiving an intra-ruminal infusion. Each sheep was allocated to only one of the following infusions: glucose (5.8 mol/L), lactic acid (1 mol/L) or acetic acid (4.3 mol/L). Infusions were continued at a rate of 2 mL/min for 6 hours in the case of glucose and lactic acid and for 1 hour in the case of acetic acid. Infusions were stopped when the rumen pH reached 4.85 except in the case of acetic acid when it reached 4.35. The purpose of the ruminal infusion was to investigate the time course and chemical changes during the development of rumen acidosis. Forty eight hours samples of rumen and caecal digesta were taken for analysis of buffering capacity measured *in vitro,* After the acidosis was induced, all sheep were euthanased and samples.

(iii) Group 3

The five ewes used were grazing lush green pasture (approximately 2.8 tons dry matter per hectare until 3 h before the experiment.

(iv) Group 4

Three ewes were used. They were prevented from feeding and drinking for 24 h before the experiment. These fasted ewes grazed the same green pasture as the sheep in Group 3.

The fluid volumes of the rumen and caecum of these sheep were from 3 to 5 litres and from 300 to 600 mL, respectively. The contents were less in the rumen and caecum of the fasted sheep, compared to the animals not fasted.

All sheep were euthanased at different times and all rumen and caecal digesta were collected and filtered/strained into different buckets in 38°C water bath with cheese cloth to remove raw dietary residue. The buckets with rumen and caecal digesta in 38°C water bath were covered with films of plastic and rapidly moved into a 37°C room to determine buffering capacity by titration as described below. About 10 minutes elapsed from euthanasia to titration of the fluid. In order to investigate

chemical buffering methods to prevent acidosis, the rumen and caecal digesta of sheep fed different diets were examined to determine the buffering capacity with four levels (0, 12.5, 25 and 50 mmol/L) of different additional buffers. The additional buffers used were as follows: $NaHCO_3$, Na_2CO_3, Na_2HPO_4 (A.R., AJAX Chemicals PTY LTD, Australia); NaH_2PO_4 (A.R., BDH Chemicals, Australia) and all buffers were tested in all rumen digesta samples except control ones. The quantity of caecal digesta was much less than that of rumen digesta and, therefore, few caecal digesta samples could be tested with additional buffers.

4.2.2 Measurement of buffering capacity

The initial pHs of the rumen and caecal digesta were not adjusted prior to titration. The buffering capacities of the rumen and caecal digesta were determined by titration with 1 mol/L of lactic, acetic or hydrochloric acid. These acids were used because lactic and acetic acids are important acids in anaerobic fermentation and can be utilised by bacteria. Hydrochloric acid (HCl), on the other hand, is not involved in an anaerobic fermentation and there was no evidence of it being utilised by bacteria during titration. All titrations were carried out at 37°C. Aliquots of 50 mL of the digesta in a beaker were stirred constantly on a magnetic stirrer while acid was added. With each addition of 0.5 mL acid, the pH was allowed to stabilise for 30 seconds. The total volume of an acid added was 10 mL. When buffers were added, the digesta were stirred for 5 minutes before titration to ensure that the additional buffers had dissolved and were mixed thoroughly.

The buffering capacity was defined as the amount of acid (mmol) required to change the pH of 1 mL of digesta by 1 unit. However, a figure of 2 x the value for a half pH change is used as a convent index in the following text and not as an estimate of the change one would get for one pH unit.

4.2.3 Statistical methods

Data were analysed using an analysis of variance (ANOVA), and Student Newman-Keuls Multiple Comparison Methods.

4.3 Results

There were two peaks of buffering capacity for the rumen and caecal digesta with or without additional buffers. Although the initial pHs of the rumen (pH 6.15 - 6.63) and caecal digesta (pH 6.9 - 7.38) were different. However, a more pronounced peak existed in buffering capacity between pH 6.0 and 6.5 in both rumen and caecal digesta over all diets for all the three titration acids. Typical changes in buffering capacity with pH for the samples of rumen and caecal digesta titrated with lactic acid are shown in Figure 4-1.

The buffering capacity of the digesta varied depending on which acid was used in the titration (Table 4-1). Using HCl as the titrant, the buffering capacities of both caecal and rumen digesta were always lower than those when either lactic or acetic acid was used, for all pH values in the range tested (Figure 4-2).

The buffering capacities of rumen and caecal digesta of sheep on different diets are summarised in Table 4-2. The data in Table 4-2 indicate that the buffering capacity of caecal digesta was nearly double that of rumen digesta in all sheep ($P < 0.001$). The same pattern of higher buffering capacity in caecal digesta was seen for all acids and for all dietary treatments. The buffering capacity of rumen digesta from sheep fed oaten chaff was 21% higher than that from sheep grazing green pasture ($P < 0.05$). The buffering capacity of rumen digesta of sheep recovering from acidosis was reduced to nearly two-third that of normal sheep fed the same oaten chaff diet ($P < 0.05$). There was no significant difference in the buffering capacity of caecal digesta of sheep on different diets. However, in Group 4, the buffering capacities of rumen and caecal digesta from the fasted sheep were significantly higher than those from the sheep in Group 3 when digesta were taken immediately after animals stopped grazing the same green pasture (62% higher for rumen digesta, $P < 0.001$; 18% higher for caecal digesta, $P < 0.05$).

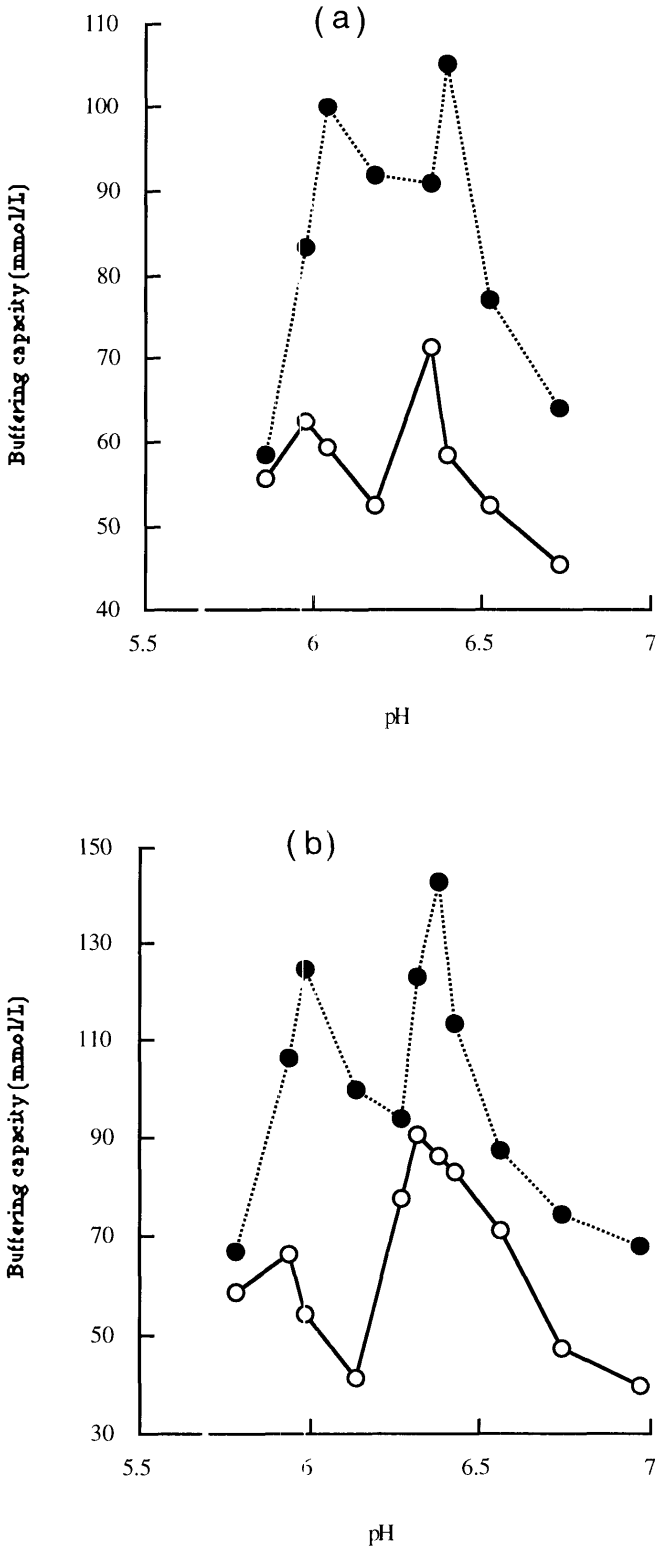

Figure 4-1. Buffering capacity of rumen (○) and caecal (●) digesta for sheep grazing green pasture. Titration was with lactic acid. (a) Live digesta and (b) with additional 25 mmol/L of $NaHCO_3$.

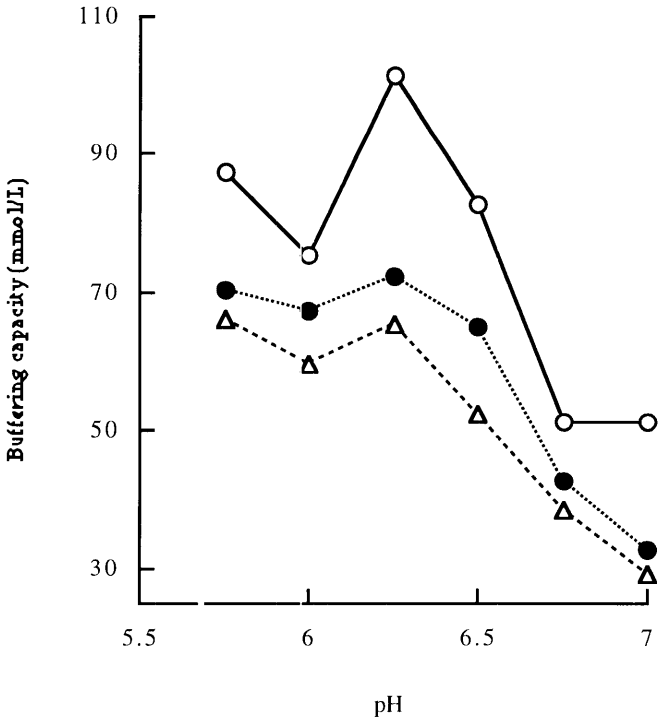

Figure 4-2. Effect of pH on buffering capacity of caecal digesta from sheep grazing green pasture. Titration was with acetic (○), lactic (●) and hydrochloric (Δ) acids.

Table 4-1. Mean buffering capacity (mmol/L) of caecal and rumen digesta from a total of 17 sheep in four groups. Rumen and caecal row comparison was very significantly different ($P < 0.001$). Both within and between row comparisons, means followed by the same letter are not significantly different ($P > 0.05$).

	Acid used for titration			s.e.m.[A]
	Acetic	Lactic	HCl	
Rumen	46.7a	44.6a	36.5b	2.0
Caecal	81.8c	73.2d	60.7e	4.1

[A] s.e.m., standard error of the mean for rows.

Table 4-2. Mean buffering capacity (mmol/L) of caecal and rumen digesta of the sheep on different dietary regimes in four groups. Titration was with lactic, acetic and hydrochloric acids. Rumen and caecal row comparison was very significantly different ($P < 0.001$). Both within and between row comparisons, means followed by the same letter are not significantly different ($P > 0.05$).

	Chaff (6 sheep)	Chaff then acidosis (3 sheep)	Pasture (5 sheep)	Fasted pasture (3 sheep)	s.e.m.[A]
Rumen	44.5a	30.0b	36.7b	59.3c	2.5
Caecal	72.5d	67.3d	67.4d	79.8c	2.4

[A] s.e.m., standard error of the mean for rows.

The buffering capacity of the rumen digesta of sheep recovering from lactic acidosis increased significantly ($P < 0.05$) after adding 50 mmol/L of $NaHCO_3$ (Figure 4-3).

As shown in Figure 4-3, it required 15 (a) and 25 (b) mmol/L of HCl to reduce 0.5 unit pH (from pH 6.5 to 6.0) in the rumen digesta containing 0 and 50 mmol/L additional $NaHCO_3$, respectively. That is to say that 50 mmol/L additional $NaHCO_3$ increased buffering 25 - 15 = 10 mmol/L HCl within 0.5 unit pH. Therefore, value, 10 x 2 = 20 mmol/L HCl, represented the increased buffering capacity for the rumen digesta associated with 50 mmol/L additional $NaHCO_3$ within 1 pH unit. Alternatively, the effect of the buffer can be estimated by the additional acid which can be added without changing the pH of the fluid when a buffer is present. Values, 22 (z) and 32 (z') mmol/L of HCl, were the effects of 50 mmol/L additional $NaHCO_3$ at pH 6.5 and 6.0, respectively, i.e. 22/50 = 0.44 mmol of HCl at pH 6.5 and 32/50 = 0.64 mmol of HCl at pH 6.0 represented per mmol additional $NaHCO_3$. However, per mmol additional $NaHCO_3$ could buffer 1.66 mmol of acetic acid or 1.15 mmol of lactic acid in the pH 6.0 rumen digesta experiments.

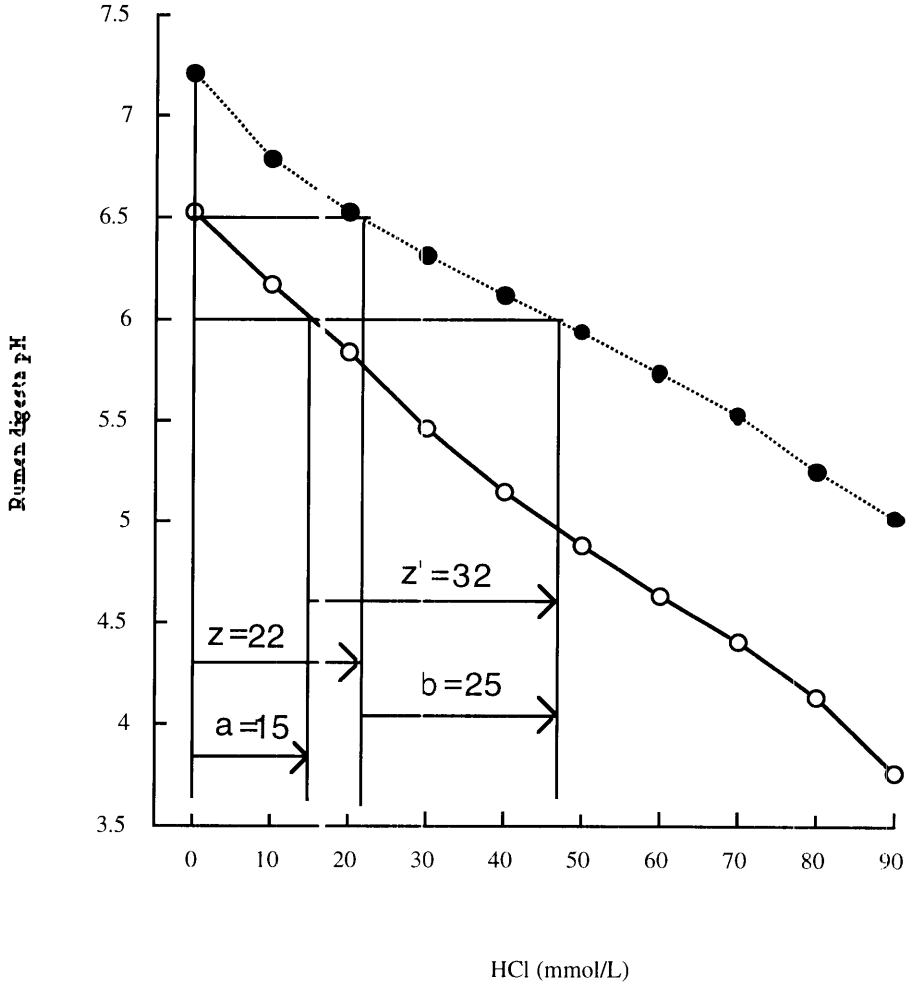

Figure 4-3. Relationships between pH and the amount of HCl added to 50 mL of rumen digesta taken from the sheep recovering from lactic acidosis and mixed with additional 0 (○) and 50 (●) mmol/L of $NaHCO_3$. The measurements a (15) and b (25) show the different amounts (mmol/L) of HCl required to reduce the pH from 6.5 to 6.0 in the rumen digesta containing 0 and 50 mmol/L $NaHCO_3$. Z (22 mmol/L of HCl) and z' (32 mmol/L of HCl) is for the effects of 50 mmol/L of $NaHCO_3$ at pH 6.5 and 6.0, respectively.

The changes in buffering capacity of the rumen digesta containing different additional buffers varied considerably. The results for rumen digesta from sheep recovering from acidosis with various concentrations of different additional buffers are summarised in Table 4-3.

As shown in Table 4-3, the increment of buffering capacity of rumen digesta containing differe it concentrations of additional buffers was significant ($P < 0.05$). The mean increment in buffering capacity for the rumen digesta with 12.5, 25 and 50 mmol/L of the various additional buffers was 18%, 42% and 75%, respectively. Within the range of concentrations tested, buffering capacity of rumen digesta increased linearly with increasing buffer concentration. The relative abilities of each buffer to increase the buffering capacity of rumen digesta were ranked as follows: CBS > Na_2CO_3 > $NaHCO_3$ > Na_2HPO_4 > PBS > NaH_2PO_4.

Table 4-3. Mean buffering capacity (mmol/L) of rumen digesta of three sheep recovering from acidosis with various concentrations of different additional buffers. Titration was with lactic acetic and hydrochloric acids and the values shown are the mean one for the three acids. Different buffer row comparisons were significantly different ($P < 0.05$) except Na_2CO_3 and $NaHCO_3$. Different buffer concentration group comparisons were significantly different ($P < 0.05$). Both within and between row comparisons, means followed by the same letter are not significantly different ($P > 0.05$).

	Buffer concentration (mmol/L)				s.e.m.[3]
	0	12.5	25	50	
CBS[1]	30.0a	37.4b	47.3c	61.9c	6.9
Na_2CO_3	30.0a	37.7b	45.0c	60.3c	6.5
$NaHCO_3$	30.0a	36.7b	45.3c	59.3c	6.3
Na_2HPO_4	30.0a	34.7b	40.5d	49.1c	4.1
PBS[2]	30.0a	35.1b	39.5d	47.9c	3.6
NaH_2PO_4	30.0a	33.3b	38.8d	45.2c	3.3
s.e.m.[3]	0	0.7	1.5	3.0	

[1]CBS (carbonate buffer system) = $NaHCO_3$ and Na_2CO_3 (1 : 1; mmol : mmol) with total buffer concentration of 0, 12.5, 25, and 50 mmol/L.
[2]PBS (phosphate buffer system) = NaH_2PO_4 and Na_2HPO_4 (1 : 1; mmol : mmol) with total buffer concentration of 0, 12.5, 25, and 50 mmol/L.
[3]s.e.m., standard error of the mean for rows or columns.

In rumen digesta $NaHCO_3$ and Na_2CO_3 provided a more effective buffering function than NaH_2PO_4 and Na_2HPO_4 (Figure 4-4).

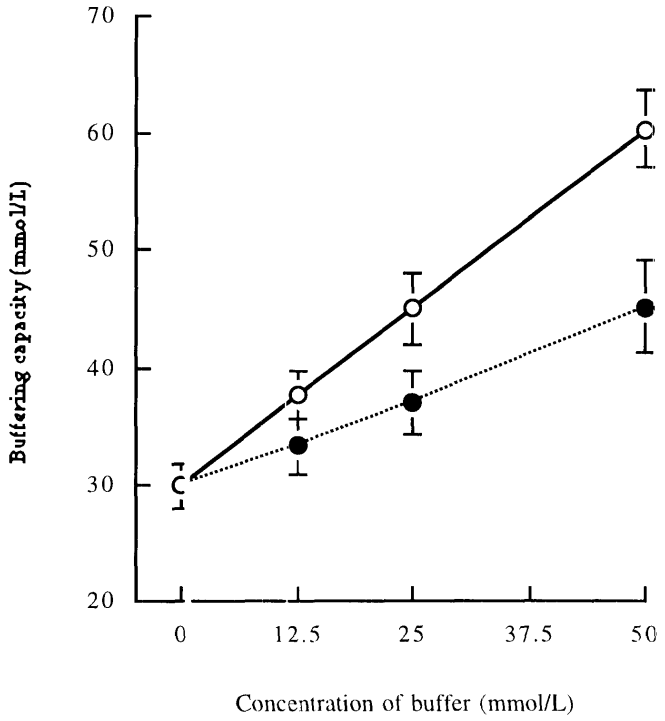

Figure 4-4. Comparison of mean buffering capacities of rumen digesta for three sheep recovering from acidosis with additional $NaHCO_3$ and Na_2CO_3 (○), and NaH_2PO_4 and Na_2HPO_4 (●). The figure is shown using the mean buffering capacity titrated with lactic, acetic and hydrochloric acids.

4.4 Discussion

Maintenance of relatively stable pH in the rumen and caecum is essential to fermentation and to maintain animal well-being. This pH is largely controlled by physiological buffering systems. The rumen has more scope for control of pH of its contents than the caecum since it receives saliva, which is rich in carbonate and phosphate buffers. In addition, the animal can readily reduce substrate entering the rumen by

simply not eating, and can increase the rumen turnover rate with the influx of water across the rumen wall to dilute the digesta by a voluntary process. The caecum, on the other hand, receives no saliva input, and the influx of digesta is largely controlled by the small intestine. Despite there being no input of saliva into the caecum, in these studies the buffering capacity of caecal digesta was much higher than that for the rumen digesta. This could be a result of the higher dry matter content of the caecal digesta resulting in the greater concentration of buffering agents. Results of this study suggest that the buffering capacities of rumen and caecal digesta may be affected by feed and water intake before slaughter. The buffering capacities of rumen and caecal digesta for the fasted sheep were much higher than those of the sheep grazing the same green pasture (Table 4-2). This was largely because the digesta from the sheep prevented from feeding and drinking for one day probably contained less water and would therefore be more concentrated. The second factor could be reduced acid production in the absence of fresh substrate for fermentation. The buffering capacities of rumen and caecal digesta of sheep fed oaten chaff were higher than those for sheep grazing green pasture because fermentable substrate for the sheep fed oaten chaff would have been lower in the amount and drier in the quality leading to reduced acid production. Thirdly, caecum has a higher proportion of finer feed particles than the rumen. The fine particles may provide greater surface area for absorption of protons, therefore, higher buffering capacity. The higher buffering capacity of the caecal digesta compared to the rumen digesta may be an important adaptive mechanism to help compensate for the reduced strategies that this organ has in coping with acidosis.

The buffering capacity determined using HCl was always lower than that for lactic or acetic acid. Firstly, this is due to the pKa for HCl being much lower (HCl = -0.47, lactic acid = 3.86, acetic acid = 4.76) and secondly, because of the potential inter-conversion of lactic and acetic acids with other VFAs during microbial fermentation, whereas there is no evidence to suggest this occurs with HCl.

At a wider range of pH in the titration, there were two peaks in buffering capacity of normal digesta with or without additional buffers, especially with $NaHCO_3$ and Na_2CO_3 (Figure 4-1). The chemical equilibria for the bicarbonate buffer system can be summarised as:

$$H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \quad (1)$$

In (1), $Ka' = [H^+][HCO_3^-]/[H_2CO_3] = 4.2 \times 10^{-7}$, $pKa' = 6.38$.

$$HCO_3^- \Leftrightarrow H^+ + CO_3^{2-} \quad (2)$$

In (2), $Ka'' = [H^+][CO_3^{2-}]/[HCO_3^-] = 4.8 \times 10^{-11}$, $pKa'' = 10.32$.

Refer to Chang (1977) for pKa and its calculation. When the digesta were titrated with acid, the equilibria moved from the right to the left and from (2) to (1). Thus, peaks 2 and 1 were formed (Figure 4-1). Peak 2 was between pH 6.5 and 6.0 which is the important range within which to maintain pH in order to prevent the accumulation of lactic acid and rumen dysfunction. This result is consistent with the calculation of Counotte *et al.* (1979) who showed the maximum buffering capacity of rumen fluid was at pH 6.25.

A pH of 5.5 is considered a critical point for physiological control in the rumen. Below this, lactic acid is not converted to the more readily absorbed fatty acids and rumen stasis rapidly develops leading to profound acidosis. As acidosis develops, pH and buffering capacity fall. The more severe the acidosis, the lower the pH and the buffering capacity. The actual buffering capacity of rumen fluid at pH 6.0 in these studies measured by titration indicated that 1 mmol of $NaHCO_3$ neutralised 0.64 mmol of HCl but neutralised 1.66 mmol of acetic acid or 1.15 mmol of lactic acid in the same experiments. These differences between HCl, lactic acid and acetic acid are perhaps due to their different Ka values and the live system of rumen digesta. They may also be due to the conversion of acetic and lactic acids to non-acidic forms by the microbes. Hydrochloric acid was used for calculating the standard in Figure 4-3 because there is no evidence for HCl being converted in the rumen and caecal digesta during titration. It should be noted that the buffering capacity of $NaHCO_3$ varies depending on the rumen pH as shown in Figure 4-1b. In practice, if acidosis in grain-fed animals is to be controlled, then prevention of a fall in rumen pH below 6.0 is highly desirable.

As expected in these experiments, the pH and buffering capacity of the rumen digesta increased after adding $NaHCO_3$ (Figure 4-3 and Table 4-3). The response to $NaHCO_3$ was similar to that previously reported by Bigham *et al.* (1973), Counotte *et al.* (1979), Rogers and Davis (1982a, b),

and Kovacik *et al.* (1986) who found the pH of the rumen digesta increased after adding $NaHCO_3$. However, an *in vivo* experiment reported by Xu *et al.* (1994) indicated no significant differences in rumen fluid pH or molar percentage of VFAs between cows fed diets containing 2.2 % sodium bicarbonate and those fed regular diets. Similarly, Zinn and Borques (1993) found no measurable effect of bicarbonate (0.75 % DM) on rumen pH in cattle fed a high corn diet. These differences may be explained by the differences in experimental techniques and the quantities of buffer used. The two studies described above were done *in vivo*, whereas the experiments in this study were done *in vitro*. The results between *in vivo* and *in vitro* might be different due to any of the following factors:

- added dietary buffers may have an effect on the production of salivary buffers and ruminal metabolism;
- the actual concentration of $NaHCO_3$ in the rumen digesta may vary due to the flow of digesta, HCO_3^- from the rumen wall, and the absorption of HCO_3^-;
- ratio of HCO_3^- to fermentable substrate.

In summary, the caecal contents are more effectively buffered against acid than rumen contents and the buffering in both organs is maximal at about pH 6.0 - 6.5. The buffering capacity of the caecal digesta is less affected by the nature of the diet than that of the rumen digesta.

Chapter 5 A Model of Rumen Fermentation to Predict Lactic Acid Concentration

5.1 Introduction

Lactic acidosis arising from rapid fermentation of carbohydrate is widespread in ruminant production systems and is a condition with severe consequences for the animal. There is a complex range of factors leading to the development of lactic acidosis. As our understanding of these improves, a computer model of fermentation in the rumen of sheep fed grain as a dietary supplement has a potential role in predicting animal responses to different feeds processing techniques and feeding patterns.

Mechanistic modelling is increasingly being used as a research tool. Following the work of Baldwin *et al.* (1970), some models of whole rumen function have been developed (Reichl and Baldwin 1975; Murphy *et al.* 1986; Baldwin *et al.* 1987a, b, c; Dijkstra *et al.* 1992; Russell *et al.* 1992; Pitt and Pell 1997). These models have endeavoured to represent the digestion and passage of ingested nutrients, microbial metabolism and the formation of end-products of digestion. The use of modelling in optimisation of rumen processes has been reviewed recently (Sauvant *et al.* 1995; Dijkstra and France 1996). Although a number of issues related to rumen modelling requires further research, modelling of whole rumen function has advanced greatly over the last few decades. The whole rumen function models provide a framework to integrate knowledge on various features of rumen fermentation from which to predict the supply of nutrients to ruminants. However, so far the rumen models do not focus on the development and prevention of lactic acidosis.

The objective of the project described in this chapter was to develop a computer model of rumen fermentation integrating knowledge on the development and control of lactic acidosis in the rumen. This chapter defines the model and the data used in developing the model before

summarising the sensitivity of the model to key parameters and its performance against experimental results. The model was also used to determine the relative effectiveness of four different ways of controlling acidosis in the rumen.

5.2 The model

The model was developed using the software "STELLA II" (High Performance Systems Inc. 45 Lyme Road, Suite 300, Hanover, NH) and was run on a Macintosh computer.

5.2.1 Overall structure and notation

The model is presented schematically in Figure 5-1. The abbreviations used to denote entities in the model are given in Table 5-1. A rumen size of five litres was assumed as being representative of an adult sheep. Many of the features of this model are based on experimental data described in Chapters 3 and 4 of this thesis. These data have been supplemented by and checked against a wide range of additional information described in the scientific literature as indicated in the following sections. The key features include: the rate of buffering capacity in controlling lactic acid build up; and the relative rates of absorption of VFAs and lactic acid from the rumen.

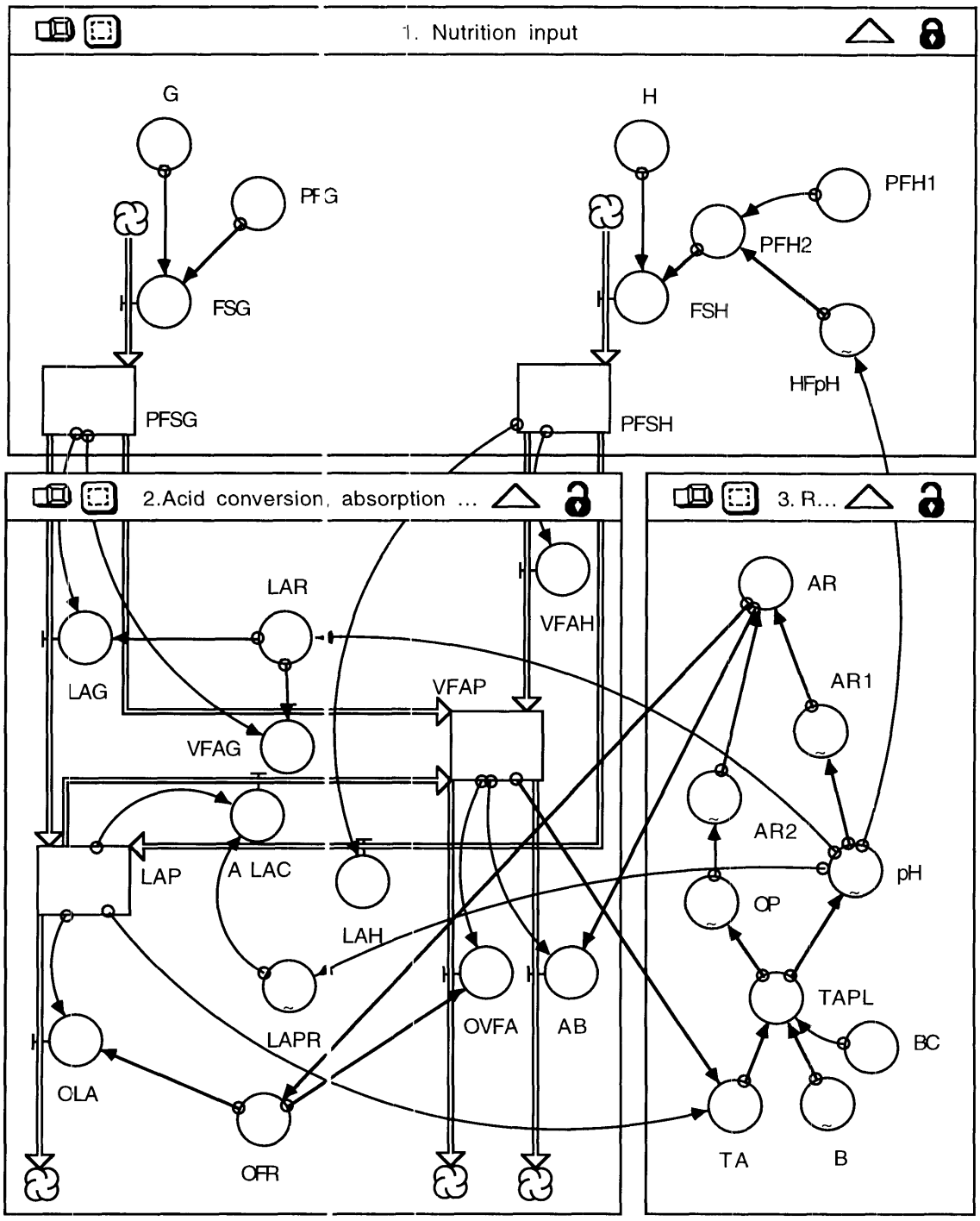

Figure 5-1. Diagrammatic representation of the model for the rumen fermentation of sheep. The model includes 3 major sections: (i) nutritional input; (ii) acid conversion, absorption and outflow; and (i i) regulation. The rectangular boxes indicate pools or state variables. The circles indicate metabolites, absorption and outflow of acids, and regulating factors. Spirals indicate beginnings or ends. Arrows indicate fluxes.

Table 5-1. General notation used in the model

Notation	Translation	Unit
G	Grain "intake"	g/min
PFG	Potential fermentability of grain	mmol acids produced/g
FSG	Fermentable substrate produced from grain	mmol acids/min
PFSG	Pool of fermentable substrate producing acids from grain	mmol acids
H	Hay "intake"	g/min
PFH1	Potential fermentability 1 of hay	mmol acids produced/g
HFpH	Effect of pH on hay fermentability	Proportion of PFH1
PFH2	PFH1 multiplied by HFpH	mmol acids produced/g
FSH	Fermentable substrate produced from hay	mmol acids/min
PFSH	Pool of fermentable substrate producing acids from hay	mmol acids
LAR	Proportion of PFSG converted to lactic acid	proportion of PFSG
LAG	Lactic acid (LA) produced from grain	mmol/min
LAH	Lactic acid produced from hay	mmol/min
VFAG	VFAs produced from grain	mmol/min
VFAH	VFAs produced from hay	mmol/min
LAP	Lactic acid pool	mmol
VFAP	VFA pool	mmol
LAPR	Proportion of LAP converted to VFAs	Proportion of LAP
ALAC	Amount of lactic acid converted to VFAs	mmol/min
TA	Total amount of lactic acid and VFAs in 5 litre rumen	mmol acids
TAPL	Total acid concentration per litre (TA/5)	mmol acids/L
B	Buffer "intake"	g/min
BC	Buffering capacity for 1 g $NaHCO_3$	mmol acids
OP	Osmotic pressure	mOsmol/kg
AR1	Absorption rate 1 (pH effect on VFA absorption rate)	Proportion of VFAP/min
AR2	Absorption rate 2 (Osmotic pressure effect on VFA absorption rate)	Proportion of VFAP/min
AR	Absorption rate of VFAs	Proportion of VFAP/min
AB	Absorption of VFAs	mmol/min
OFR	Outflow rate of LA & VFAs in	

	rumen fluid	Proportion of VFAP/min
OLA	Outflow of lactic acid in rumen fluid	mmol/min
OVFA	Outflow of VFAs in rumen fluid	mmol/min

The following description of the model involves the three parts shown in Figure 5-1: 1) nutrition input; 2) acid conversion, absorption and outflow; and 3) regulation.

5.2.2 Nutrition input

The "diet" in the mode can be set for any ingredients and the period over which the model runs can be set for any duration. However, for basic modelling, dietary nutrients entering the "rumen" included grain and hay, and running time was normally 24 hours (1440 minutes). The iteration interval was set at 1 minute Therefore, all flows of material are described in g/min or mmol/min. "Intake" levels for the basic investigation of the model were set at 300 g/d grain and 700 g/d hay. The pattern of "intake" of grain (G) and hay (H) can be altered in the model. For the basic modelling, the grain component of the ration of 300 g/d was "fed" separately and "consumed" over a 3-hour period and therefore was assumed to enter the "rumen" at a constant rate of 1.667 g/min during this time. The hay ration of 700 g/d entered the "rumen" over the full 24-hour period at a constant rate of 0.486 g/min (Figure 5-2).

The grain and hay in the rumen are "fermented" and converted to metabolites, such as acids (VFAs, lactic acid etc.). These "precursors" or "products" are described as follows:

$$FSG = G * PFG \quad (1a)$$

$$FSH = H * PFH2 \quad (1b)$$

where FSG (mmol acids/min) and FSH (mmol acids/min) are the fermentable substrates from grain and hay capable of producing organic acids. Their values are the mass of grain (G) and hay (H) multiplied by potential fermentability of grain (PFG) and potential fermentability 2 of hay (PFH2), respectively.

The potential fermentability 2 of hay (PFH2, mmol acids produced/g) was estimated from the potential fermentability 1 of hay (PFH1, mmol acids produced/g) multiplied by the effect of pH on hay fermentability (HFpH, proportion of PFH1) as described in Figure 5-3. The potential fermentability 1 of hay (PFH1) was modified in this way to take account of the negative effect of acidic conditions on fibre digestion.

$$PFH2 = PFH1 * HFpH \qquad (2)$$

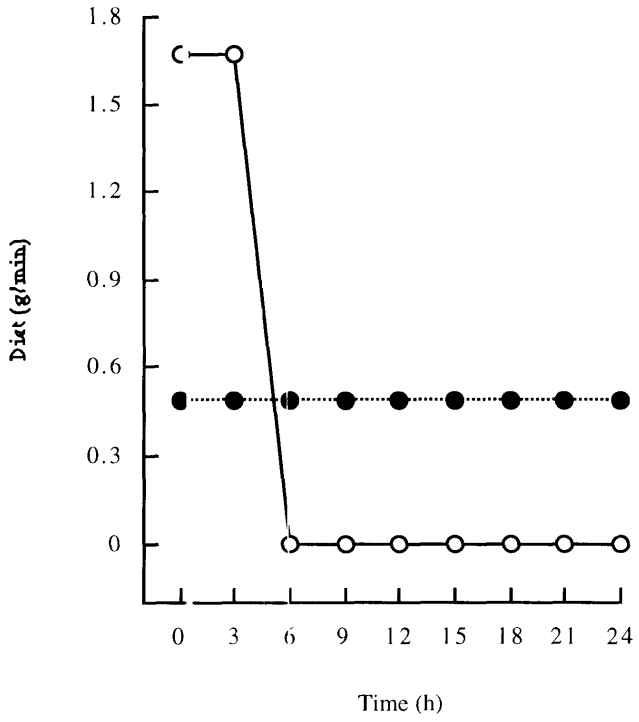

Figure 5-2. The rate of "intake" of the grain supplement of 300 g/d in a 3-hour period and the hay ration of 700 g/d over the full 24-hour period entering the rumen of sheep for the basic modelling. Grain (○) and Hay (●).

The potential fermentabilities of grain (PFG) and hay (PFH) were set at 8 mmol acids produced/g and 5.5 mmol acids produced/g, respectively. These quantitative values for fermentation were taken from the work of Leng and Leonard (1965), Bergman *et al.* (1965), Weller *et al.* (1967), Bergman (1990) and Murray *et al.* (1990). There is quite a wide range of values reported in the literature for VFA production per gram of substrate fermented. For example, values for grain vary from 4 to 12 mmol VFAs/g and those for hay from 4.5 to 6.5 mmol VFAs/g. The values depend on the

efficiency of cell production per unit of fermentable substrate. For this reason, the model was tested for sensitivity to the potential fermentability values for grain (PFG) and hay (PFH) before finalising these values. The results are presented in the results section.

One of the most important factors influencing the fermentable substrate derived from hay in the rumen is pH. The pH effect on hay fermentability (HFpH, proportion of PFH1) was included as a function by which the pH altered fermentation of hay. The data used in the model were derived from the work of Tilley *et al.* (1963), Rowe (1983) and Rowe *et al.* (1991). It was assumed that the effect of pH on fermentation rate of hay would not differ significantly between different diets of hay, and a single graph shown in Figure 5-3 was used in the model as the parameter of HFpH.

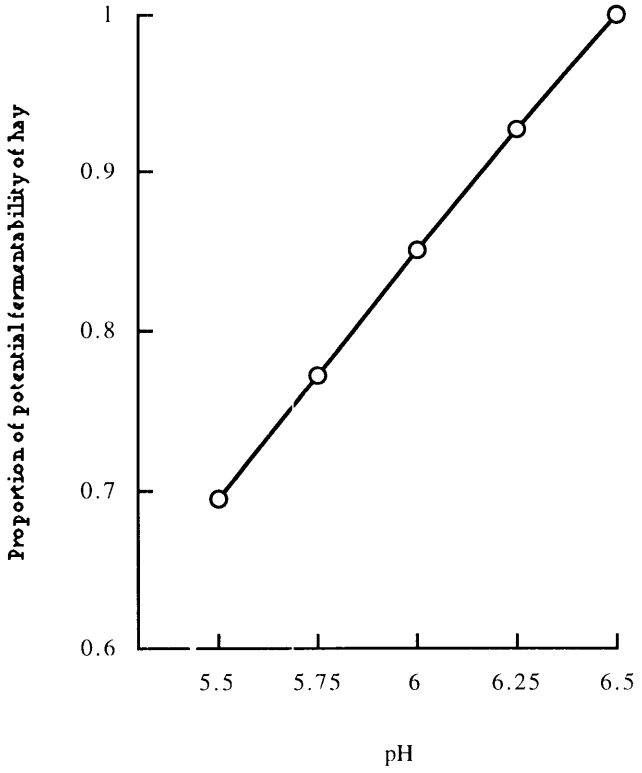

Figure 5-3. The effect of pH on the hay fermentability (HFpH, proportion of PFH1) in the rumen of sheep. The extent of fermentation at pH 6.5 was taken to be maximal (1, i.e. 100%) and was reduced to 0.685 (68.5%) of the maximum rate with decreasing pH to 5.5 (from Tilley *et al.* 1963; Rowe 1983; and Rowe *et al.* 1991).

In the model, lactic acid and VFAs are produced from the pools of fermentable substrate from grain (PFSG) and hay (PFSH). The initial values for PFSG (mmol acids) and PFSH (mmol acids) were assumed to be 0. However, the pools are the state variables calculated as follows:

$$PFSG(t) = PFSG\ (t - dt) + (FSG - LAG - VFAG) * dt \qquad (3a)$$

$$PFSH(t) = PFSH(t - dt) + (FSH - VFAH - LAH) * dt \qquad (3b)$$

where t = 1 minute. FSG and FSH have been defined in Equations 1a and 1b. LAG, VFAG, LAH and VFAH will be defined in Equations 4a, 4b, 5a and 5b, respectively.

5.2.3 Acid conversion, absorption and outflow

5.2.3.1 Acid conversion

Lactic acid and VFAs are produced by fermentation of both grain and hay. The percentages of lactic acid and VFA production are determined by rate of fermentation and pH. Lactic acid does not normally accumulate in the rumen of a sheep fed hay (Gall *et al.* 1953; Jayasuriya and Hungate 1959; Nakamura *et al.* 1971). However, in sheep fed grain, lactic acid can accumulate with rapid fermentation and reduced rumen pH. The relationship between lactic acid production and pH is an inverse one, where increases in lactic acid production lead to a reduction in pH (Dunlop 1972). The relationship between lactic acid production and pH used in the model can be seen in Figure 5-4. Fermentation of grain to lactic acid was assumed to be 1 (100%) below pH 5, 0.667 at pH 5, 0.333 at pH 5.25 and 0 above pH 5.5, where all fermentation was to VFAs (Rowe, Aitchison and McDonald unpublished).

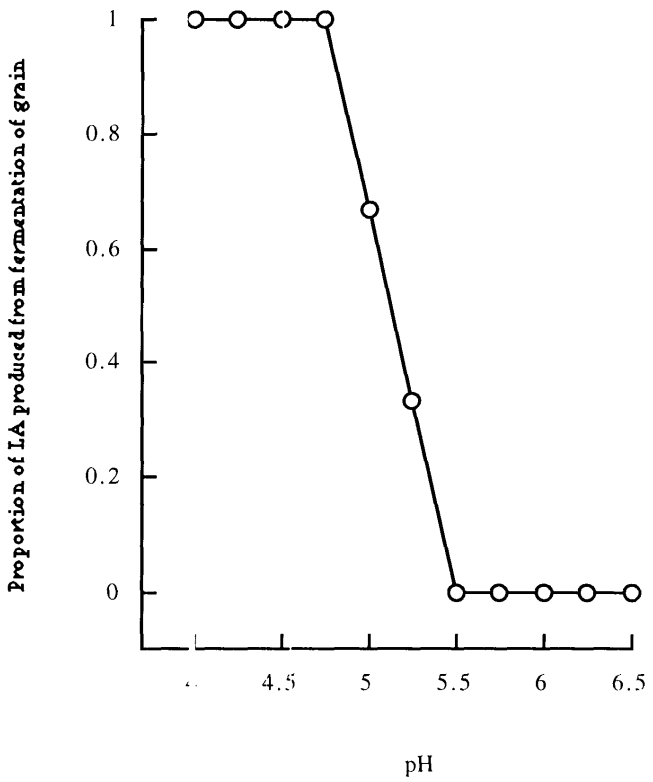

Figure 5-4. The relationship between the proportion of lactic acid production (LAR) and pH in the rumen of sheep. A level of 1 (100%) indicates lactic acid as the sole end-product of fermentation; while 0 indicates fermentation of grain through to VFAs (adapted from Rowe, J. B., Aitchison, E. M., and McDonald, C. L. M., unpublished).

Equations 4a and 4b describe the production of lactic acid from grain and hay, respectively.

$$LAG = PFSG * LAR \quad (4a)$$

$$LAH = PFSH * 0.01 \quad (4b)$$

LAR (proportion of PFSG) is the proportion of the pool of fermentable substrate from grain (PFSG) converted to lactic acid and varies depending on pH. LAG (mmol/min) is the amount of lactic acid produced from the pool of fermentable substrate from grain (PFSG) and is mostly influenced by pH via LAR. LAG is calculated by multiplying PFSG by LAR. However, LAH (mmol/min) is the amount of lactic acid produced from the pool of fermentable substrate from hay (PFSH) and is not affected by pH.

LAH produced from PFSH was assumed to be negligible and constant at 0.01 (1%) (Jayasuriya and Hungate 1959; Nakamura *et al.* 1971). The remaining PFSG and PFSH are converted to VFAs using Equations 5a and 5b, respectively.

$$VFAG = PFSG * (1 - LAR) \quad (5a)$$
$$VFAH = PFSH * 0.99 \quad (5b)$$

VFAG (mmol/min) is the amount of VFAs produced from the pool of fermentable substrate derived from grain (PFSG) and is most influenced by pH via LAR which depends on pH. VFAH (mmol/min) is the amount of VFAs from the pool of fermentable substrate produced from hay (PFSH). A further two state variables, namely LAP (LA pool, mmol) and VFAP (VFAs pool, mmol), are used in the model and their initial values were assumed to be 0 and 500 mmol, respectively. However, their values at any time (t) are expressed as:

$$LAP(t) = LAP(t - dt) + (LAG + LAH - OLA - ALAC) * dt \quad (6a)$$
$$VFAP(t) = VFAP(t - dt) + (VFAH + VFAG + ALAC - AB - OVFA)*dt \quad (6b)$$

where t = 1 minute. LAG and LAH have been defined in Equations 4a and 4b, respectively. OLA (mmol/min) and OVFA (mmol/min) are the amount of outflow of lactic acid and VFAs from the rumen in the fluid phase, respectively. Further details are provided later in this chapter. ALAC (mmol/min) is the amount of lactic acid converted to VFAs and is calculated as follows:

$$ALAC = LAP * LAPR \quad (7)$$

LAP has been defined above and LAPR (proportion of LAP) is the proportion of lactic acid pool converted to VFAs. Again the LAPR value depends on pH and is calculated according to the relationship shown in Figure 5-5.

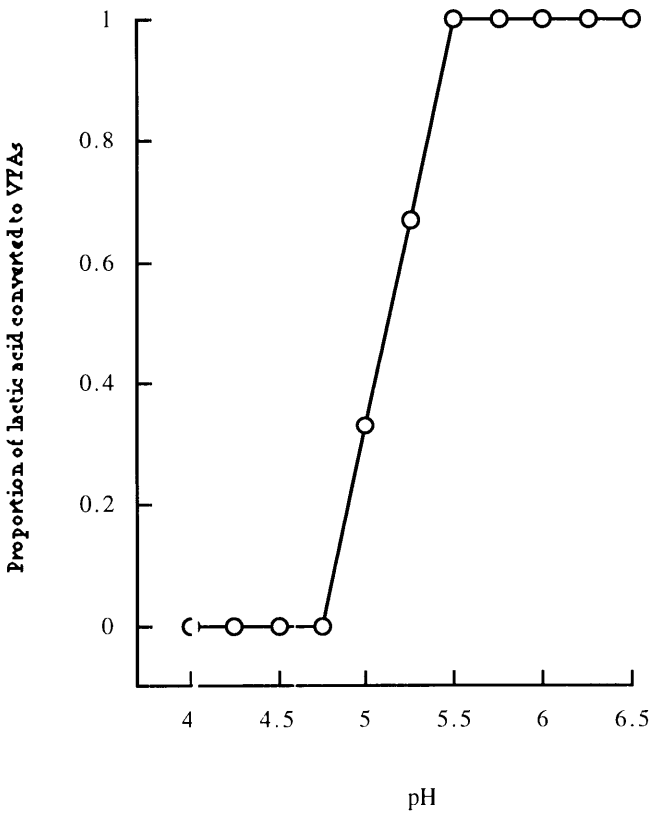

Figure 5-5. The relationship between the proportion of the lactic acid pool converted to VFAs (LAPR) and pH in the rumen of sheep. A level of 1 (100%) indicates conversion of all lactic acid to VFAs, while 0 indicates no conversion of lactic acid to VFAs (adapted from Rowe, J. B., Aitchison, E. M., and McDonald, C. I. M., unpublished; Chapter 4).

5.2.3.2 Acid absorption

VFAs are mainly absorbed from the rumen, however, lactic acid is apparently not absorbed from the rumen (Chapter 3). Lactic acid is either converted to VFAs or flows out of the rumen. Absorption (AB) (mmol/min) of VFAs is calculated using Equation 8 where VFAP (mmol) is the VFA pool and AR (proportion of VFAP) is the absorption rate. The absorption rate (AR) is mainly influenced by pH and osmotic pressure (OP). The calculation of AR, pH and OP are described more fully in the regulation section of this chapter (Equation 13).

$$AB = VFAP * AR \qquad (8)$$

5.2.3.3 Acid outflow

In addition to fermentation and absorption in the rumen, some nutrients flow to the omasum, abomasum and small intestine. The outflow of VFAs and lactic acid from the rumen depends on the VFA pool (VFAP), the lactic acid pool (LAP) and outflow rate (OFR). According to the work of Weston and Hogan (1968) about 24% of VFAs produced in the rumen each hour flow out of the rumen in the fluid phase. Therefore, a value of 0.4%/min for VFAs and lactic acid was taken as the outflow rate (OFR) in Equation 9 used in the model. Again this value was dependent on the pH and osmotic pressure.

$$OFR = (1 - AR)/155.6 \qquad (9)$$

where OFR (proportion of VFAP and LAP/min) is outflow rate of VFAP and LAP from the rumen. OFR is mainly affected by pH and osmotic pressure (OP) via absorption rate (AR) which varies depending on pH and OP. The constant 155.6 is calculated on the basis of AR described in Equation 13.

$$OVFA = VFAP * OFR \qquad (10)$$

where OVFA (mmol/min) is outflow of VFAs from the rumen and its value equals the product of the VFA pool (VFAP) and the outflow rate (OFR).

$$OLA = LAP * OFR \qquad (11)$$

OLA (mmol/min) is the outflow lactic acid from the rumen determined as the product of lactic acid pool (LAP) and outflow rate (OFR). OLA and OVFA change with pH since the formation of lactic acid (LA) and VFAs from the pool of fermentable substrate from grain (PFSG) is affected by pH (Equations 4a and 5a)

5.2.4 Regulation

The regulating system in the model includes buffer, pH and osmotic pressure (OP).

The inclusion of additional buffer (B) is a decision variable in the model which can be altered over time. Sodium bicarbonate ($NaHCO_3$) was used as a standard buffer in a series of experiments in this PhD study and was therefore chosen for use in this model. The buffering capacity (BC) used in the model was 15 mmol VFAs per gram of $NaHCO_3$. This is 76% of the theoretical value determined by titration in Chapter 4 (24% of additional $NaHCO_3$ was assumed to flow out of the rumen with the fluid or solid phase). In Chapter 4, it was shown that 1 g $NaHCO_3$ can buffer 20 mmol acetic acid at pH 6.

The total amount of lactic acid and VFAs present in the rumen directly affects pH. The total acids (TA, mmol) in 5 litres of "the rumen" were considered together with the amount of additional buffer (B) to calculate the total effective acid concentration (TAPL, mmol/L) as described in Equation 12.

$$TAPL = (TA - B * BC)/5 \qquad (12)$$

TA (mmol) is total acids, i.e. the sum of lactic acid pool (LAP) and VFA pool (VFAP). If no additional buffer (B) is added, B * BC will be 0 and TAPL will equal TA/5.

The relationships between TAPL (mmol/L), pH and OP are shown in Figures 5-6 and 5-7, respectively.

The pH and osmotic pressure (OP) were found to be very important factors affecting the absorption of VFAs both in the experiments of this thesis (Chapter 3) and the work of Williams and Mackenzie (1965). The effects of pH and osmotic pressure (OP) on the absorption of VFAs from the VFAP in the rumen are included in the model through absorption rate 1 (AR1) and absorption rate 2 (AR2), respectively. These absorption rates are defined by Figures 5-8 and 5-9.

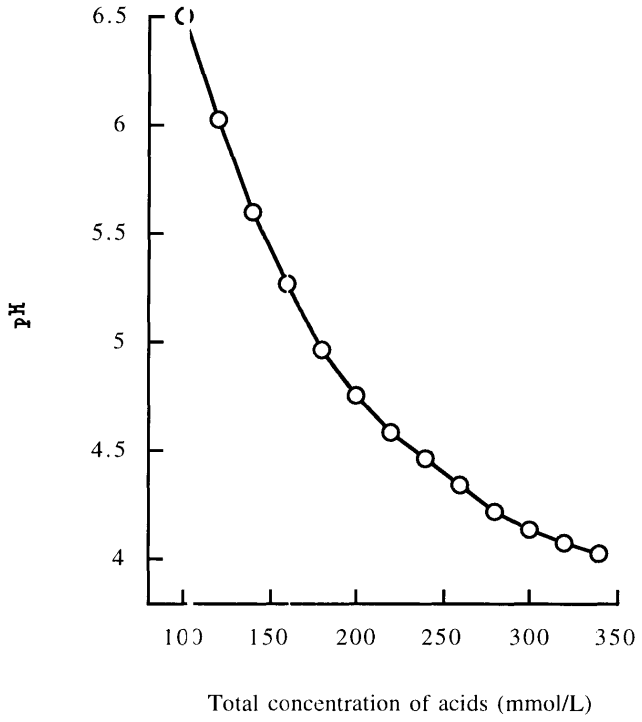

Figure 5-6. The relationship between pH and total concentration of acids (mmol/L) in rumen digesta (from titration data of buffering capacity for normal ruminal digesta).

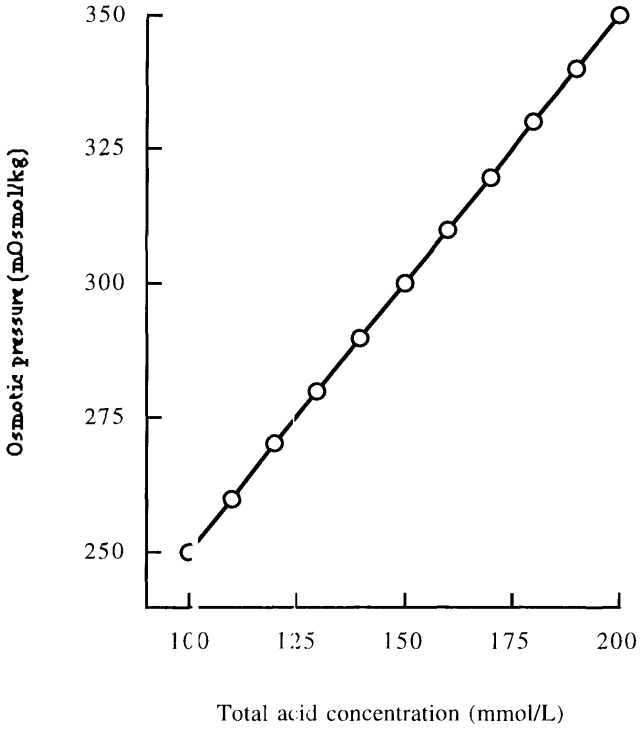

Figure 5-7. The relationship between osmotic pressure (OP) and total concentration of acids (mmol/L) in rumen digesta (measured in solutions prepared for the absorption test).

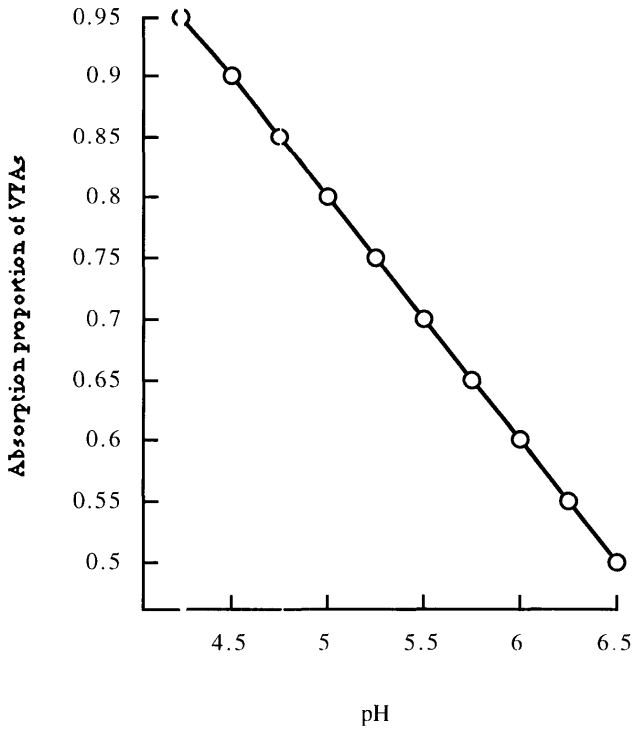

Figure 5-8. The relationship between VFA absorption rate 1 (AR1) and pH in the rumer of sheep (from Chapter 3).

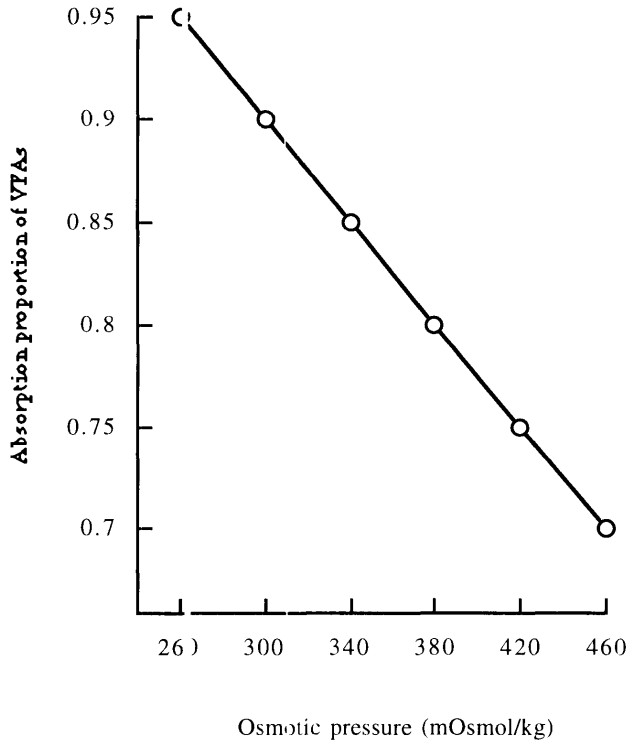

Figure 5-9. The relationship between VFA absorption rate 2 (AR2) and osmotic pressure (OP) in the rumen of sheep (from Chapter 3).

The absorption of VFAs depends on VFAP (VFA pool) and VFA absorption rate (AR). AR (proportion of VFAP/min) was calculated in the model using Equation 13.

$$AR = AR1 * AR2/31.25 \qquad (13)$$

where AR1 (proportion of VFAP/min) is absorption rate 1 (pH effect on VFA absorption rate), and AR2 (proportion of VFAP/min) is absorption rate 2 (Osmotic pressure effect on VFA absorption rate). The constant 31.25 is calculated on the basis of outflow rate (OFR) described in Equation 9. Equation 13 represents base rate of absorption of approximately 76%/h of VFA production in the rumen of sheep (Weston and Hogan 1968), but it is dependent on the pH and osmotic pressure.

5.2.5 Summary of model

All aspects of the model are represented in Equations (1) to (13) and Figures 5-1 to 5-9. The differential equations can be solved numerically for a given set of initial conditions and parameter values. The model was developed using the software "STELLA II" and run on a Macintosh computer.

5.3 Results

The model has been subjected to sensitivity and general behavioural tests. It has been used to compare the output with the results from published studies. The model was used to simulate experiments to create and control lactic acidosis.

5.3.1 Sensitivity and behavioural tests

The model was tested for sensitivity to two key parameters, potential fermentability of grain (PFG) and potential fermentability 1 of hay (PFH1). All tests in the model were run for 24 hours (1440 minutes). However, the x axis (time) in the figures has been truncated to 210 or 240 minutes since each variable presented maintained a somewhat steady state after that

time. The effect of altering the values of parameters in the model was tested with respect to pH, the pools of lactic acid and VFAs, and VFA absorption. The results presented in Figure 5-10 illustrate five levels of potential fermentability of grain (PFG) to VFAs (PFG = 4, 6, 8, 10, 12 mmol acids produced/g grain consumed). Rumen pH (a), VFA pool (c) and VFA absorption (d) varied signif cantly depending on potential fermentability of grain (PFG). The higher potential fermentability of grain (PFG), the greater VFA pool and VFA absorption, but the lower the pH. The lactic acid pool increased with increasing PFG, however, lactic acid accumulation only occurred when PFG was 12 mmol acids produced/g. The values of PFG below 12 mmol acids produced/g were not high enough for lactic acid accumulation on the basis of the diet consumed.

Figure 5-11 presents the results which express three levels of assumed potential fermentability 1 of hay (PFH1) to VFAs (PFH1 = 4.5, 5.5, 6.5 mmol acids produced/g hay consumed). The results in Figure 5-11 are similar to those of Figure 5-10: the higher potential fermentability 1 of hay (PFH1), the greater the VFA pool (c) and VFA absorption (d), but the lower the pH (a). However, the changes were limited because hay is fermented more slowly than grain. Therefore, only traces of lactic acid (b) were observed even at the highest levels of PFH1.

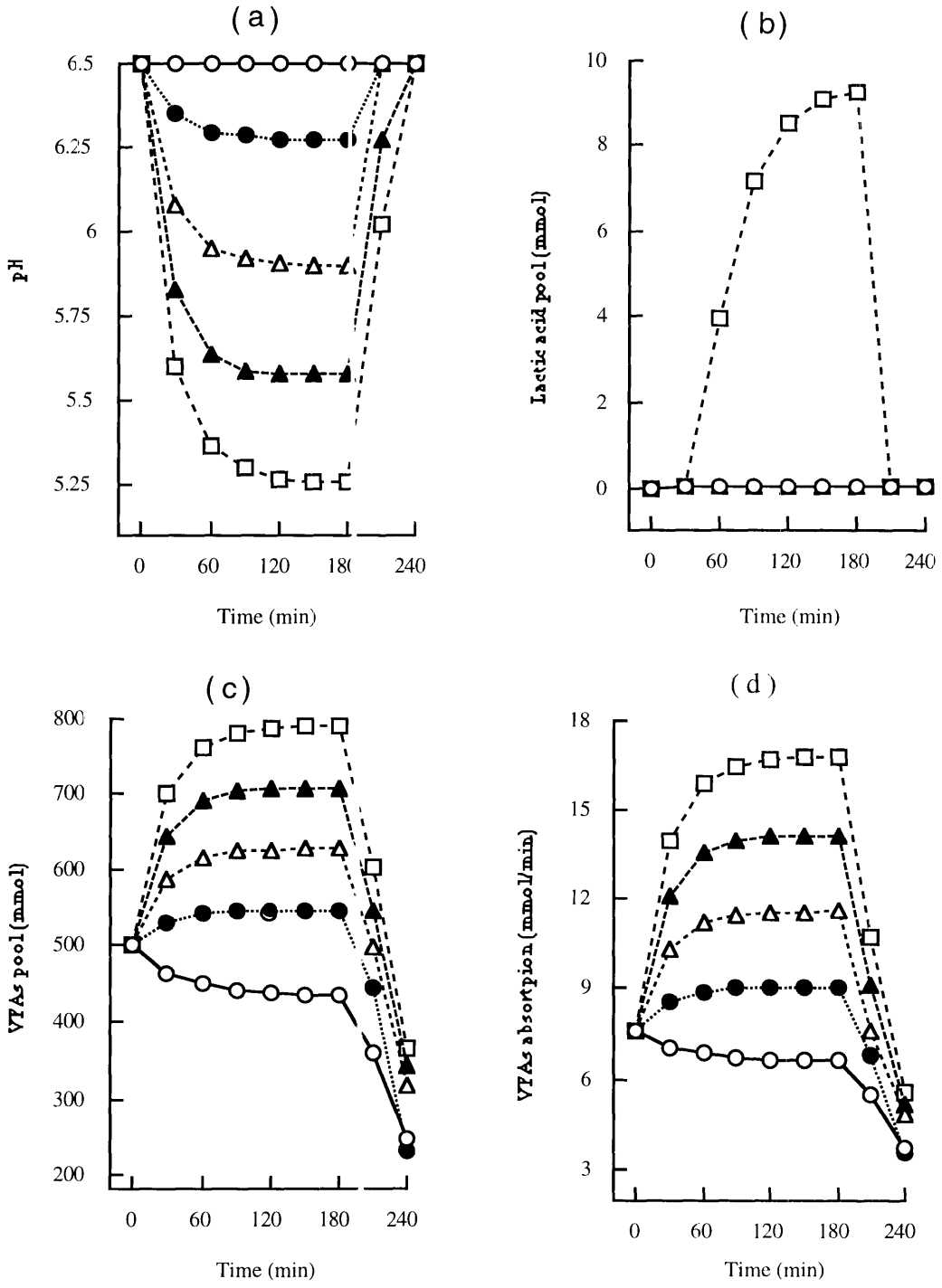

Figure 5-10. Effects of varying potential fermentability (mmol/g) of grain (PFG) on model behaviour. (a) pH, (b) lactic acid pool, (c) VFA pool, and (d) VFA absorption. PFG = 4 mmol acids produced/g (○), PFG = 6 mmol acids produced/g (●), PFG = 8 mmol acids produced/g (△), PFG = 10 mmol acids produced/g (▲) and PFG = 12 mmol acids produced/g (□).

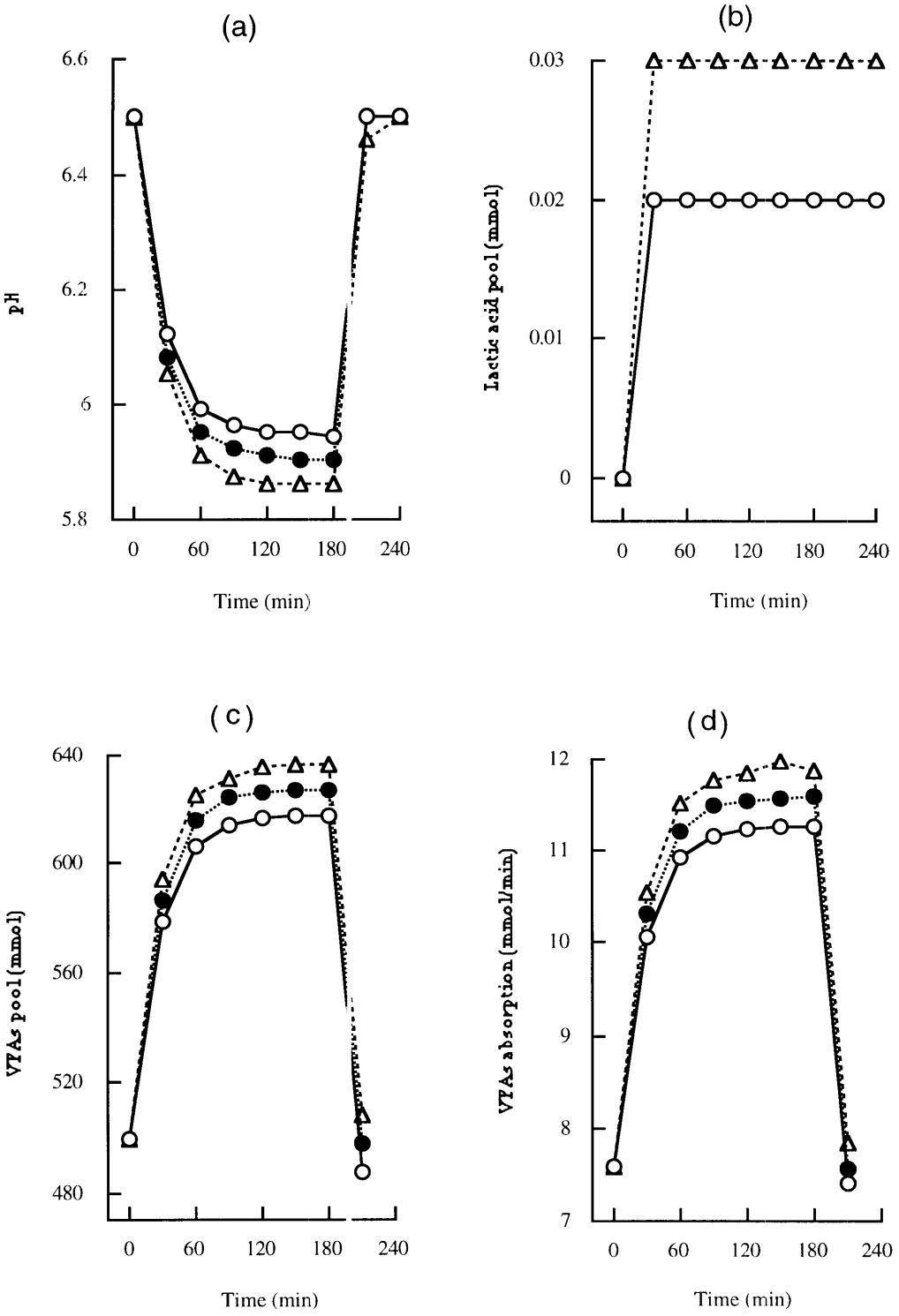

Figure 5-11. Effects of varyi ıg potential fermentability 1 of hay (PFH1) on model behaviour. (a) pH, (b) lactic acid pool, (c) VFA pool, and (d) VFA a)sorption. PFH1 = 4.5 mmol acids produced/g (○), PFH1 =5.5 mmol acids produced/g (●) and PFH1 =6.5 mmol acids pr(duced/g (Δ).

5.3.2 Comparisons with experimental data

There are few studies in the literature which provide ideal data for comparison with the model predictions. One set of data suitable for comparison is from the study of Reid *et al.* (1957) in which sheep consumed a diet of 320 g lucerne chaff and 480 g grain in about three hours. These data were compared with the predictions of the model using a potential fermentability for grain of 8 mmol acids produced/g and 5.5 mmol acids produced/g for hay. The peaks for the model predictions and the experimental observations were "displaced" with the peaks for rumen pH and VFAs concentration (Figure 5-12) being approximately 2 h earlier in the model predictions.

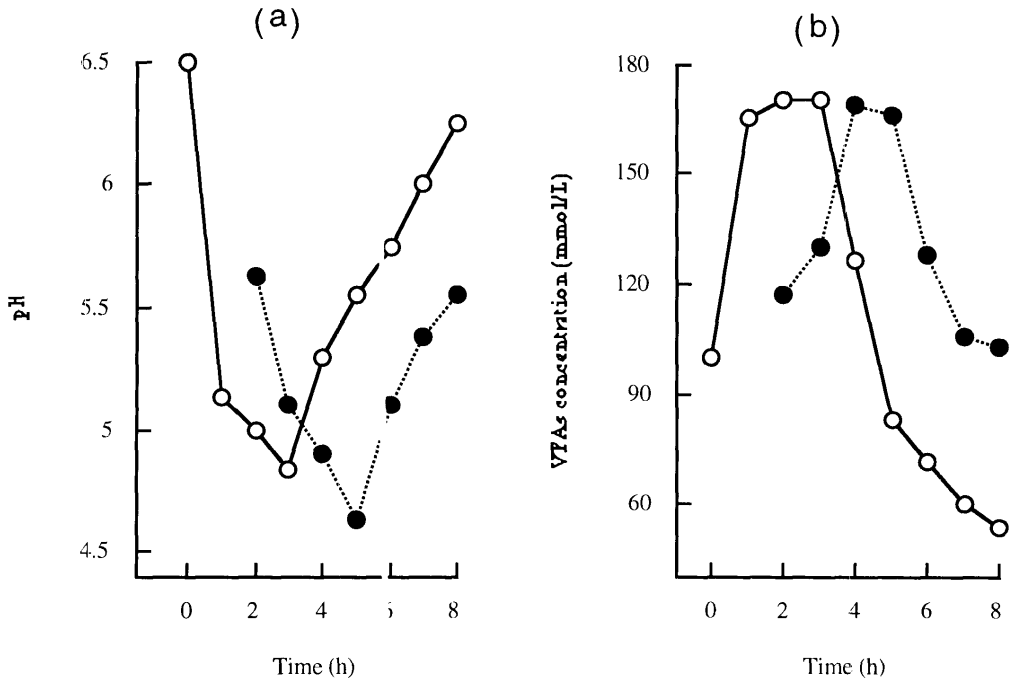

Figure 5-12. Comparisons of model predictions with experimental observations of Reid *et al.* (1957) in the rumen after feeding sheep on a diet of 320 g hay and 480 g grain. (a) pH and (b) VFA concentration (mmol/L). Prediction (○) and observation (●).

5.3.3 Simulated experiments of lactic acid production and its control

A series of experiments was designed to simulate the production of lactic acid and its treatment in the model, including:

(i) control of pH using different levels of buffer;

(ii) blocking lactic acid production;

(iii) enhancing the conversion of lactic acid to VFAs; and

(iv) gradual "intake" of grain.

In order to compare the effects of these treatments, the ration in all these experiments was standardised at 1000 g/d consiting of 460 g/d hay and 540 g/d grain in order to produce fermentation conditions for "mild" fermentative acidosis in the "rumen". The grain ration of 540 g/d entered the "rumen" over a three-hour period at a constant rate of 3 g/min. The hay ration of 460 g/d entered the "rumen" over the full 24-hour period at a constant rate of 0.319 g/min. The results in sensitivity tests indicated a stable pattern of fermentation with diurnal fluctuations when the potential fermentability of grain (PFG) was set at 8 mmol acids produced/g and for the potential fermentability 1 of hay (PFH1) 5.5 mmol acids produced/g. The model predictions with these potential fermentabilities were also similar to those observed in practice (Reid *et al.* 1957). These values of parameters were therefore used for simulated experiments as well as for the basic model. The experimental results are described as follows.

(i) Different levels of buffer and effect on pH

There were two ways in which the buffer, sodium bicarbonate ($NaHCO_3$), could be "fed" to the animal to control pH in the model experiments. One way was to add $NaHCO_3$ at a constant rate with the grain over a three-hour period. Another way was to add $NaHCO_3$ at a constant rate over the full 24-hour period with the hay. Sodium bicarbonate played a greater buffering role when added with the grain and less of a role when "fed" with the hay based on the same total amount of $NaHCO_3$ (Figure 5-13). Although all recorded parameters, including pools of VFAs and lactic acid, VFA absorption, and pH, changed at their peaks, the greatest effect was on lactic acid and then on pH. Lactic acid pool decreased 14% with the addition of 20 g $NaHCO_3$ in 3 h; 28% for addition of 50 g $NaHCO_3$ in 3 h; 2% for addition of 20 g $NaHCO_3$ in 24

h; and 5% for addition of 50 g $NaHCO_3$ in 24 h. The pH increased between 0.01 and 0.04 uni s in response to the buffer. The model at that stage did not include HCO_3^- and $HCO_3^- \Leftrightarrow H+ + CO_3^{2-}$ in the saliva and the movement of bicarbonate and CO_2 across the gut wall *in vivo*.

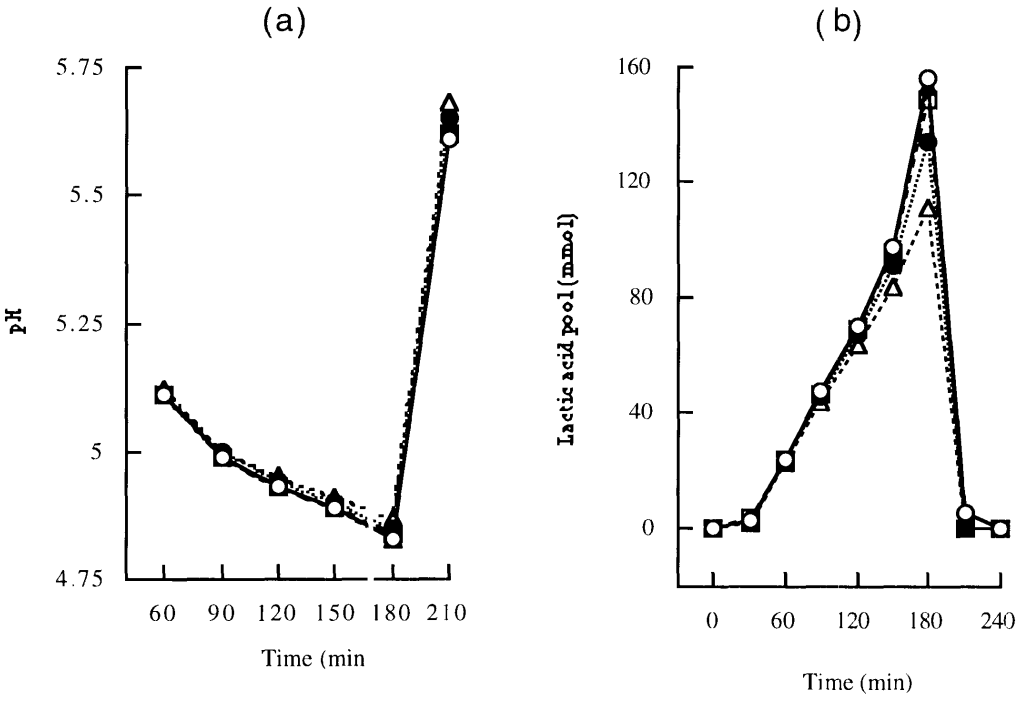

Figure 5-13. Effects of buffer ($NaHCO_3$) on model behaviour. (a) pH and (b) lactic acid pool. 0 g $NaHCO_3$ (○), 20 g $NaHCO_3$ in 3 h (●), 50 g $NaHCO_3$ in 3 h (Δ), 20 g $NaHCO_3$ in 24 h (▲), and 50 g $NaHCO_3$ in 24 h (□).

(ii) Blocking lactic acid production

The effect of virginiamycin in reducing the risk of acidosis is thought to be due to its reduction of lactic acid production during rapid fermentation of carbohydrate. When lactic acid produced from grain (LAG) and from hay (LAH) was constrained to zero in the model, all fermentable substrates were converted to VFAs. Under these conditions, the VFA pool increased 11% and the pH increased 0.17 units at their peaks (Figure 5-14). The effect of the control of lactic acid production simulated in this situation produced a greater positive effect on pH than that which was achieved at high levels of additional buffer.

(iii) Enhancing conversion of lactic acid to VFAs

Another approach to controlling acidosis is to increase the rate of conversion of lactic acid to VFAs. This can be done, theoretically, by the addition of "probiotics" in the forms of Gram-positive lactic acid utilisers and live yeast cultures to the "rumen". In this experiment, the proportion of lactic acid pool converted to VFAs (LAPR) in the model was changed to 1 (100%). The response to this change was that the lactic acid pool was reduced to one-tenth of the value for basic run at its peak. At the same time, VFA pool increased 10% and pH increased 0.14 units (Figure 5-14). The change of pH was of a similar level to that observed when "blocking" lactic acid production. This is to be expected since both methods of interaction have similar effects on reducing the lactic acid pool.

(iv) Gradual "intake" of grain

The most common way of feeding grain to ruminants is through a gradual introduction followed by regular feeds. Gradual "intake" of grain was simulated by feeding grain at a constant rate over the full 24-hour period in the model. The result in Figure 5-14 showed that a gradual "intake" of grain even as high as 1950 g/d over the full 24-hour period at a constant rate of 1.354 g/min did not result in the accumulation of lactic acid and did not reduce pH (maintained at pH 6.5). At the same time, the VFA pool (round 502 mmol) and VFA absorption (7.7 mmol/min) were always maintained at higher levels.

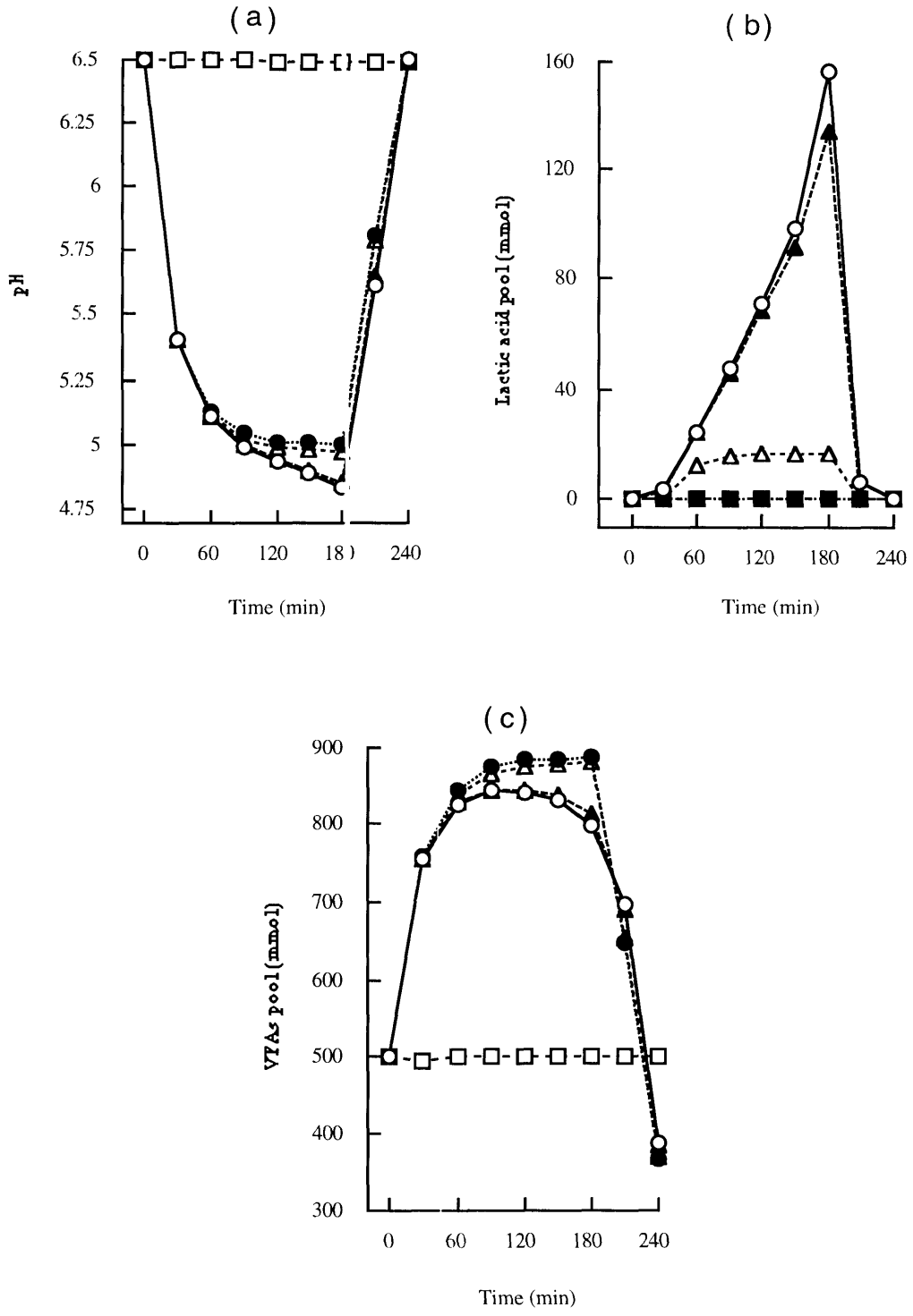

Figure 5-14. Comparisons of the different ways of controlling lactic acidosis on model behaviour. (a) pH, (b) lactic acid pool and (c) VFA pool. Base run (○), no lactic acid (●), conversion of lactic acid to VFAs (Δ), 20 g $NaHCO_3$ in 3 h (▲) and grain 1950 g in 24 h (□).

To make comparisons among the different ways of preventing lactic acidosis, a rank of the effectiveness was as follows: gradual "intake" of grain > blocking lactic acid production > conversion of lactic acid to VFAs > $NaHCO_3$. The best way of preventing acid accumulation in the rumen is to add grain gradually at a constant rate over the full 24-hour period. However, this is not practical as animals tend to consume "meals" at varying intervals throughout the day. It is only practical to feed grazing animals once or twice per week. Under these conditions, the use of virginiamycin to control acidosis may be important.

5.4 Discussion

5.4.1 Sensitivity and behavioural analyses

Fermentation of grain results in rates of VFA production within the rumen ranging from 4 to 12 mmol/g of feed, whereas fermentation of hay yields 4.5 to 6.5 mmol/g of VFAs (Leng and Leonard 1965; Leng and Brett 1966; Weller *et al.* 1967; Bergman 1990; Murray *et al.* 1990). When the sensitivity and behaviour of the model were tested by altering the potential fermentability of grain (PFG) over ranges of 4 to 12 mmol acids produced/g, the model was relatively stable and produced acceptable response patterns: the higher the potential fermentability of grain (PFG), the greater the VFA pool and VFA absorption, but the lower the pH (Figure 5-10). When the grain with higher potential fermentability was fermented in the "rumen system", greater quantities of VFAs were produced. Increased VFAs promoted the absorption of VFAs and increased total acids resulted in a fall in the "rumen" pH. When the sensitivity and behaviour of the model were tested by altering the potential fermentability 1 of hay (PFH1) over ranges of 4.5 to 6.5 mmol acids produced/g, the model produced similar response patterns to those recorded in the case of grain. However, the model was more stable than when it underwent the sensitivity test of grain and the changes were limited, i.e. the model, like the rumen, was only partially sensitive to potential fermentability 1 of hay (PFH1). This is because hay is fermented more slowly and has a lower PFH1 than grain. This, too, is consistent with the response feature of the rumen of the live sheep to feed in which the

rumen is more sensitive to grain than hay. The model is sensitive to potential fermentabilities, i e. fermentation rates, because the model, like the rumen, is able to accommodate a range of metabolic interactions.

Lactic acid production increased with increasing potential fermentability of grain (PFG), however, lactic acid accumulation only occurred when PFG was 12 mmol acids produced/g (Figure 5-10b). This is because the values of PFG below 12 mmol acids produced/g were not high enough for lactic acid accumulation on the basis of the diet consumed. When the potential fermentability of grain (PFG) was 12 mmol acids produced/g, which is the highest potential fermentability of grain in the ranges tested, the highest level of lactic acid was produced. This level exceeded the capacity for conversion of lactic acid to VFAs to be absorbed and resulted in the accumulation of lactic acid (Figure 5-10b). However, the accumulation of lactic acid did not appear to influence proportionally the pH level (Figure 5-10a) because the total acid level had not increased proportionally due to the conversion of lactic acid to VFAs and the absorption of VFAs. This result supports the theory that lactic acidosis is affected by total acids and all acids contribute to the acidosis by disturbing the acid-base status (Chapter 3).

The model "fermentation" is similar in many ways to the fermentation in the rumen of live sheep and the predictions are comparable with the observations in practice. When the model predictions using a potential fermentability for grain of 8 mmol acids produced/g and 5.5 mmol acids produced/g for hay were compared with the experimental observations of Reid *et al.* (1957), the results had similar patterns in rumen pH and VFA concentration (Figure 5-12). However, their peak times were different and this perhaps is due to the variations in hay and grain. The grain in the experiment of Reid *et al.* (1957) was the mixture of crushed oats, linseed, bran and wheaten starch, while the grain used in the model was maize. The hay in the experiment of Reid *et al.* (1957) was lucerne chaff, whereas the hay used in the model was dried grass. Different feedstuffs contain various quantities of acidic and basic substances and, therefore, differ in their contribution to the acid production and buffering system in the rumen of the live sheep. In addition, hay or forages elicit saliva production, which should have a significant effect on regulating ruminal pH directly, and indirectly through the rate of fermentation, the proportions of acids produced, and on rate of acid absorption from the

rumen of the live sheep. The rate of eating may be different between the live sheep and the model although total feed and the period of eating are the same. The fermentation of the grain in the rumen of the live sheep is also quite different from the model. There is a certain amount of time for bacteria to attach themselves to grain particles and to commence the production of VFAs.

In summary, the sensitivity and behavioural analyses indicate that the values of parameters arising from *in vitro* kinetic experiments can be employed in the model of rumen fermentation, and that the model described here can be usefully employed in the design and interpretation of experiments to study lactic acid production and the prevention of acidosis in the rumen. However, the case of the rumen in the live sheep is far more complex.

5.4.2 Features of the model

There are a number of very useful predictions in this model with respect to the potential efficacy of various methods of managing lactic acidosis. These are:

(1) relative effects of acid production and absorption of acids and pH;
(2) benefits of neutralising acids and buffering pH by addition of buffers;
(3) efficacy of blocking the production of lactic acid by addition of antibiotics; and
(4) efficacy of enhancing the conversion of lactic acid to VFAs using microbiological methods.

These features of the model are discussed in detail below.

(1) Predicting the relative effects of acid production and absorption on acids and pH.

When the model "rumen" "fermented" 540 g/d of grain in 3 h (3 g/min) with production of 8 mmol acids/g and 460 g/d of hay over the full 24-hour period (0.319 g/min) with production of 5.5 mmol acids/g, the predictions showed that the fermentation occurred rapidly with significant accumulation of lactic acid (156 mmol) and VFAs (Figure 5-14b, c) and a lower pH (pH 4.83, Figure 5-14a) at their peaks. This is

because the production rate of acids exceeded the removal rate of the acids and lactic acid could not be converted to VFAs below pH 5 (Chapter 4). The removal of acids from the rumen includes the absorption of VFAs into blood and the passage of lactic acid and VFAs to the abomasum. However, if the "rumen" was gradually "fed" grain at a constant rate over a 24-hour period, there was no accumulation of lactic acid nor a fall in "rumen" pH even when grain as high as 1950 g/d (1.354 g/min) was "fermented" (Figure 5-14). This is due to the fact that the "rumen" kept an equilibrium in terms of acid production and removal so that the total acid concentration was maintained at a constant level in the "rumen", and the pH was unchanged from its initial value of pH 6.5 in the predictions. This is similar to gradual adaptation in the animal to a carbohydrate-rich diet followed by regular feeding of small amounts of grain. Under these conditions, the microbial population adapts with a rise of lactic acid-metabolising bacteria and an ecological balance between production and utilisation so that the animal can prevent acidosis from the diets high in readily fermentable carbohydrates. In practice, however, it is not always as easy to achieve a regular pattern of intake, as it is in the model.

(2) Predicting the benefits of neutralising acids and buffering pH by addition of buffers.

Although lactic acid and VFAs accumulated and resulted in acidosis and a lower pH when the model "rumen" "fermented" 540 g/d of grain in 3 h at a rate of 3 g/min (Figures 5-13 and 5-14), the predictions showed that the addition of $NaHCO_3$ as supplement with grain could decrease the accumulation of lactic acid as well as VFAs and could increase the pH (Figure 5-13). Compared to other methods of preventing lactic acidosis in the model, the effects of $NaHCO_3$ on lactic acid and pH were minimal (Figure 5-14). Probably the additional buffer partially neutralised the acids and buffered the pH, but did not alter the rate of fermentation of starch and did not prevent the microbial changes in the gut which are responsible for rapid fermentation of readily fermentable carbohydrates and the accumulation of lactic acid. The "fermentation" produced much more acid than could be buffered by the amount of $NaHCO_3$ added. In addition, the buffering capacity of $NaHCO_3$ varies depending on the rumen pH (Chapter 4). The predicted results agree with the observations in the experiments of this thesis (Figure 4-3 and Table 4-3) and those

previously reported by Bigham *et al.* (1973), Counotte *et al.* (1979), Rogers and Davis (1982a, b), and Kovacik *et al.* (1986) in that the addition of $NaHCO_3$ resulted in an increase in pH and buffering capacity of rumen and caecal digesta.

(3) Predicting the efficacy of blocking the production of lactic acid by addition of antibiotics.

When lactic acid produced from grain (LAG) and from hay (LAH) were constrained to zero in the model, the predictions showed that VFA pool and pH increased greatly by 11% and 0.17 pH units at their peaks, respectively, as shown in Figure 5-14. This is mainly because fermentable substrates from grain and hay passed through VFAs and VFAs were absorbed from the "rumen". This was to simulate the application of antibiotics and the predicted results are consistent with those of virginiamycin application (Rowe *et al.* 1989; Godfrey *et al.* 1992; Thorniley *et al.* 1994) in that virginiamycin was found to be effective in preventing lactic acid accumulation and very low pH. Rowe *et al.* (1989) found that virginiamycin prevented lactic acid production even at a concentration of 0.5 mg/ml using an *in vitro* fermentation of rumen fluid taken from sheep. Rowe and Zorrilla-Rios (1993) observed no signs of acidosis in cattle when virginiamycin was included at a concentration of 20 mg/kg in a complete diet containing 80% barley even without a gradual increase in grain content of the diet. Antibiotics have been found to inhibit the production of lactic acid by controlling the populations of the major lactate-producing bacteria, *Streptococcus bovis* and lactobacillus. The consistent results between observations and predictions implied that the application of antibiotics can be simulated in the model to predict their effects. However, the practical consequences of using antibiotics are more complex than simulated in this model.

(4) Predicting the efficacy of enhancing the conversion of lactic acid to VFAs using microbiological methods.

If the proportion of lactic acid pool converted to VFAs (LAPR) was maintained at 100% in the model, then lactic acid pool reduced to one-tenth of the value of the control value. This was associated with increased production and absorption of VFAs and higher pH (Figure 5-14) because

of the conversion of lactic acid to VFAs. This was to simulate the use of probiotics capable of using lactic acid. In this case, the "rumen" converted all lactic acid to VFAs, however, little lactic acid still accumulated in the pool in the predictions. This means that although enhancing the conversion of lactic acid to VFAs using microbiological methods is a way to treat lactic acidosis, it is only a temporary strategy. The predicted results are consistent with those reported by Newbold (1990), Williams and Newbold (1990), Girard *et al.* (1993), and Newbold *et al.* (1996) in that the probiotic Yea Sacc (Alltech) was shown to reduce the accumulation of lactic acid in the rumen fermentation of starch.

5.4.3 The area in need of development

The fermentation, absorption and outflow of the rumen are highly complex and are difficult to quantify. Some factors are therefore difficult to include in the model. The exogenous inputs to the model are feed intake and the fermentative rate of these feeds. Van Straalen and Tamminga (1989) pointed out that information is limited or highly variable on similar foodstuffs in different papers. These differences in sheep may be due to differences in

(i) substrate utilisation (Russell 1984);
(ii) outflow rate from the rumen with the fluid or solid phase (Cheng and Costerton 1980); and
(iii) recycling of microbial matter within the rumen (Jouany *et al.* 1988).

These differences would affect accuracy of the model simulations and, hence, further efforts to standardise these techniques are required.

The model has simplified many steps and it is likely that greater accuracy could be achieved by including more detail and more pools. The purpose of the present model was to focus on the key factors associated with acidosis in order to provide a better understanding of the relative importance of the major management options. In its present form, it appears to achieve this objective.

Absorption and outflow rates are two of the key parameters for the model, which depend on pH, osmotic pressure (Williams and Mackenzie 1965; Chapters 3 and 4), particle size, density, hydration rate of the gut content as well as feed (Welch 1986). The relationships of these factors and the absorption and outflow rates need to be investigated further.

Chapter 6 General Discussion and Conclusion

The results obtained in the experiments supported the general hypothesis explored in this thesis that the rate of production and accumulation of lactic acid is more important than the buffering capacity within the gut in the development of fermentative acidosis.

The development of fermentative acidosis in the gut of the animal depends on the balance between rates of acid production and absorption, and the buffering capacity in the gut. While the production of acids depends on the fermentation of carbohydrates, the absorption and outflow of acids depend mainly on their concentrations and pH, but can be influenced by other factors. Buffering capacity of the gut depends upon the input of buffers from saliva, the nature of the feed, and the movement of HCO_3^- and other ions across the gut wall.

On the same diet, acid production and absorption as well as amount of buffers from saliva and the feed may differ between individual animals and between species, and may be influenced by various conditions. However, apart from this variation, the main factors affecting acid accumulation and buffering capacity are: (1) type of diet; (2) pattern of eating; and (3) the amount of feed consumed. Different types of diet, patterns of eating and the amounts of feed consumed largely influence the rate of acid production and the amount of buffer provided from saliva and from the feed. Examples can be seen from the predictions of the computer model described in Chapter 5. Various types of diet with different qualities possess different fermentabilities and produce different amounts of fermentable substrate. Variation in the rate and extent of fermentation results in differences in acid production and absorption, and pH. The higher the fermentation rate, the greater the production of acids, but the lower the pH (Figures 5-10 and 5-11). Various types of diet stimulate different amounts of buffers from saliva during rumination and digestion. The more buffer, the higher the buffering capacity and the pH (Figure 5-13). In addition, the pattern of eating and the amount of feed consumed result in significant differences in the production of acids, the absorption of VFAs, and the pH (Figure 5-14).

Volatile fatty acids constitute the most important acids as they provide energy substrates for metabolism in both ruminants and monogastric herbivores (Bergman 1990). Volatile fatty acids originate from anaerobic fermentation of carbohydrates in the rumen, the caecum and the colon. The volume of the rumen is about 11-fold that of the caecum. The amount of VFAs produced in the rumen is much greater than that in the caecum. However, total VFA concentrations are similar in the two fermentative compartments (Bugaut 1987) and there is still considerable risk of fermentative acidosis in the hindgut under certain conditions (Lee *et al.* 1982; Godfrey *et al.* 1992).

Volatile fatty acids absorption from the reticulo-rumen and the hindgut is important for the supply of energy in ruminants. In this study, the absorption rates of acetic, propionic and butyric acids from the caecum (0.49, 0.17 and 0.08 μmol/cm^2.min, respectively) and from the rumen (0.48, 0.16 and 0.08 μmol/cm^2.min, respectively) were very similar based on absorptive surface area of the pouches. The results provide further evidence that absorption of VFAs from the caecum is efficient and similar to absorption from the rumen (Faichney 1968; Berggren *et al.* 1993; Horspool *et al.* 1994) even though there are differences in the morphological structure between the rumen and the large intestine. These results are consistant with other reports (Argenzio *et al.* 1975; Rechkemmer *et al.* 1988; Engelhardt *et al.* 1989; Barcroft *et al.* 1944; Stevens 1970). However, the reticulo-rumen is of far greater importance than the hindgut in terms of the absorption of nutrients as well as fermentation because of its greater volume and its position in the digestive tract. About 88% of the rumen VFAs are directly absorbed from the rumen (Sutherland *et al.* 1963, 1964) and this can constitute around 70% of the metabolisable energy available to the animal (Church 1988).

In addition to VFAs, acid fermentative production includes lactic acid. Normally, there is very little or no lactic acid accumulation in the rumen or hindgut of animals consuming roughages or constant levels of high-grain diets (Doetsch *et al.* 1953; Waldo and Schultz 1956; Jayasuriya and Hungate 1959). Only traces of lactic acid have been reported for the rumen of sheep (Ryan 1964a, b) and dairy cows fed high-roughage diets (Balch and Rowland 1957). However, if there is a sudden increase in the intake of grain or starch or other feed that is rich in readily fermentable

carbohydrates, then significant amounts of lactic acid can accumulate in the rumen and/or the caecum.

The fact that no lactic acid is apparently absorbed from either rumen or caecal pouches of sheep (Figure 3-1) has been one of the most important findings in this investigation. Under normal conditions, absorption of lactic acid from the rumen is not likely to occur simply because lactic acid is not present. Lactic acid is normally all converted through acetyl-CoA to VFAs and is absorbed in this form. Dobson and Phillipson (1956) found no evidence of absorption of lactate from buffered solutions containing lactic acid in the isolated rumen of sheep. There were also no changes in blood pH and blood lactic acid levels reported by Juhász and Szegedi (1968) after infusion of lactic acid or administration of grain meal or glucose into the rumen of sheep. Gill *et al.* (1986) observed that even when lactic acid constituted over 13% of the dry matter in silage diets, no lactic acid was absorbed from the rumen of sheep.

There are, however, other studies which suggest that lactic acid can be absorbed from the rumen. The results of Williams and Mackenzie (1965) show that the mean absorption rate of lactic acid is about 8% that of VFAs from a solution of approximately pH 5 in ligated fore-stomach of sheep. Argenzio and Whipp (1980) found the rate of lactic acid absorption to be 10% that of VFAs in swine. Hueter *et al.* (1956) observed an increase in the concentration of lactic acid in blood after intra-ruminal administration of sodium lactate. These were probably due to absorption of lactic acid from abomasum and/or intestine.

Lactic acid has been found to be absorbed from the abomasum of sheep (Herden 1980) and cattle (Huntington and Reynolds 1986). Dunlop (1970) also reported that absorption of both isomers of lactic acid occurs more rapidly from the small intestine than the fore-stomach. It is possible therefore that blood lactic acid arising from carbohydrate overload may be due to the absorption of lactic acid from the small intestine, the abomasum and the colon. The retention time of digesta in the colon is longer than that in the caecum (Warner 1981) and lactic acid may also be absorbed from the colon. The flow of lactic acid from the rumen and its absorption from the abomasum and intestine could explain the results reported by Hueter *et al.* (1956), Williams and Mackenzie (1965), and Argenzio and Whipp (1980).

Acidity (pH 4.0 - 4.5) of the rumen fluid during lactic acidosis may inhibit rumen motility (Shinozaki 1958). This will reduce the flow of rumen fluid to the small intestine and therefore limit the post-ruminal absorption of lactic acid. Under conditions of very severe lactic acidosis, gross structural changes occur in the rumen wall in sheep (Lee *et al.* 1982) and similar changes have been reported in the caecal wall in horses (Krueger *et al.* 1986). It is likely that this damage may make it possible for lactic acid to be absorbed directly from the rumen and caecum. This suggestion is supported by the results of Godfrey *et al.* (1992, 1995) who found that high levels of blood D-lactate only occurred under conditions of very low pH in the rumen.

The different views of lactic acid absorption may be explained by the differences in experimental conditions. Usually lactic acid is considered to be absorbed by diffusion (Dobson and Phillipson 1956) and is transported in free (undissociated) acid (Giesecke and Stangassinger 1980; Newbold *et al* 1984; Gill *et al* 1986). Free lactic acid is more readily absorbed than the anion because the free form is less polar (Williams and Mackenzie 1965; Dunlop 1972; Giesecke and Stangassinger 1980). The pH of gut fluid may therefore be a factor affecting the absorption of lactic acid. The pKa of lactic acid at 25° C is 3.86 and it is known that the anion fraction of lactic acid predominates in the rumen when it occurs. Under conditions of lactic acidosis, rumen pH values are often in the range of 3.9-4.5 (Dunlop 1972; Lee 1977) and there is an associated increase in the concentration of free lactic acid and, in turn, increased absorption of lactic acid. This is supported by the results of Williams and Mackenzie (1965) who found that increasing pH of test solutions introduced into the rumen from pH 5.0 to 7.6 had little effect on the rate of lactate absorption, however, decreasing the pH from pH 5.0 to 4.0 increased the rate of lactate absorption by an average of 54%. Therefore, changes in the pH can result in great differences in the absorption of lactic acid under conditions of pH below 5.

The pH in the fermentation compartments of the gut is normally relatively constant and this stability is very important to maintain normal functions of the gut and the growth of microbes in the gut. Although respiratory and excretory systems also contribute to pH regulation via blood, the maintenance of relatively stable pH in the gut depends mainly

on the balance between rates of acid production and absorption and the buffering capacity in the gut. Buffering capacity in the gut is mainly provided by buffers entering via saliva and feed, and by passage of bicarbonate through the gut wall. Buffers such as bicarbonate (HCO_3^-) and phosphates (PO_4^{3-}) are important in the gut. Saliva is rich in mineral ions, particularly Na^+, HCO_3^-, HPO_4^{2-}, K^+, and Cl^-, which provide buffering capacity. While saliva enters the rumen with feed to perform a buffering function, no saliva is secreted into the caecum. This is a major difference between the buffering systems of the caecum and the rumen. In the caecum and the rumen, as well as other parts of the gut, urea also plays a buffering role through its conversion to ammonium by microbial ureases. Feed proteins and non-protein nitrogen (NPN) fractions of forage are rich in glutamate, aspartate, glutamine and asparagine and may also contribute significantly to buffering capacity.

The results in this study show that the sodium bicarbonate and sodium carbonate ($NaHCO_3$ and Na_2CO_3) system plays a more important buffering role than the sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate (NaH_2PO_4 and Na_2HPO_4) system (Figure 4-4). This result agrees with that of Turner and Hodgetts (1955) who found that, in the fasting animal, rumen bicarbonate was more important than phosphate. This was also found to be the case with parotid saliva. As ruminal fermentation proceeded and VFAs accumulated, bicarbonate and pH decreased and the buffering capacity depended more and more upon phosphate. Studies by Counotte *et al.* (1979) suggested that bicarbonate and VFAs are the main components of the buffering system in the rumen fluid of dairy cattle under a range of feeding conditions, and that saliva phosphates are of little importance as a buffer.

Another important finding in this study is the difference in buffering capacity between the caecal digesta and the rumen digesta. The buffering capacity of caecal digesta was found to be nearly double that of rumen digesta ($P < 0.001$) of sheep grazing green pasture or fed roughage diets (Table 4-2) despite there being no input of saliva into the caecum. This difference could be a result of the higher dry matter content of the caecal digesta resulting in greater concentration of buffering agents. The results in this study suggest that the buffering capacities of rumen and caecal digesta may be affected by feed and water intake before slaughter. The buffering capacities of rumen and caecal digesta of sheep fasted for 24

hours were much higher than those of the sheep grazing the same green pasture (Table 4-2). This was because the digesta in the sheep prevented from feeding and drinking for one day may have contained less water and were therefore more concentrated. The second factor could be the reduced acid production in the absence of fresh substrate for fermentation. The buffering capacities of rumen and caecal digesta of sheep fed oaten chaff were higher than those of sheep grazing green pasture. It is possible that this was because fermentable substrate in the sheep fed oaten chaff was lower leading to reduced acid production. The higher buffering capacity of caecal digesta compared to rumen digesta may be an important adaptive mechanism to help compensate for the reduced strategies that the caecum has in coping with the build-up of acid.

In the *in vitro* experiments in this study, the pH and buffering capacity of the caecal and rumen digesta significantly increased ($P < 0.05$) after adding bicarbonate and/or phosphate (Table 4-3 and Figure 4-3). The effect of $NaHCO_3$ was similar to that previously reported in sheep (Bigham *et al.* 1973; Kovacik *et al.* (1986), in cattle (Counotte *et al.* 1979; Rogers and Davis 1982a, b; Boerner *et al.* 1987; Zinn 1991; Bigner 1996) and in goats (Cetinkaya and Unal 1992) in that the pH of the rumen digesta increased after adding $NaHCO_3$. This suggests that $NaHCO_3$ can be used as a tool in the prevention of acidosis. However, there are reports in the literature which consider that $NaHCO_3$ supplement in the diets of cattle by 0.75-2.2% of DM has no effect on acidosis (Russell et al. 1980; Haaland and Tyrell 1982; Zinn and Borques 1993) or no significant effect (Xu *et al.* 1994; Clayton *et al.* 1997) on ruminal pH. These differences may be accounted for by the differences in experimental techniques and the quantities of buffer used. The fermentation of 1000 g grain produces 4 - 12 moles of acids (Leng and Leonard 1965; Murray *et al.* 1990). If the fermentation is rapid, and even if only one-tenth of the fermentative acids accumulate in the gut, 27 - 80 g $NaHCO_3$ (2.7 - 8% of grain) is needed to neutralise the accumulated acids, according to the experimental data in this study. When $NaHCO_3$ supplements are included in the diet, it normally constitutes 0.75-2.2% of DM as mentioned above. This is clearly not enough to neutralise the acids which accumulate in the acidotic animals. This helps to explain why $NaHCO_3$ supplement does not appear to play a major role in the treatment of acidosis. In addition, the latter five studies were done in cattle *in vivo*,

while the experiments in th s investigation were done *in vitro*. The results between *in vivo* and *in vitro* might be different. The effects of added dietary buffers on the p oduction of salivary buffers and ruminal metabolism is not known. Furthermore, it is difficult to estimate the actual increase in $NaHCO_3$ conce ıtration of the rumen digesta at any one point as feed intake is at irregulai intervals throughout the day.

Although the addition of buffer can neutralise part of the gut acids and increase buffering capacity and pH, the effects of bicarbonate application in the model on buffering l:ctic acid and pH were minimal (Figures 5-13, 5-14). It is possible that buffer can not alter the rate of fermentation of starch and does not appear :o prevent microbial changes in the gut which are responsible for rapid fermentation of readily fermentable carbohydrates and the accumulation of lactic acid. As we know, the buffering capacity is less important than the balance between the rates of acid production and absc rption. When the rate of acid production exceeds that of acid absorption and buffering capacity in the gut of the animal, lactic acidosis will occur. This is supported by the predictions of the computer model in this study. The higher potential fermentability of grain, the greater the acid p oduction, but the lower the pH (Figures 5-10, 5-11). If the "rumen" "fermented" 540 g/d of grain in 3 h (3 g/min) with potential fermentability of 8 mmol acids/g and 460 g/d of hay over the full 24-hour period (0.319 g/min) with potential fermentability of 5.5 mmol acids/g, the fermentation c ccurred rapidly and acid production largely exceeded that of acid abso ption and resulted in acidosis (Figure 5-14). However, when the "rumen" was "fed" grain at a constant rate over a 24-hour period, and even w ıen 1950 g/d of grain (1.354 g/min) was "fermented", there was no a:cumulation of lactic acid nor a fall in "rumen" pH (Figure 5-14). This is b:cause the rate of acid production was always balanced by that of acid ab: orption.

In the case of lactic acidosis, it appears that lactic acid has its primary adverse effect on the anima through direct local effects within the gut, as stated in Chapter 3. This s consistent with the studies of Juhász and Szegedi (1968) who attributed the adverse effects of lactic acidosis to the local effect of pH and exce ;s lactic acid in the rumen. At the same time, excess CO_2 formed in the rumen during rapid fermentation can be associated with bloat. Syste mic effects may change the balance between acid and base through acid absorption from the gut. Acidosis is affected

by total acid production, not only lactic acid production, and the contribution of all acids to he disturbance of the acid-base status leads to acidosis (Harmon 1983).

It is concluded that the balance between the rate of acid production and the rate of absorption s more important than the buffering capacity within the gut in the development of fermentative acidosis. This conclusion supports the general hypothesis explored in this thesis.

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Appendix: Publications Arising from This Thesis

1. Ding, Z., Rowe, J. B., Godwin, I. R., Xu, Y., Ball, F., and Atkinson, S. (1998). No lactic acid absorbed from the caecum and rumen of sheep. *Australian Journal of Agricultural Research* **49,** 293-301.

2. Ding, Z., Rowe, J. B., Godwin, I. R., and Xu, Y. (1997). The buffering capacity of caecal digesta exceeds that of rumen digesta from sheep fed pasture or roughage diets. *Australian Journal of Agricultural Research* **48**, 723-728.

3. Ding, Z., Rowe, J. B., Godwin, I. R., and Xu, Y. (1996). Buffering capacities of rumen and caecal digesta from sheep. *Animal Production in Australia, Proceeding of Australian Society of Animal Production* **21,** 343.