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A model for exploring lactic acidosis : 2. Model evaluation and validation

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ABSTRACT. For exploring lactic acidosis, a computer model based on incorporating our experimental data with information in the literature was evaluated its sensitivity to key parameters and validated by performing against experimental results in sheep. The model produced reasonable, interesting responses in the concentrations of lactic acid and volatile fatty acids (VFAs) as well as pH and the predicted data was relatively stable over the range of the values of the parameters tested. The results suggest that the rate of fermentation and the amount of substrate are important factors in terms of the development of fermentative acidosis. 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.

KEY WORDS : model, lactic acid, acidosis, volatile fatty acids, rumen, sheep.

INTRODUCTION

As our understanding improves, some models of whole rumen function have been developed (PITT & PELL, 1997; KYRIAZAKIS, 2001; KEUNEN et al., 2002; KNOTT & KERNER, 2003). Although a number of issues related to rumen model require further research, the whole rumen function models have endeavoured to represent the digestion and passage of ingested nutrients, microbial metabolism and the formation of end products of digestion (ZIEMER et al., 2000; POPPI et al., 2001; KEUNEN et al., 2002; KOHN, 2003).

Lactic acidosis arising from rapid fermentation of carbohydrate is a condition with severe consequences for the animal and wide-spread in ruminant production systems (ROWE, 1997; DING et al., 1998; MULLER et al., 2002; DING & XU, 2003; BECKER et al., 2004). The control of the concentrations of lactic acid and volatile fatty acids (VFAs) as well as pH in the rumen during rumen fermentation is becoming the focus of more and more research in this area. Based on incorporating our experimental data with information in the literature, we have developed a computer model on the development and prevention of lactic acidosis in sheep and entitled 'a model for exploring lactic acidosis : 1. model description' in the same issue of this journal. However, the model was not valuated and validated.

This paper is a continuance of the model to valuate it to key parameters and validate the model by performance against experimental results in sheep. The model was also used to determine the relative effectiveness of four different ways of controlling acidosis in the rumen.

MATERIALS AND METHODS

Experimental design

Eighteen (18) wether (Merino sheep), weighing 30 to 50 kg and aged approximately 2 years, were nocked in the rumen and penned individually. The sheep were originally fed 900 g/day hay containing 1% urea for other experiments. They were fed the same diet for 4 weeks before this experiment and the feed was given hourly in equal amounts. Then the sheep were randomly divided into six groups for two experiments as follows :

(1) Experiment 1

Each rumen-nocked wether of Group 1 to 3 (N = 9) was fed a diet of 460 g hay in 24 h and 540 g wheat with 20 g $NaHCO_3$ in 3 h. The feed was given hourly in equal amounts.

(2) Experiment 2

Every rumen-nocked wether of Group 4 to 6 (N = 9) was fed a diet of 320 g hay and 480 g wheat in 3 h. The feed was given hourly in equal amounts.

Collection and analysis of samples

During 0-8 h in the experiments, the rumen digestive liquid was hourly collected from the rumen-nock of experimental sheep and filtered/strained separately into tube in 38ºC water bath with cheese cloth to remove raw dietary residue. The tubes with rumen digestive liquid in 38ºC water bath were covered with films of plastic and rapidly moved into a 37ºC room to determine pH, lactic acid, and VFAs.

The pH of samples was measured immediately after sampling, using a pH-meter with a glass electrode (0-14 pH ± 0.01 pH£¨Beckman, USA).

Concentrations of VFAs were determined using a gas-liquid chromatography (GLC, Model 304, Pye Unicam Ltd, Cambridge, Cambs) based on the method of ERWIN et al. (1961).

Lactic acid concentrations were assayed using a Cobas Mira Auto-analyser (Roche Diagnostics Inc., Frenchs Forest, NSW) and enzyme kits (D-Lactic acid/L-Lactic acid kit, Cat. No. 1112821, Boehringer-Mannheim, Mannheim, Germany).

Statistical methods

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

RESULTS AND VALIDATION OF THE MODEL

The experiments were conducted for validating the model so that we can perform the model effectively to predict and treat lactic acidosis. Therefore, the experimental results are present here by comparing to the predictions of the model.

In experiment 1, every rumen-nocked sheep consumed a diet of 460 g hay in 24 h and 540 g wheat with 20 g $NaHCO_3$ in 3 h. The same in the model, 540 g wheat with 20 g $NaHCO_3$ entered the 'rumen' over a 3 h period at a constant rate of 3 g/min wheat with 0.111 g $NaHCO_3$. The 460 g hay entered the 'rumen' over the full 24 h period at a constant rate of 0.319 g/min. The results predicted by the model were similar to those of the experimental sheep fed the same diets (P >0.05). The greatest effect was on lactic acid and pH, however, the recorded parameters changed at their peaks that appeared in 2 h after sheep had $NaHCO_3$ (Fig. 1). The experimental lactic acid and pH changed a little narrower and lighter than those predicted by the model (P >0.05).

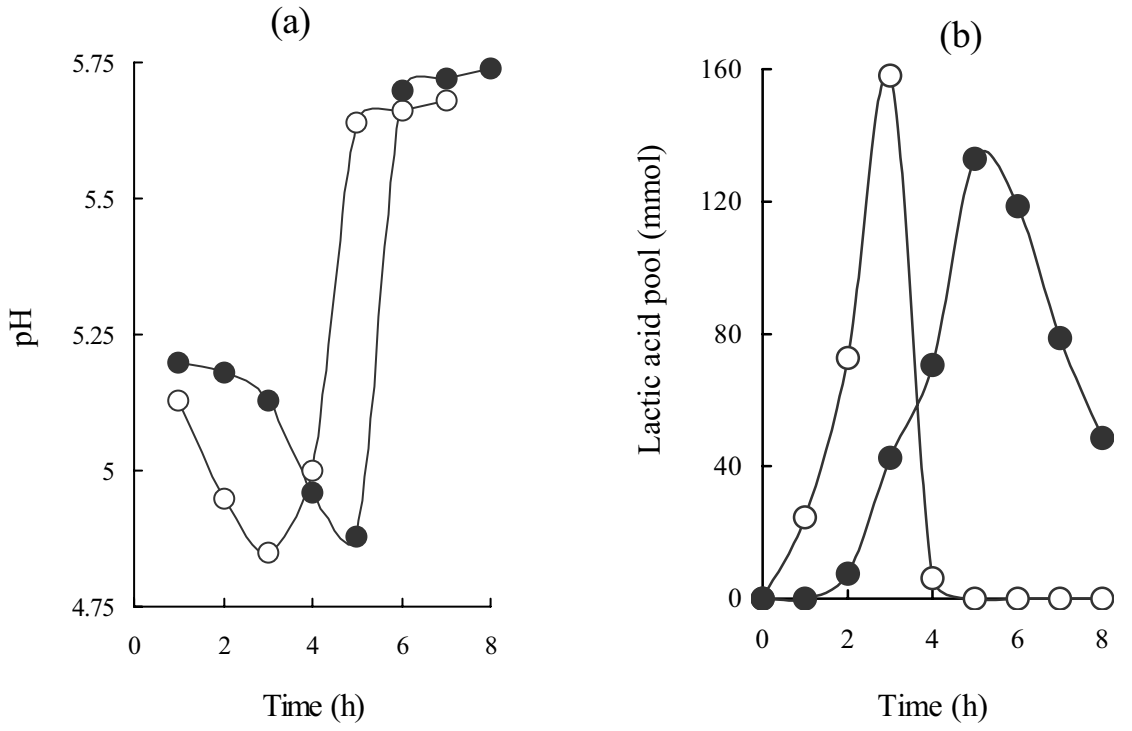

Fig. 1. – **Comparisons of model predictions with mean results from the experimental sheep (N = 9) fed a diet of 460 g hay in 24 h and 540 g wheat with 20 g $NaHCO_3$ in 3 h.** (a) pH and (b) lactic acid pool. Predictions (O), experimental observations (●).

In experiment 2, each rumen-nocked sheep consumed another diet of 320 g hay and 480 g wheat in 3 h. The feed was given hourly in equal amounts. In the model, 'feeding' 320 g hay and 480 g wheat was over a 3 h period at constant rates of 1.778 g and 2.667 g, respectively. The results from sheep were compared to the predictions of the model using a potential fermentability for grain of 8 mmol acids produced/g and hay 5.5 mmol acids produced/g. The two supposed potential fermentabilities are mean quantitative values for fermentation of grain and hay, respectively, from the work of LENG & LEONARD (1965), BERGMAN et al. (1965), WELLER et al. (1967),

BERGMAN (1990) and MURRAY et al. (1990). The peaks for the model predictions and the experimental observations were 'displaced' with the peaks for rumen pH and VFAs concentration (Fig. 2) being approximately 2 h earlier in the model predictions. The prediction VFAs and pH varied a little wider and deeper than those of *in vivo* experimental observations (P >0.05).

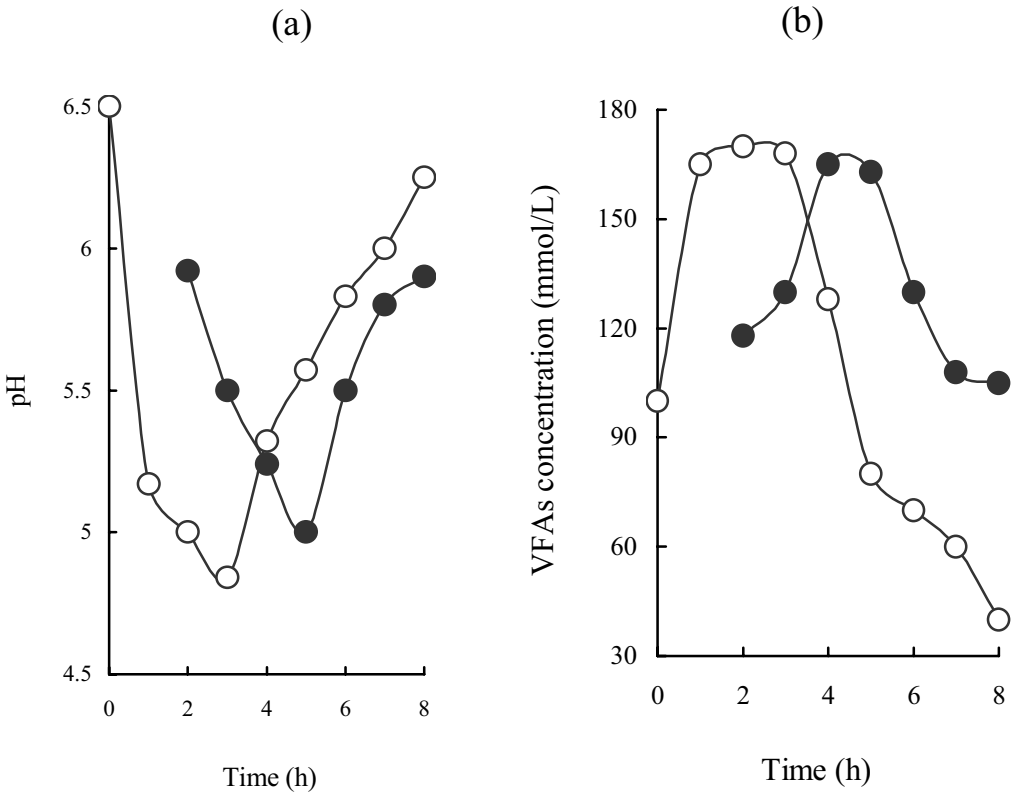

Fig. 2. – **Comparisons of model predictions with mean results from the experimental sheep (N = 9) fed a diet of 320 g hay and 480 g wheat in 3 h (N = 9).** (a) pH and (b) VFAs concentration (mmol/L). Prediction (O) and experimental observation (●).

In two experiments, the lactic acid, pH, and VFAs changed a little narrower and lighter than those predicted by the model (P >0.05). These may be due to a complicated living organism that is capable of preventing strong changes in certain ways in the cases.

VALUATION OF THE MODEL

The model was subjected to sensitivity and general behavioural tests and the tests were compared the output to the results from published studies. The model was also used to simulate experiments to create and control lactic acidosis in sheep.

Sensitivity and behavioural tests

The model was tested for its sensitivity to two key parameters, potential fermentability of grain (PFG) and potential fermentability of hay (PFH1). All tests in the model were run for 24 h (1440 min). However, the X axis (time) in the figures has been truncated to 210 or 240 min or 24 h 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 VFAs absorption. The results presented in Fig. 3 illustrate five levels (4, 6, 8, 10, 12 mmol acids produced/g grain consumed) of potential fermentability of grain (PFG) to VFAs. In the work of LENG & 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 for VFA production per gram of substrate fermented and values for grain vary from 4 to 12 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 before finalising the value. Rumen pH (a), VFAs pool (c) and VFAs absorption (d) varied significantly depending on potential fermentability of grain (PFG). The higher potential fermentability of grain (PFG), the greater VFAs pool and VFAs 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.

Fig. 4 presents the results which express three levels (4.5, 5.5, 6.5 mmol acids produced/g hay consumed) of

assumed potential fermentability of hay (PFH1) to VFAs since the values for VFAs production of hay are from 4.5 to 6.5 mmol VFAs/g in the literature (LENG & LEONARD, 1965; BERGMAN et al., 1965; WELLER et al., 1967; BERGMAN, 1990; MURRAY et al., 1990). The results in Fig. 4 are similar to those of Fig. 1 : the higher potential fermentability of hay (PFH1), the greater the VFAs pool (c) and VFAs 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. This is consistent with the natural life of sheep.

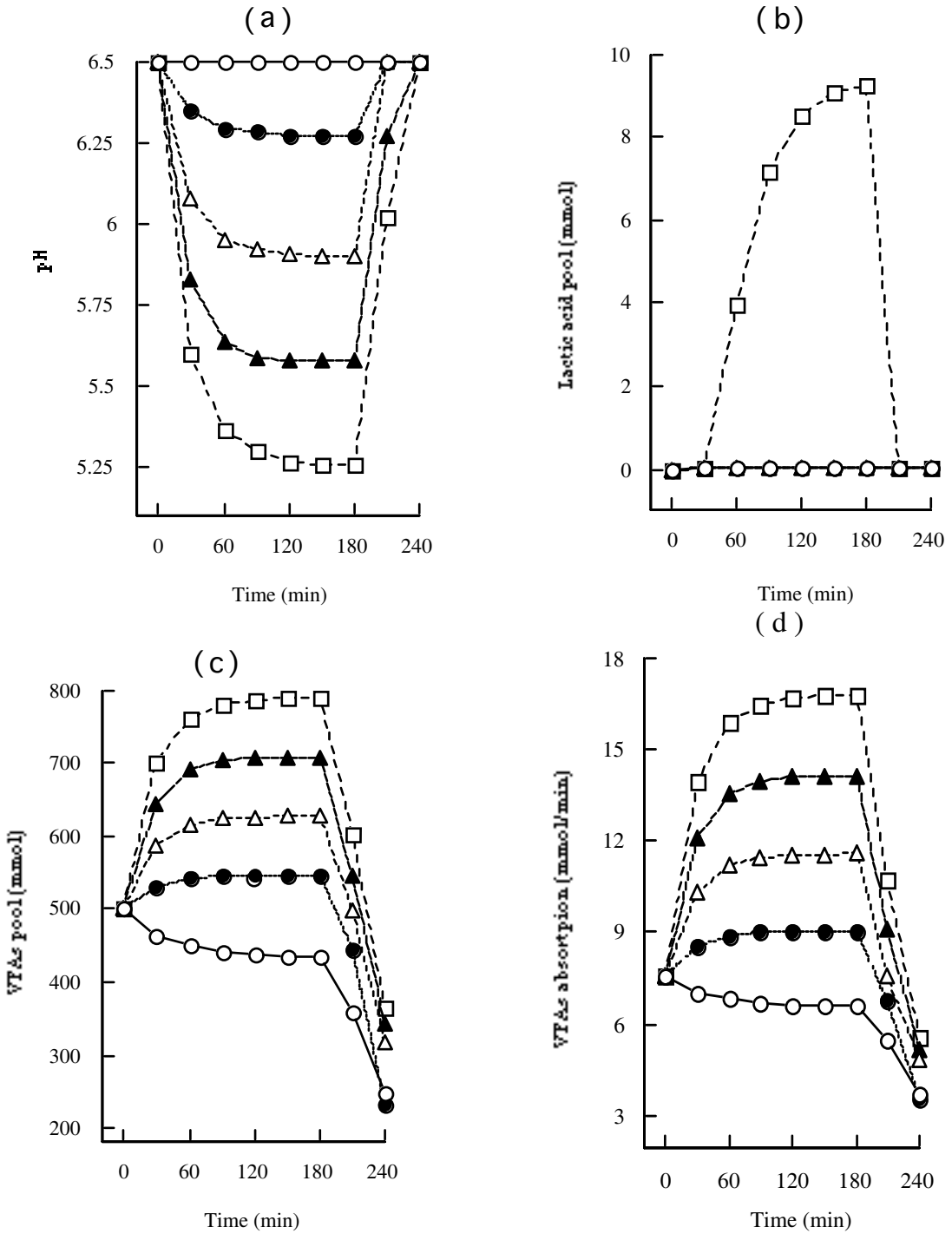

Fig. 3. – **Effects of varying potential fermentability (mmol/g) of grain (PFG) on model behavior.** The X axis (time) in the figure has been truncated to 240 min since each variable presented maintained a somewhat steady state after that time. Four lactic acid pools for different PFGs in the Y axis in b were overlaid except a lactic acid pool for PFG = 12 mmol acids produced/g. (a) pH, (b) lactic acid pool, (c) VFAs pool, and (d) VFAs absorption. PFG = 4 mmol acids produced/g (O), PFG = 6 mmol acids produced/g (●), PFG = 8 mmol acids produced/g (Δ), PFG = 10 mmol acids produced/g (▲) and PFG = 12 mmol acids produced/g (□).

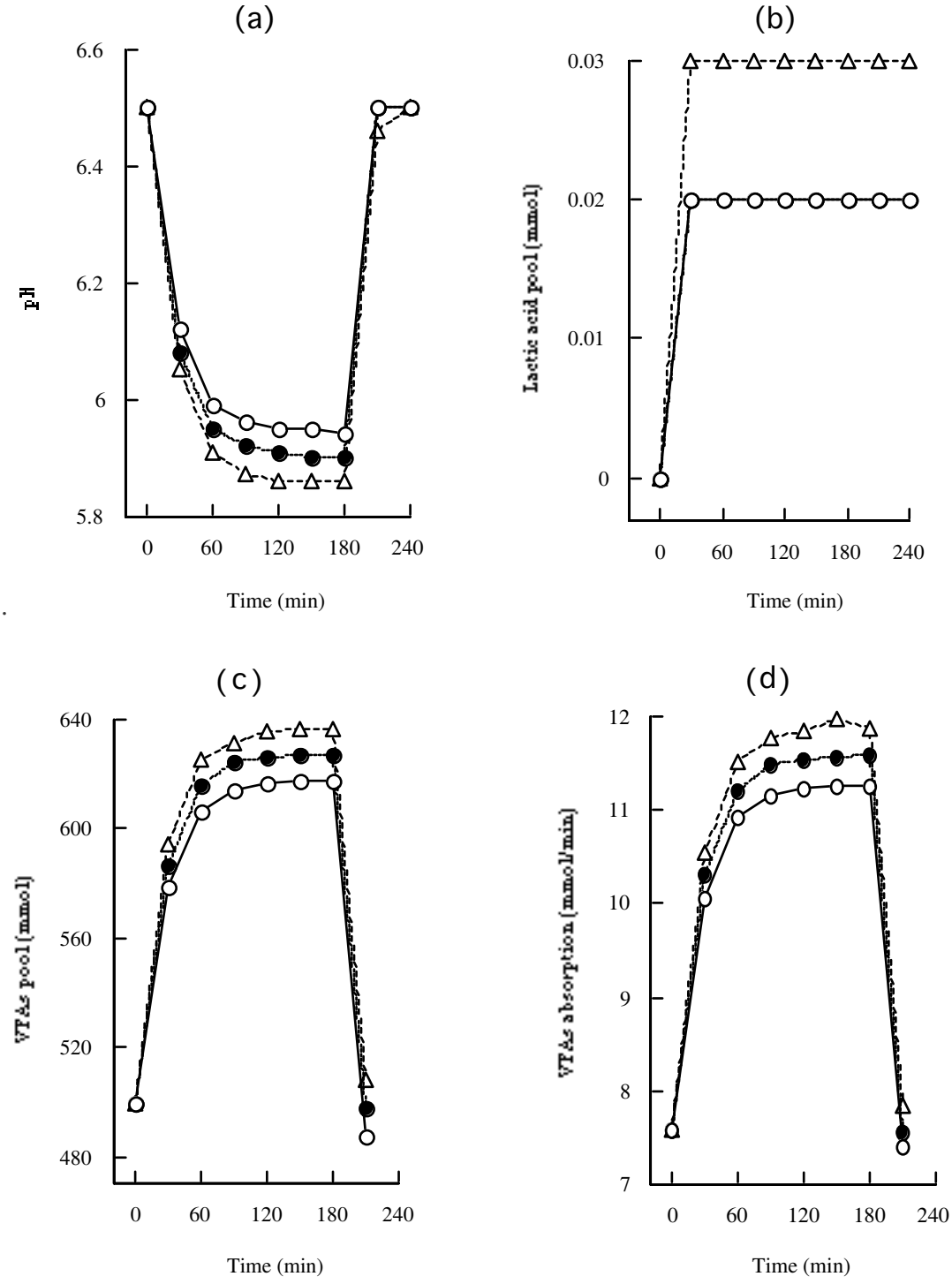

Fig. 4. – **Effects of varying potential fermentability of hay (PFH1) on model behaviour.** The X axis (time) in the figure has been truncated to 240 min since each variable presented maintained a somewhat steady state after that time. Two lactic acid pools for different PFH1s in the Y axis in b were overlaid except a lactic acid pool for PFH1 = 6.5 mmol acids produced/g. (a) pH, (b) lactic acid pool, (c) VFAs pool, and (d) VFAs absorption. PFH1 = 4.5 mmol acids produced/g (O), PFH1 =5.5 mmol acids produced/g (●) and PFH1 = 6.5 mmol acids produced/g (Δ).

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 standardized at 1000 g/d consisting of hay 460 g/d and grain 540 g/d 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 3 h period at a constant rate of 3 g/min. The hay ration of 460 g/d entered the 'rumen' over the full 24 h 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 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; LENG & LEONARD, 1965; WELLER et al., 1967; BERGMAN, 1990; MURRAY et al., 1990). These values of parameters were therefore used for simulated experiments as well as for the basic model. The results of simulated experiments are described as follows.

Different levels of buffer and effect on pH

Buffer is often used to prevent lactic acidosis because of its buffering capacity and sodium bicarbonate ($NaHCO_3$) is a good buffer in practice (ROGERS & DAVIS, 1982a; KOVACIK et al., 1986; DING et al., 1997; CUMBY et al., 2001). There were two ways in which the buffer, $NaHCO_3$, 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 3 h period. Another way was to add $NaHCO_3$ at a constant rate over the full 24 h period with the hay. $NaHCO_3$ 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 $NaHCO_3$ (Fig. 5). Although all recorded parameters, including pools of VFAs and lactic acid, VFAs 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 units in response to the buffer. The model did not include HCO_3^- and $HCO_3^- H^+ + CO_3^{2-}$ in the saliva and the movement of bicarbonate and CO_2 across the gut wall *in vivo* that need to be studied.

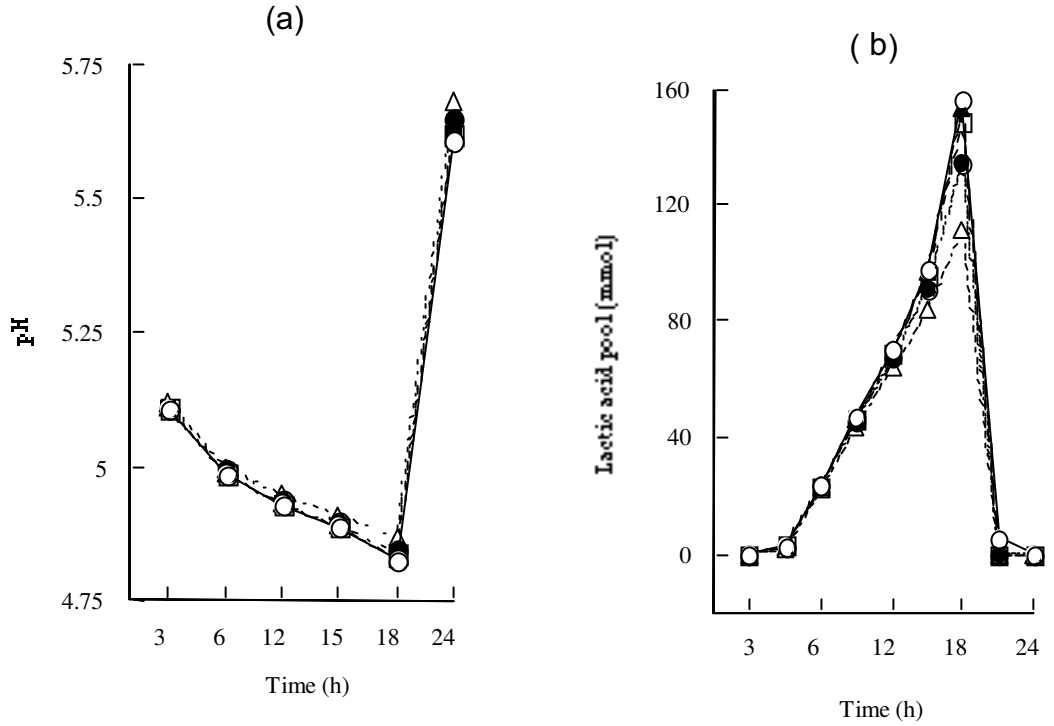

Fig. 5. – **Effects of buffer ($NaHCO_3$) on model behavior.** The X axis (time) in the figure has been truncated to 24 h since each variable presented maintained a somewhat steady state after that time. The pHs in a and lactic acid pools in b for different amounts of $NaHCO_3$ in the Y axis were overlaid. (a) pH and (b) lactic acid pool. 0 g $NaHCO_3$ (O), 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 (□).

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 (ROWE & ZORRILLA-RIOS, 1993; ROWE et al., 1995; GODFREY et al., 1995a, b; NAGARAJA et al., 1995). 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 VFAs pool increased 11% and the pH increased 0.17 units at their peaks (Fig. 6). 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.

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 form of Gram-positive lactic acid utilizers to the 'rumen'. The use of probiotics is based on the theory that bacterial pre-treatment can prevent acidosis by increasing lactate utilization in animals. In practice, the probiotic Yea Sacc 1026 (Alltech) was shown to reduce the accumulation of lactic acid in the rumen fermentation of starch (NEWBOLD, 1990; WILLIAMS & NEWBOLD, 1990; GIRARD et al., 1993; NEWBOLD et al., 1996). 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, VFAs pool increased 10% and pH increased 0.14 units (Fig. 6). 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.

Gradual 'intake' of grain

Current practices to prevent acidosis in livestock depend largely on gradual adaptation to diets high in readily fermentable carbohydrates and careful management while feeding such diets (HUNTINGTON, 1988; GODFREY et al., 1992; WIRYAWAN & BROOKER, 1995). Adaptation is based on changes in microbial species and relative population densities in response to changes in substrate. The most common way of feeding grain to ruminants is through a gradual introduction followed by regular feeds. In the model gradual 'intake' of grain was simulated by feeding grain at a constant rate over the full 24 h period. The result in Fig. 6 showed that a gradual 'intake' of grain even as high as 1950 g/d over the full 24 h 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 VFAs pool (round 502 mmol) and VFAs absorption (7.7 mmol/min) were always maintained at higher levels.

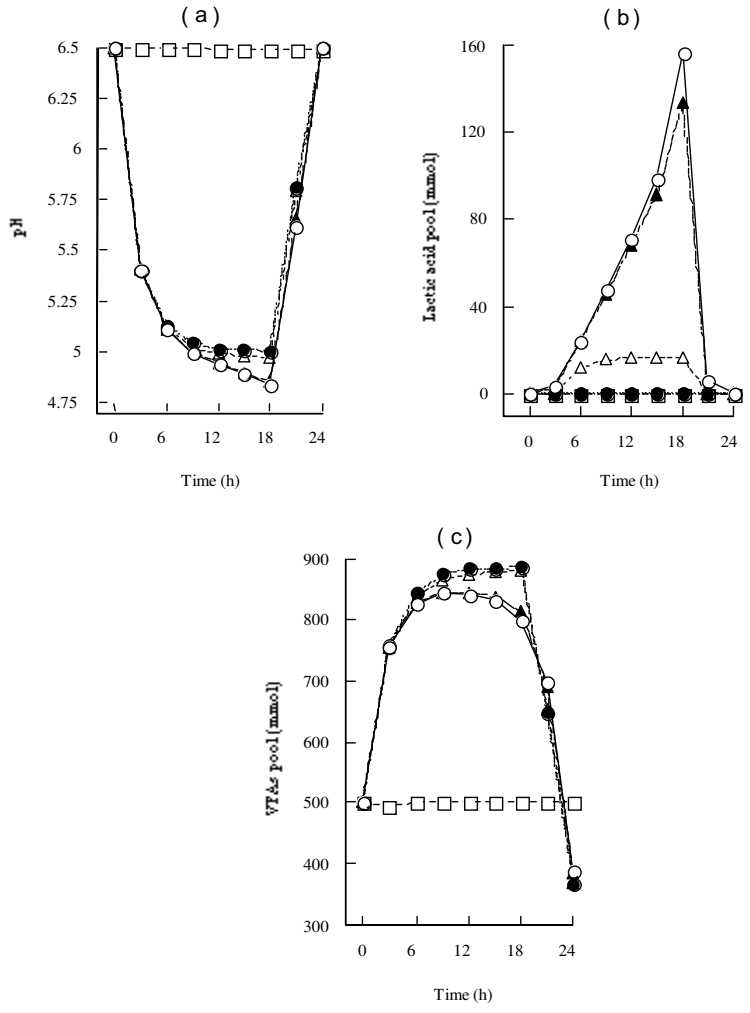

Fig. 6. – **Comparisons of the different ways of controlling lactic acidosis on model behavior.** The X axis (time) in the figure has been truncated to 24 h since each variable presented maintained a somewhat steady state after that time. Some of pHs in a, lactic acid pools in b and VFAs pools in c for different amounts of $NaHCO_3$ in the Y axis were overlaid. (a) pH, (b) lactic acid pool and (c) VFAs pool. Base run (O), 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 h 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.

DISCUSSION

Sensitivity and behavioural analyses

Fermentation of grain results in rates of VFAs production within the rumen ranging from 4 to 12 mmol/g of feed, whereas fermentation of hay yields VFAs 4.5 to 6.5 mmol/g (LENG & LEONARD, 1965; LENG & 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 VFAs pool and VFAs absorption, but the lower the pH (Fig. 3). 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 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 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 ruminant rumen 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 (Fig. 3b). 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 and the level exceeded the capacity for conversion of lactic acid to VFAs to be absorbed and resulted in the accumulation of lactic acid (Fig. 3b). However, the accumulation of lactic acid did not appear to influence proportionally the pH level in Fig. 1a 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 (ROWE, 1997; DING et al., 1998; DING & XU, 2003).

The model 'fermentation' is similar in many ways to the fermentation in the ruminant rumen and the predictions are comparable with the observations in practice. If a potential fermentability 8 mmol acids produced/g for grain and 5.5 mmol acids produced/g for hay were used, the model predictions had similar patterns in rumen pH and VFAs concentration as the experimental sheep consumed the same diet of 320 g hay and 480 g wheat in 3 h (Fig. 2). The results from sheep consumed a diet of 460 g hay in 24 h and 540 g grain with 20 g $NaHCO_3$ in 3 h were also similar to the predictions of the model 'fed' the same diet (Fig. 1). The predictions of the model were comparable with experimental results of the ruminant rumen because the parameters employed in the model were derived from experimental observations. The observation results appeared approximately 2 h after sheep fed experimental diets and changed a little narrower and lighter. These are reasonable since the experimental diets need to be ingested, absorbed and reacted with complicated systems *in vivo* while they are immediately proceeded in the model; there are many control systems *in vivo* that lack in the model.

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.

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 neutralizing 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 different from those of published models of rumen function and discussed in detail below.

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 h 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 (Fig. 6b, c) and a lower pH (pH 4.83, Fig. 6a) 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 (DING, 1997; DING et al., 1997; ROWE, 1997). 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 h period, there was no accumulation of lactic acid nor a fall in 'rumen' pH even as high as 1950 g/d of grain (1.354 g/min) was 'fermented' (Fig. 6). 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-metabolizing bacteria and an ecological balance between production and utilization 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.

Predicting the benefits of neutralizing acids and buffering pH by addition of buffers.

Lactic acid and VFAs accumulated to result 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 (Figs 5 and 6). However, the predictions showed that the addition of $NaHCO_3$ as supplement with grain could decrease the accumulation of lactic acid and VFAs as well as could increase the pH (Fig. 5). Compared to other methods of preventing lactic acidosis in the model, the effects of $NaHCO_3$ on lactic acid and pH were not very great (Fig. 6). Probably the additional buffer partially neutralized 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. The effects *in vivo* for the supplement of $NaHCO_3$ may be more greatly associated with other reactions (BIGHAM et al., 1973; COUNOTTE et al., 1979; CUMBY et al., 2001) and the buffering capacity of $NaHCO_3$ varies depending on the rumen pH. Experiments showed that 1 g $NaHCO_3$ can buffer 20 mmol acetic acid *in vitro* at pH 6 (DING, 1997; DING et al., 1996, 1997). When the rate of buffering capacity and the relative rates of absorption of VFAs and lactic acid from the rumen were used to control lactic acid build-up in the model, the predicted results agreed with the observations in the experiments in that the addition of $NaHCO_3$ resulted in an increase in pH and buffering capacity of rumen and caecal digesta (ROGERS & DAVIS, 1982a, b; KOVACIK et al., 1986; DING et al., 1997; CUMBY, 2001).

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 VFAs pool and pH were increased greatly by 11% and 0.17 pH units at their peaks, respectively, as shown in Fig. 6. 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; NAGARAJA et al., 1995) 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 & 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.

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 was reduced to one-tenth of the control value. This was associated with increased production and absorption of VFAs and higher pH (Fig. 6) 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, a little lactic acid was 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 & 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.

The area in need of development

For the model, the fermentation, absorption and outflow of the rumen are three of the key parameters which are highly complex and hard to quantify. The fermentation depends on substrate, animal, animal state, microorganisms and pH etc (WESTON & HOGAN, 1968; BIGHAM et al., 1973; JOUANY et al., 1988). Absorption and outflow rates depend on pH and osmotic pressure (WILLIAMS & MACKENZIE, 1965; DING et al., 1997, 1998), particle size, density, hydration rate of the gut content as well as feed (WELCH, 1986; DIJKSTRA et al., 1992). The relationships

of these factors and the absorption and outflow rates need to be investigated further. Some factors are therefore difficult to be included in the model. VAN STRAALEN & 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 (like sort of carbohydrates and chain length of fatty acids) utilization (RUSSELL, 1984);

(ii) outflow rate from the rumen with the fluid or solid phase (CHENG & COSTERTON, 1980); and

(iii) recycling of microbial matter within the rumen (JOUANY et al., 1988);

(iv) effects on digesting bacteria (ZIEMER et al., 2002; GALBRAITH et al., 2004).

These differences would affect accuracy of the model simulations and, hence, further efforts to standardize these techniques and determine 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.

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