

**RUMINAL ACIDOSIS IN SOUTHERN AUSTRALIAN DAIRY  
CATTLE**

**Elizabeth Bramley**

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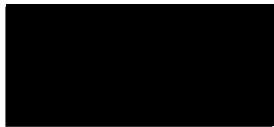
University of Sydney

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## **DECLARATION**

The study presented in this discussion were completed by the author while a postgraduate student in the Faculty of Veterinary Science, University of Sydney, Camden, NSW, Australia. Assistance given by others is indicated in the Acknowledgements. All cited references are listed in the reference section of each chapter. The work is otherwise original.

I hereby certify that the substance of this thesis has not already been submitted for any other degree, and is not currently being submitted for any other degree.



Elizabeth Bramley

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## ABSTRACT

A number of areas of deficiency in knowledge of acidosis, especially acidosis in pasture fed cattle, were identified through a critical evaluation of literature. Previous research examined in detail acidosis experimentally induced by feeding large amounts of concentrates. The Australian dairy industry is strongly pasture-based and there is controversy over the prevalence and significance of acidosis in cattle primarily fed on pasture. Therefore, amounts of starch in Australian dairy diets are not generally as great as diets of cows fed grain with a maize silage forage base, the effectiveness of pasture as a fibre source has not been defined. Consequently, there are marked differences between the Australian feed base and the feed base on which the vast majority of studies of acidosis have been conducted.

There have also been no extensive studies examining the dietary risk for acidosis for feeds commonly present in diets for Australian dairy cattle. Little research has been conducted into herd outcomes of acidosis, particularly in establishing a direct link between acidosis and outcomes such as lameness, scouring and rumen fill. The effectiveness of rumen buffers, modifiers and antibiotics in preventing acidosis, although examined in controlled trials, have not been examined in extensive surveys of the industry.

Ruminal acidosis in pasture-fed dairy cattle in Southern Australia was investigated using a cross-sectional study design. One hundred dairy herds were selected from lists supplied by veterinary practices in five prominent dairy areas of NSW and Victoria. A survey of feeding practices and other relevant health disorders was conducted on each farm. The lactating herds were examined and samples of pasture grazed on the sampling day taken. Eight fresh cows (< 100 days in milk), consisting of three primipars and five multipars, were randomly selected from each herd. Rumen fluid was sampled from each cow by both rumenocentesis and stomach tube. The rumenocentesis samples were tested for pH. Stomach tube rumen fluid samples were tested for pH and analysed for VFA, ammonia and D-lactate concentration.

Feeding practices of the study herds were summarised. Observations were made on type of feeding system used, amount and species of grains fed were recorded, protein meals and commodities fed and type of pasture grazed were also determined. Amount of buffers, modifiers and antibiotics fed in each sampling area were also summarised. Seventy nine percent of study herds fed pasture in combination with grain or pellets twice daily at milking. All but one herd fed concentrate and the percentage of concentrate in the diet ranged from a mean of  $25.1 \pm 11.64\%$  in the Western Districts to  $44.1 \pm 12.04\%$  in Taree. Sixty percent of study herds fed monensin and 24% fed virginiamycin, the most used rumen modifiers, however, many herds fed these below rates recommended by manufacturers.

Rumen biochemical measures were used in Chapter 4 to categorise cows into three groups using K-means cluster analysis. It was ascertained using a Bayesian logistic regression model that random effect of herd was not a significant factor in determining group category. Discriminant analysis was subsequently used to categorise cows ( $n = 114$ ) that were not categorised by cluster analysis because of missing data. All rumen variables measured were significant in predicting group allocation ( $P < 0.01$ ). L-lactate was not used in developing predictive models, given an extremely high correlation (0.9) between D- and L- lactate. Propionate ( $F = 332.38$ ) and valerate ( $F = 332.62$ ) were the most important factors in determining cluster groups. Interestingly, rumen pH and D-lactate were least important in prediction of the groups with  $F = 54.95$  and  $F = 5.02$ , respectively, indicating that future research may be better focused on propionate and valerate concentrations as an indication of acidosis, particularly for pasture-based diets. These findings are consistent with the known biology, however, valerate is a product of lactate metabolism in the rumen and pH is an outcome measure influenced by VFA, ammonia and lactic acid concentrations. Rumen pH obtained from 686 samples using rumenocentesis was predicted as  $\text{pH} = 6.226 (\pm 0.0278) + 0.00378 (\pm 0.002350)$  acetic acid  $- 0.0257 (\pm 0.00271)$  propionic acid  $+ 0.594 (\pm 0.0989)$  isobutyric acid  $- 0.0359 (\pm 0.00823)$  butyric acid  $+ 0.153 (\pm 0.0683)$  caproic acid  $+ 0.0693 (\pm 0.02192)$  D-lactate. L-lactate was included in a second model using 169 samples summarised in Chapter 4.

Ten percent of cows were found in category 1, 30% in category 2 and 60% in category 3. Biochemical measures of category 1 cows were consistent with acidosis, category 2 cows consistent with sub-optimal rumen function and production and category 3 cows not acidotic with adequate production levels. Milk and milk solids production were determined for each category, by using a random effects analysis of variance model to control for effects of herd, parity, days in milk and days to last herd test. Category 1 cows were characterised by slightly higher milk production ( $30.0 \pm 0.97$  L/cow/day) but low milk fat: protein percentage ( $1.06 \pm 0.036\%$ ), category 2 cows low milk production ( $27.9 \pm 0.52$  L/cow/day) with higher milk fat: protein percentage ( $1.20 \pm 0.020\%$ ) and category 3 cows a milk production of ( $29.7 \pm 0.36$  L/cow/day) with a milk fat: protein percentage of  $1.18 \pm 0.010\%$ . Dietary composition associated with the three cow categories was examined on a herd level using Kruskal-Wallis One-way ANOVA and Wilcoxon rank sum test. In order to compare cow level results with herd level results, herds were categorised into three categories corresponding to the three cow categories. For example, herds were categorised as 'ACID' if  $\geq 3/8$  cows from category 1 were found in the herd. Similarly, 'SO' herds related to category 2 cows and 'NA' herds related to category 3 cows. Diets of category 'ACID' herds were significantly lower in neutral detergent fibre percentage ( $30.4 \pm 4.27\%$ ,  $P < 0.05$ ) than category 'SO' herds ( $35.7 \pm 5.98\%$ ) or category 'NA' herds ( $36.1 \pm 5.18\%$ ). Category 'ACID' herds also had diets significantly higher in non-fibrous carbohydrate percentage ( $40.3 \pm 4.44\%$ ,  $P < 0.01$ ) than category 'SO' herds ( $33.4 \pm 6.45\%$ ) or category 'NA' herds ( $33.5 \pm 5.71\%$ ).

In Chapter 5 outcomes associated with the different categories of rumen function were evaluated. These outcomes included body condition, locomotion, rumen fill and faecal score assessed on a herd level using the same categories; ACID, SO and NA as determined in Chapter 4. The effect of district was incorporated into final logistic normal regression model developed as a random effect using Egret (Version 2.03.1 Cytel Statistical Software).

Categories based on rumen function were associated with biological outcomes consistent with acidosis. While cows categorised as acidotic (category 1) had higher milk production than cows in category 2 or 3, herds that had a higher risk of lameness also had an 83% higher risk of being categorised ACID than SO and 118% higher risk

of being categorised ACID than NA herds. Herds with a high prevalence of low rumen scores were also 75% more likely to be category ACID than SO and 57% more likely to be category NA than SO when the random effect of district was evaluated in the model predicting rumen fill score.

Dietary risk factors associated with being in a herd category were identified in Chapter 6. Presence and amounts of constituents fed and total diet analysis variables were examined using multinomial logistic regression and Pearson Chi-square (SPSS version 11.0). Prevalence of feeding wheat and amount of wheat fed tended to be greater in herd category ACID than NA herds ( $P = 0.083$ ,  $P = 0.055$  respectively). Legume-dominant pastures (clover, shaftal or lucerne) were significantly more prevalent in ACID herd diets than NA herd diets ( $P = 0.008$ ) whilst ryegrass dominant pastures were significantly less prevalent in SO herd diets than NA herd diets ( $P = 0.02$ ). Pasture texture was also scored on a 1 to 5 scale where 1=soft lush pastures with minimal effective fibre and 5=forage sorghum or maize with very high effective fibre. Prevalence of score 1 pastures was significantly greater in ACID herds than NA herds ( $P = 0.004$ ), confirming pasture type results. Importantly, neutral detergent fibre %, non-fibrous carbohydrate % and carbohydrate B1 % were all important predictors of ACID herds compared with NA herds. ACID herd diets contained a significantly higher non-fibrous carbohydrate % ( $P = 0.002$ ) and carbohydrate B1 % ( $P = 0.008$ ) but lower neutral detergent fibre % ( $P = 0.004$ ) than NA herds. There was no significant difference in neutral detergent fibre %, non-fibrous carbohydrate % and carbohydrate B1% between SO herds and NA herds ( $P > 0.6$ ), however SO herds tended to feed more fibrous by-product ( $P = 0.159$ ) and straw ( $P = 0.144$ ) than NA herds. The final logistic regression model weighted by number of acidotic cows in a herd simply described the decrease in risk of a herd being category ACID as a function of the ratio of percentage of NDF to percentage of NFC in the diet of herds.

In Chapter 7, one of the study herds with severe clinical acidosis was documented in a case study. Diets of the two lactating cow herds (less than 7 days in milk, between 7 and 100 days in milk) and transition herd were documented and rumen fluid samples from eight randomly selected cows from each of the two lactating cow herds taken. Rumen fluid was analysed for VFA, ammonia, D-lactate and L-lactate concentration. Clinical acidosis was diagnosed using rumen biochemistry and confirmed with

physical outcomes of severe lameness and scouring in the very fresh herd. Diet history indicated an abrupt change from a highly fibrous transition diet with very little dry matter intake to a lactating cow diet high in non-fibrous carbohydrate, poor buffering and minimal effective fibre. Suggestions for treatment, control and prevention of the disease were highlighted. This case study showed clearly the production and health related issues acidosis can cause on both an cow and herd level.

This study introduced several new tools for assessing herd management and feeding including rumen scores, faecal staining and pasture scoring that had biological significance consistent with that expected from the use of these. It is the first epidemiologic study to examine rumen function in pasture fed cattle and identified a prevalence of 10% of cows less than 100 days after calving with acidosis. While, these cows had slightly higher milk production than cows in other categories, a higher prevalence of lameness in herds was strongly associated with acidosis at the herd level. These findings demonstrate that acidosis is both a prevalent and important condition of dairy cows on pasture diets. The lactational incidence of acidosis is likely to be considerably higher than the prevalence, given that 10% of cows had acidosis on the single day of assessment.

Risk factors associated with 'ACID' herds, included pastures that were low in NDF, especially clover based and soft ryegrass pastures and feeding wheat. Importantly, the risk of acidosis at the herd level could be described by the NDF: NFC ratio in the diet. This ratio should be used in diet formulation to reduce the risk of this disorder.

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“Apply your heart to instruction,  
and your ears to words of knowledge”

Proverbs 23vs12

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## LIST OF ABBREVIATIONS

<b>ADICP%CP</b>	acid detergent fibre in crude protein (as a % crude protein)
<b>ADF</b>	acid detergent fibre
<b>BCS</b>	body condition score
<b>BW</b>	body weight
<b>CHO</b>	carbohydrate
<b>CP</b>	crude protein
<b>CTAB</b>	cetyltrimethylammonium bromide
<b>DIM</b>	days in milk
<b>DM</b>	dry matter
<b>DMI</b>	dry matter intake
<b>eNDF</b>	effective NDF
<b>F:C</b>	forage: concentrate
<b>GC</b>	gas chromatograph
<b>IV</b>	intravenous
<b>KW ANOVA</b>	Kruskal-Wallis analysis of variance
<b>LDA</b>	left displaced abomasum
<b>LR</b>	logistic regression
<b>ME</b>	metabolisable energy
<b>NAD(H)</b>	Nicotinamide-adenine dinucleotide
<b>NADP(H)</b>	Nicotinamide-adenine dinucleotide phosphate
<b>NDF</b>	neutral detergent fibre
<b>NDICP%CP</b>	neutral detergent fibre in crude protein (as a % crude protein)
<b>NPN</b>	non-protein nitrogen
<b>NFC</b>	non-fibrous carbohydrate
<b>NSC</b>	non-structural carbohydrate
<b>peNDF</b>	physically effective NDF
<b>PMR</b>	partial mixed ration
<b>RDA</b>	right displaced abomasum
<b>RDP</b>	rumen degradable protein
<b>SC</b>	subcutaneous
<b>TMR</b>	total mixed ration

<b>UDP</b>	undegradable dietary protein
<b>VFA</b>	volatile fatty acid

## Chapter 1.

# LITERATURE REVIEW

## 1.1 General Introduction

Lactic acidosis induced by grain feeding is an important clinical issue in the dairy herd that has been well documented and reviewed (Dirksen 1970; Huber 1976; Nocek 1997; Owens *et al.* 1998; Slyter 1976; Underwood 1992). However, sub-clinical acidosis and secondary effects such as depressed milk production, milk fat content and laminitis have not been well documented, especially in predominantly pasture-fed cows. Sub-clinical acidosis in cattle needs further definition under Australian conditions.

This issue is particularly important where good quality pasture, combined with twice daily grain feeding in the bail, is the preferred feeding practice in the industry. New pasture species and cultivars are now available that contain increased concentrations of water soluble carbohydrates and less effective fibre, increasing the potential risk of sub-clinical and clinical acidosis. It is also important to know the sequence and type of changes occurring in the rumen when cattle are changed suddenly from low quality to high quality pasture. More research is needed in defining effectiveness of fibre and methods to provide a quantitative index of fibre efficacy to use in the development of grasses and in nutritional models.

Rumen modifiers provide a partial solution for sub-clinical acidosis in a herd situation, but aspects of use need further investigation. Studies in Australia on the effects of rumen modifiers and antibiotics have already been reported with favorable results (Abe *et al.* 1994; Clayton *et al.* 1999; Lean *et al.* 2001), however there have been no large scale studies conducted to evaluate the effectiveness of these in controlling acidosis in the field.

This review examines carbohydrate metabolism and factors leading to acidosis in the rumen, providing a proposed aetiology for ruminal acidosis in cattle on diets with high starch concentrations. Rumen pH and the physiological buffering capacity of the rumen and practical techniques for rumen fluid collection are discussed. Practical methods of defining acidosis both clinically and by use of indirect markers will be examined. Methods of preventing ruminal acidosis, including adequate fibre intake, the importance of gradual adaption to diets and the feeding of buffers/ neutralising agents, rumen modifiers and antibiotics will be presented.

## **1.2 The Australian dairy industry**

The Australian dairy industry is in a period of dynamic change. The number of dairy farms in Australia has halved in the last two decades from 21,994 herds milking on average 85 cows in 1979/1980 to 10,654 herds in 2002/2003 milking an average of 195 cows (Dairy Australia 2003). The state with the highest number of dairy farms and herds in 2002/2003 was Victoria, followed by New South Wales (NSW) and Queensland. The decrease in farm gate milk prices has encouraged larger, more efficient operations with average annual milk production per cow increasing from 2,848 L in 1979/1980 to 4,800 L in 2002/2003 (Dairy Australia 2003). The majority of farms remain family owned and operated, however, share farming is becoming more common with approximately 15% of enterprises using share farmers. Seventy percent of dairy cows in Australia are Holstein-Friesian. Other breeds represented include Jerseys, Friesian-Jersey cross, Illawarra Shorthorns, Guernseys, Brown Swiss and Ayreshires (Dairy Australia 2003).

Australian milk production remains strongly seasonal, reflecting the pasture-based nature of the industry. However, the need to increase milk production is reflected in increased feeding of supplementary grains and fodder (Dairy Australia 2003). The majority of supplementary fed herds are offered concentrate twice daily in the bail at milking. Some herds feed a total mixed ration (TMR) or partial mixed ration (PMR) where access to feeds and facilities allow.

### 1.3 Rumen ecology associated with rumen fermentation

The rumen forms an anaerobic environment that hosts bacteria, fungi and protozoa. The microbial ecosystem in the rumen is complex and is highly dependent on diet (Preston and Leng 1987). The extent to which protozoa and fungi contribute directly to the microbial protein available for protein supply to the ruminant is unclear, however, both make significant contributions in the breakdown of plant material. Bacteria provide the largest percentage of the biomass of microbial crude protein available to the animal (Hungate 1966; Preston and Leng 1987), making up over 50% of the total microbial population. Concentrations of bacteria in a healthy rumen have been recorded between  $10^9$  and  $10^{11}$  /ml (Hungate 1966; Preston and Leng 1987).

Bacteria can be compartmentalized into a number of groups including;

- i. bacteria free in the liquid medium (30% of total)
  - ii. bacteria attached to feed particles (70% of total)
  - iii. bacteria attached to the epithelial lining of rumen (<1% of total)
  - iv. bacteria attached to protozoa (mainly methanogens)
- (Preston and Leng 1987)

Hungate (1966) suggested that approximately 200 different species of bacteria could be isolated from rumen fluid, concentrations of these being dependant on diet. With the advent of molecular biology, some existing rumen species have been found to encompass a large amount of genetic variation with several strain groupings in the rumen possible (Stewart *et al.* 1997). The number of species of rumen bacteria therefore, remains unknown given the frequent reclassification of species. Most ruminal bacteria are cocci or short rods ranging from 0.4 to 1  $\mu\text{m}$  in diameter and 1 to 3  $\mu\text{m}$  long (Hungate 1966). In a ruminant fed a hay or forage ration, the majority of the bacteria are gram negative. As the concentration of grain fed increases, so do the numbers of gram positive bacteria (Hungate 1966; Latham *et al.* 1971).

In the past, rumen bacteria have been divided into eight major classes, according to the classification of Hungate (1966) and based on substrate affinity:

- i. cellulose digesters
- ii. starch digesters
- iii. hemicellulose digesters
- iv. sugar fermenters
- v. acid utilizers
- vi. proteolytic digesters
- vii. lipolytic bacteria
- viii. the rumen methanogens (Hungate 1966)

Although this classification is still commonly used, it does not recognise the complexity of the rumen ecology system. Many classes of bacteria exist together on different diets. Some bacteria are more predominant than others and any dietary shift can change the proportions of classes of bacteria present. Table 1.1 summarises the major bacteria present in the rumen, their substrates and fermentation products.

**Table 1.1** Classification of rumen bacteria (adapted from Russell and Rychlik 2001)

Major classification	Major substrates <sup>a</sup>	Fermentation products ( <i>in vitro</i> ) <sup>b</sup>	Sensitivity
<b>Cellulolytic</b>			
<i>Fibrobacter succinogenes</i>	CU	S, F, A	
<i>Ruminococcus albus</i>	CU, HC	A, F, E, H <sub>2</sub>	Monensin Low pH
<i>Ruminococcus flavefaciens</i>	CU, HC	S, F, A, H <sub>2</sub>	Monensin pH<6.1
<b>Amylo- and Dextrinolytic</b>			
<i>Ruminobacter amylophilus</i>	ST	S, F, A, E	
<i>Streptococcus bovis</i>	ST, SU	L, A, F, E	Virginiamycin
<i>Succinomonas amylolytica</i>	ST	S, A, P	
<i>Succinivibrio dextrinosolvans</i>	PC, DX, SU	S, A, F, L	
<b>Saccharolytic</b>			
<i>Prevotella ruminicola</i>	ST, PC, XY, SU	S, A, F, P NH <sub>3</sub>	

<i>Butyrivibrio fibrisolvens</i>	ST, CU, HC, PC, SU	B, F, A, H <sub>2</sub>	
<i>Selenomonas ruminantium</i>	ST, DX, SU, L, S	L, A, P, B, F, H <sub>2</sub> , NH <sub>3</sub>	
<i>Megasphaera elsdenii</i>	L, SU	P, A, B, V, Br, H <sub>2</sub> , NH <sub>3</sub>	pH<5.5
<b>Sugar fermenters</b>			
<i>Lactobacillus spp</i>	SU	L	Variable virginiamycin sensitivity
<b>Hydrogen Utilisers</b>			
<i>Methanobrevibacter ruminantium</i>	H <sub>2</sub> , CO <sub>2</sub> , F	CH <sub>4</sub>	
<i>Methanomicrobium mobile</i>	H <sub>2</sub> , CO <sub>2</sub> , F	CH <sub>4</sub>	

<sup>a</sup>CU, cellulose; HC, hemicellulose; DX, dextrins; SU, sugars; ST, starch; PC, pectin; XY, xylans; L, lactate.

<sup>b</sup>S, succinate; H<sub>2</sub>, hydrogen; F, formate; CO<sub>2</sub>, carbon dioxide; A, acetate; E, ethanol; B, butyrate; L, lactate; P, propionate; Br, branched-chain volatile fatty acids; V, valerate; NH<sub>3</sub>, ammonia; and CH<sub>4</sub>, methane.

### 1.3.1 *Fibrobacter succinogenes*

*F. succinogenes* (previously *Bacteroides succinogenes*), is one of the most widespread cellulolytic bacteria in the rumen, and accounted for around 20% of the bacteria recovered in one study from a cow fed wheat straw compared to other diets where it accounted for about 5% of all isolates (Bryant and Burkey 1953). The predominant substrates for *F. succinogenes* are acids derived from cellulose, cellobiose and glucose. However, some strains have also been known to use starch, pectin, maltose and lactose. Major fermentation products in pure culture are acetate and succinate with some strains also producing formate, propionate and isovalerate (Hungate 1966).

### 1.3.2 *Prevotella ruminicola*

*P. ruminicola*, a gram negative rod or coccobacillus, is one of the most numerous groups of rumen bacteria found on many different diets, and accounts for up to 60% of isolates in cattle fed silage in some studies (Stewart *et al.* 1997; Van Gylswyk 1990). There is wide genetic divergence between strains in this group (Hudman and Gregg 1989), hence the change in division between groups. *P. ruminicola* cannot degrade cellulose. Probable roles of the organism are to degrade and utilise starch and plant cell wall polysaccharides,

for example xylans and pectins (Stewart *et al.* 1997). Major substrates on pure culture include cellobiose, glucose, fructose, galactose, mannose and lactose and fermentation products include acetate, succinate, formate, propionate and isobutyrate (Hungate 1966; Stewart *et al.* 1997). *P. ruminicola* is also probably one of the most important  $\text{NH}_3$  producers in the rumen (Dehority 2003).

### 1.3.3 *Streptococcus bovis*

*S. bovis*, a gram positive, non-motile coccoid is capable of very rapid growth when diet changes suddenly from hay to cereal grain (McAllister *et al.* 1990). Normally, when rumen pH declines, bacteria can accumulate toxic concentrations of anions such as acetate. However, under these conditions as extracellular pH declines, intracellular pH of *S. bovis* is also reduced, avoiding this outcome (Russell 1991a; Russell 1991b). There are relatively few strains of *S. bovis* found in the rumen with good homogeneity within the genus (Al Jassim and Rowe 1999; Nelms *et al.* 1995). The major substrates in pure culture include cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, raffinose, starch and sucrose with isolates very sensitive to virginiamycin (Al Jassim and Rowe 1999). *S. bovis* was unable to tolerate concentrations of 2  $\mu\text{g/ml}$  virginiamycin in a broth of Basal medium 10 with glucose (0.5%). Although *S. bovis* is most often discussed in relation to starch-based diets, it is able to grow on water-soluble cellodextrins from crystalline cellulose and therefore may still be present on forage based diets (Hungate 1966; Russell and Hino 1985). The major fermentation product of *S. bovis* is L-lactate, although some strains also produce formate and acetate (Al Jassim and Rowe 1999; Stewart *et al.* 1997).

### 1.3.4 *Selenomonas ruminantium*

*S. ruminantium*, a gram negative rod, is also found in high numbers in the rumen of animals fed cereal grains, constituting 22 to 51% of the total count in steers fed cracked corn and urea (Caldwell and Bryant 1966; Hungate 1966). Major substrates in pure culture include maltose, cellobiose, D-xylose, L-arabinose, glucose, fructose, galactose, mannose, lactose and mannitol. Some strains also utilise D-L lactate (Henderson 1975). During growth in high glucose environments, D and L - lactate is the major fermentation

product, but this is replaced by acetate and propionate in low glucose environments (Al Jassim and Rowe 1999; Melville *et al.* 1988a; Melville *et al.* 1988b). Those strains able to utilize lactate and grown on glucose can convert accumulated lactate to acetate and propionate. Virginiamycin does not affect growth of *S. ruminantium* in the rumen, with isolates being resistant to virginiamycin concentrations of 8 µg/ml (Al Jassim and Rowe 1999).

### 1.3.5 *Lactobacilli species*

Sugar fermenters are very important as many forage plants contain appreciable concentrations of sugars during rapid growth. This factor is becoming more important with continued development of higher quality pasture varieties by seed companies, maximizing growth rates in pastures. Thomas (1960) found that ryegrass, for example, contains 1% glucose, 1% fructose, 9% sucrose and 19% fructan. Recent research indicates that new tetraploid ryegrasses may contain approximately 20% sugars with NIRS analysis (Bramley *et al.*, unpublished). A number of species of *Lactobacillus* are important sugar fermenters and are present in the rumen. Lactobacilli are common in the rumen of young animals on milk rations and also when high grain diets are fed to older animals. Often, lactobacilli are present in the rumen as normal commensals, including oxygen-tolerant species such as *L. acidophilus*, *L. casei*, *L. fermentum*, *L. plantarum*, *L. buchneri*, *L. cellobiose*, *L. helveticus* and *L. salivarius* (Hungate 1966). Two gram positive rod anaerobic species, *L. ruminus* and *L. vitulinus* were first isolated from the rumen and produce predominantly L-lactic acid and D-lactic acid respectively (Al Jassim and Rowe 1999; Stewart *et al.* 1997). *L. vitulinus* in pure culture ferments cellobiose, fructose, glucose, mannose, galactose, maltose, raffinose, melibiose, lactose, sucrose, salicin, amygdalin and aesculin, but are unable to utilize starch or xylose (Al Jassim and Rowe 1999; Sharpe *et al.* 1973). Al Jassim *et al.* (1999) isolated 5 strains of *L. vitulinus* from the rumen, four of which produced D-lactate and the fifth which produced both D and L- lactate in equal proportions. *L. ruminus* ferments the same substrates as *L. vitulinus* with the exception that lactose was only fermented by some strains (Al Jassim and Rowe 1999; Sharpe *et al.* 1973).

### 1.3.6 *Megasphaera elsdenii*

*M. elsdenii* is normally found in the rumen in conjunction with starch fermenting bacteria such as *Streptococcus bovis* when rumen conditions precipitate the formation of lactate (Hungate 1966), taking five to seven days to establish rumen populations of  $5.5 \times 10^{11}$  in concentration after introduction of grain to the diet (Klieve *et al.* 2003). Major substrates in pure culture include lactate, glucose and fructose and the fermentation products produced vary according to substrate used. Lactate is mainly fermented to butyrate, propionate, isobutyrate and valerate (Stewart *et al.* 1997). *M. elsdenii* is important in the production of branched chain volatile fatty acids and ammonia, essential as growth factors for other bacteria in the production of microbial protein (Wallace 1986).

The identification and quantification of rumen bacterial population on various diets at varying rumen pH values is only one step in the understanding of sub-clinical acidosis in predominantly pasture fed animals. Rumen function, including carbohydrate and nitrogen metabolism and fibre digestion and metabolism is also critical in this complex problem.

## 1.4 Carbohydrate metabolism and requirements

### 1.4.1 Hydrolysis of carbohydrates

The majority of carbohydrates in ruminant feed are polymers; cellulose, hemicellulose, pectin and starch. The first step in the fermentation of dietary carbohydrates is the breakdown of polysaccharides to oligosaccharides and disaccharides (Baldwin and Allison 1983). Bacteria achieve this hydrolysis of polysaccharides by secreting extracellular enzymes.

Cellulose and hemicellulose are the most abundant of the plant carbohydrates. Cellulose can be present in the rumen as both an amorphous loosely associated form and a highly crystalline form which is much more difficult to degrade (Prins 1977). Hydrolysis to the disaccharide, cellobiose occurs by the secretion of an extracellular cellulase. *Ruminococcus albus* and *R. flavefaciens* both have the ability of producing extracellular cellulase in response to the presence of cellulose (Baldwin and Allison 1983). The

cellulase produced by *R. albus* is able to hydrolyse amorphous cellulose but is relatively inefficient against the crystalline form, which is able to be degraded by *R. flavefaciens*. This explains the increased concentration of *F. succinogenes* on diets high in crystalline cellulose such as wheat straw (Baldwin and Allison 1983). Hemicellulose, forming xylobiose is hydrolysed to xylose by the intracellular enzyme xylosidase. Xylobiose can also be used by some non-cellulolytic bacteria also present in rumen fluid. Both cellulose and hemicellulose are fermented slowly in the rumen.

Pectins are an important group of structural carbohydrates found in the plant cell wall. These are fermented much more rapidly than cellulose or hemicellulose. Pectins are hydrolysed by *F. succinogenes*, *P. ruminicola*, *B. fibrisolvans* and *S. bovis* by secreting methylesterase and polygalacturonidase.

Starch (amylose and amylopectin), a non-structural carbohydrate, is hydrolysed by microbial amylases to maltotriose, maltose and some glucose (Baldwin and Allison 1983; van Houtert 1993). Starches and other storage carbohydrates are present in higher concentrations in grains and other concentrated feed sources than in fibrous feeds. The fermentation rate of starch depends on the type of starch being degraded and on physical form. Opatpatanakit *et al.* (1994) found that grains were fermented in the order from most rapid to least rapid of wheat > triticale > oats > barley > maize > rice, sorghum. Processing of grain also increases efficiency of starch fermentation and microbial degradation of starch in the rumen (Hale 1973; Theurer 1986). Heating cereal grains is known to break the crystalline structure of cereal starches and increase rates of hydrolysis and fermentation (Theurer 1986; Zinn 1993). Zinn (1993) found that steam-rolling barley as opposed to dry rolling increased the net energy for maintenance value of barley by 2.8 to 7%. This is probably not economical in many situations, however the routine process of cracking, rolling and crushing cereal grains is commonly practiced in the dairy industry to increase availability and rumen degradability resulting in increased milk yield of cows (Yang *et al.* 2001).

The disaccharides, i.e. cellobiose, maltose, xylobiose and glucuronides are broken down into their constituent monosaccharides; xylose and hexoses. Most microbes ferment monosaccharides (sugars) through anaerobic glycolysis, a key pathway in bacteria.

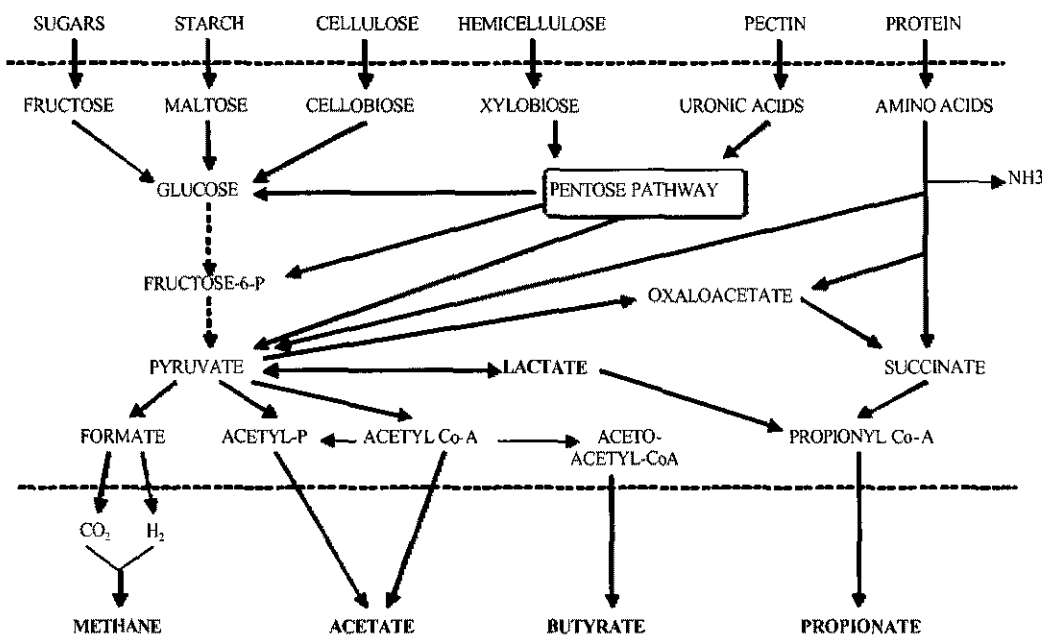
#### 1.4.2 *Fermentation of sugars in the rumen*

Fermentation of sugars is the primary source of energy for the formation of the high energy phosphate bonds of ATP that are utilised by rumen protozoa, bacteria and fungi for maintenance and growth (Baldwin and Allison 1983). The main end products from this fermentation process are methane gas, carbon dioxide and volatile fatty acids (Figure 1.1). The volatile fatty acids present in highest concentration in the healthy rumen are the three volatile fatty acids associated with carbohydrate fermentation; acetic acid, propionic acid and butyric acid, although concentrations vary with the nature of the carbohydrate source in the diet (Hussein *et al.* 1991). Annison (1954a) demonstrated the presence of isobutyric, isovaleric and 2-methylbutyric acid, in the rumen of sheep maintained on various diets. Traces of formic acid were occasionally observed (Annison 1954b) constituting a maximum of only 5% of the total volatile fatty acids present in the rumen (Gray *et al.* 1951). D and L- lactate can also be present in the healthy rumen in small quantities (Kuncharapu *et al.* 2000). Kuncharapu *et al.* (2000) reported that sheep maintained on lucerne pellets had concentrations of only 1.54 +/- 0.59 mM D-lactate and 0.78 +/- 0.15 mM L-lactate present in the rumen. However, rumen concentrations of D and L-lactate increase with the increase in concentration of starch in the diet (Al Jassim and Rowe 1999; Lee *et al.* 1982).

The biochemical pathways involved in carbohydrate fermentation in the rumen have been extensively reviewed (Baldwin and Allison 1983; Czerkawski 1986; Hungate 1966; van Houtert 1993). The primary pathway (Figure 1.1) fermenting fructose, maltose and cellobiose to hexose, glucose and then pyruvate is the Emden-Meyerhof-Parnas pathway of anaerobic glycolysis (Baldwin and Allison 1983). The net products from this pathway are 2 ATP/ mole of hexose fermented to pyruvate, 2 pyruvate and 2 NAD<sup>+</sup>. There is a need for an electron sink product in this pathway to regenerate NAD<sup>+</sup> from NADH in order to maintain ATP production. Electron sink products are critical for the formation of

ATP, which is used by bacteria for maintenance and growth. Electron sink products include propionate, methane, butyrate, lactate, ethanol and hydrogen; the two most important are propionate and methane, where there seems to be an inverse relationship between the two products (Wolin 1960). The other pathway is the pentose pathway converting xylobiose and uronic acids. This pathway feeds into the pathway of anaerobic glycolysis with the net ATP yield being 1.67 /mole of pentose used when forming an electron sink product.

**Figure 1.1** Fermentation of carbohydrates in the rumen (adapted from Baldwin and Allison 1983; Hungate 1966; Lean 1987; van Houtert 1993)



The fate of pyruvate and NADH formed in the rumen depend greatly on the substrates available in the rumen and therefore the microbes involved. The most common pathway of pyruvate conversion to propionate is the succinate pathway where pyruvate is converted to oxaloacetate, succinate and then propionate. This pathway is cobalt and vitamin B12 dependent. The minor of the two pathways is the acrylate pathway converting pyruvate to lactate and then propionate via a number of intermediary steps. The acrylate pathway is used by *M. elsdenii* and *P. ruminicola* (Baldwin and Allison 1983). Pyruvate can also be converted to acetate through acetyl CoA with the ATP yield

being 1 ATP /mole and is used by *Clostridia*, *M. elsdenii* and *V. parvula*. Butyrate is converted from pyruvate through the additional step to aceto-acetyl CoA, regenerating  $\text{NAD}^+$  from NADH, therefore resulting in an electron sink product. Formate is effectively a by-product of the conversion of pyruvate to acetyl-CoA when producing acetate. The formate released is converted to  $\text{CO}_2$  and  $\text{H}_2$ , which are converted to methane as a waste product.

In ruminants fed predominantly roughage diets high in cellulose, intermediate in soluble sugars and low in starch, cellulolytic and saccharolytic bacteria predominate. Cellulose is hydrolysed primarily to acetate and methane via anaerobic glycolysis. Methane is the electron sink product. Propionate amounts produced on cellulose predominant diets are 15 to 20% of the total VFA pool. When cereal grains containing high levels of starch are fed in increasing amounts, amylolytic bacteria fermenting this substrate produce a higher proportion of propionate (35 to 45% of total VFA pool) (Orskov 1986). Cows fed 90% concentrate had lower ruminal pH values and acetate to propionate ratios (4.1) than did cows fed 100% hay diets (acetate: propionate = 2.2) (Lana *et al.* 1998; Russell 1998). One explanation for the lower acetate to propionate ratio in concentrate fed cattle has been to ascribe this to substrate preferences of rumen bacteria. Favorable conditions for amylolytic bacteria such as *Selenomonas ruminantium*, *Streptococcus bovis*, *Succinomonas amylolytica* and *Prevotella ruminicola* result in production of propionate as well as acetate. Cellulolytic bacteria in pure culture such as *Ruminococcus albus* produce large amounts of acetate. However, *Fibrobacter succinogenes* and *Ruminococcus flavefaciens*, also cellulolytic bacteria, produce mainly succinate, an intermediate in the production of propionate (Hungate 1966). More plausible, therefore is the theory proposed by Lana *et al.* (1998) suggesting that the ratio of ruminal acetate to propionate was substantially influenced by the capacity of the bacteria to produce methane *in vitro*. Lana *et al.* (1998) found that cattle with low acetate to propionate ratios also had low ruminal pH values, contributing to reduced methanogenesis *in vitro*. In comparison to propionate and acetate, butyrate is quantitatively the least important of the three major VFAs in the rumen fluid of animals fed roughages, usually contributing only

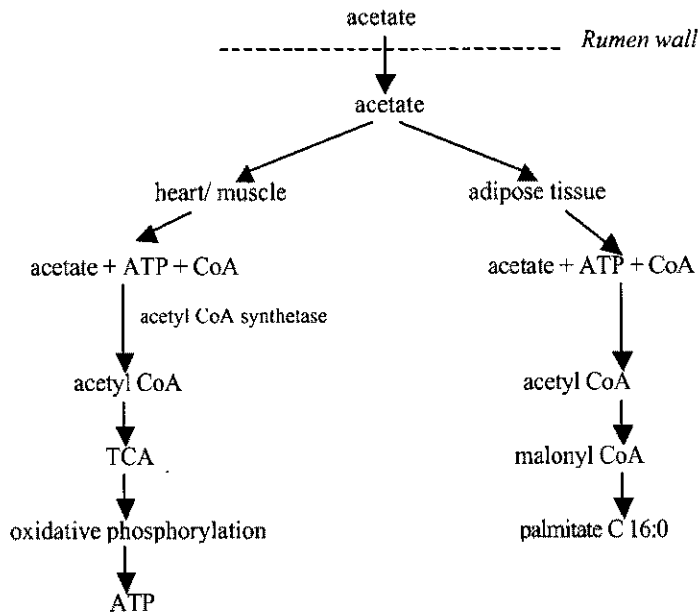
0.05 to 0.1 M to the total VFA pool (van Houtert 1993). The longer-chain volatile fatty acids are present in smaller quantities.

#### ***1.4.3 Absorption and metabolism of volatile fatty acids in the ruminant***

Acetic acid, propionic acid and butyric acid are absorbed across the rumen wall and are the major energy supply for ruminants. A number of factors influence the relative rates of absorption of the three major VFAs. These include the total concentration of and the ratios of the VFAs present, rumen pH and rate of blood flow through the rumen epithelium (Annison *et al.* 1957; Stevens 1970). Unlike non-ruminants, glucose is not absorbed from the rumen or small intestine of ruminants and they are reliant on gluconeogenesis, using predominantly propionate for energy, even in a well-fed animal. The concentrations of the three major VFAs in blood are low in comparison with rumen concentrations, indicating rapid utilisation by the animal (van Houtert 1993).

Acetate is absorbed across the rumen wall as acetic acid and is converted back to the salt acetate in the portal blood. It is first transported via the portal blood to the liver where it is the only VFA not to be metabolised to a large extent, possibly due to the low activity of acetyl-CoA synthetase under physiological conditions (Smith 1971). Both propionate and butyrate have been found to suppress activity of acetyl-CoA synthetase (Ash and Baird 1973). Pethick *et al.* (1981) found increased acetate uptake by the liver in sheep fed forage-based diets with lower concentrations of propionate present. Acetate predominantly supplies energy to the heart and muscle and can be used by adipose tissue to form palmitic acid. When acetate is used by the heart and muscle it is converted to acetyl CoA by acetyl CoA synthetase in the mitochondria and is catabolised via the TCA cycle and oxidative phosphorylation to form ATP (van Houtert 1993) (Figure 1.2). When acetate is involved in fat synthesis, it is converted to acetyl CoA in the cytosol by acetyl CoA synthetase and malonyl CoA.

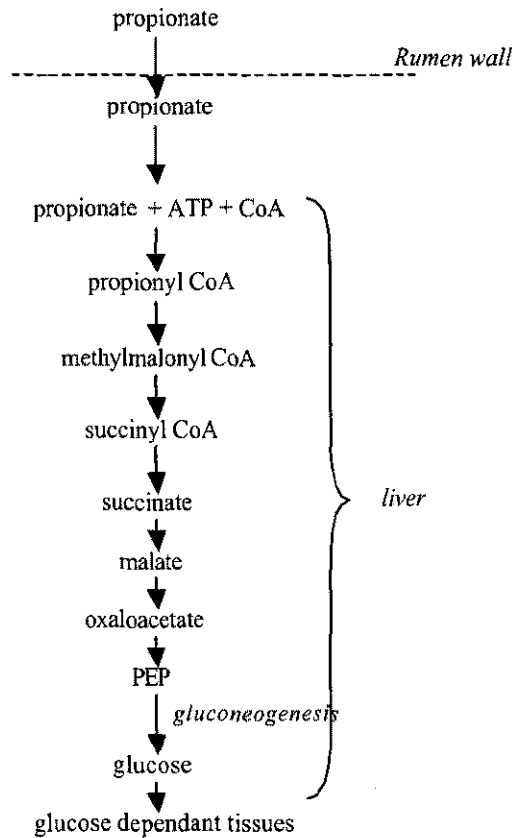
Figure 1.2. The fate of acetate in the ruminant



Propionate is absorbed across the rumen wall almost in its entirety with a small, insignificant portion (less than 5%) converted in the rumen wall to lactate by rumen epithelium (Weigand *et al.* 1972). Ash and Baird (1973) found that butyrate strongly inhibits the metabolism of propionate by rumen epithelium *in vitro* through the inhibition of propionyl-CoA synthetase. *In vivo* studies have shown that 30 to 50% of all propionate is metabolised in the rumen wall (Weekes and Webster 1975), however this may be less in cattle than sheep. In roughage-fed animals, a significant proportion of ruminal propionate reaches the liver via the portal vein where it is converted to glucose by gluconeogenesis in the mitochondria and cytoplasm of cells (Figure 1.3). Glucose, essential in cellular metabolism, is then transported to the glucose dependent tissues of the body; the brain, blood cells, spermatozoa, mammary gland, uterus and foetus, via the hepatic vein. It is also used in muscle metabolism and fat synthesis. Small amounts of propionate not metabolized in the liver in roughage-fed ruminants are used by various peripheral tissues, particularly the mammary gland. High propionate concentrations in the portal blood exceed the liver's capacity to convert propionate to glucose. This stimulates insulin production and propionate is oxidized to fatty acids via the TCA cycle. The

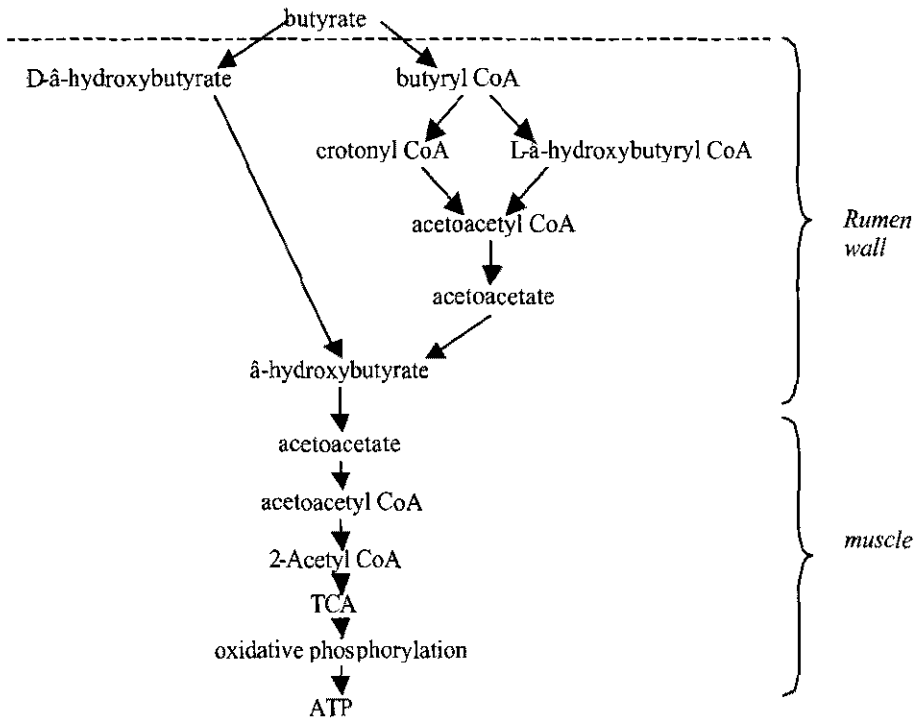
resulting increased uptake of nutrients by the tissues and reduction in lipolysis reduces milk yield, particularly milk fat (Orskov 1986).

**Figure 1.3.** The fate of propionate in the ruminant



Butyrate is not absorbed directly across the rumen wall as in the case of acetate and propionate. Although large amounts of butyrate were shown to occur in the rumen on some diets, a low concentration or complete absence of butyrate from the portal blood occurs (Annison *et al.* 1957). Ketone body concentrations in the portal blood have also been shown to be significantly higher than in arterial blood. As butyrate is absorbed across the rumen wall, it is converted to the ketone bodies  $\beta$ -hydroxy-butyrate and to a lesser extent acetoacetate by rumen epithelial cells (Annison *et al.* 1957) (Figure 1.4). In this form, it passes through the liver to the muscle and heart tissue where it is converted in the mitochondria and cytoplasm to aceto-acetylCoA. It is then catabolised via the TCA cycle and oxidative phosphorylation to form ATP for energy.

**Figure 1.4.** The fate of butyrate in the ruminant



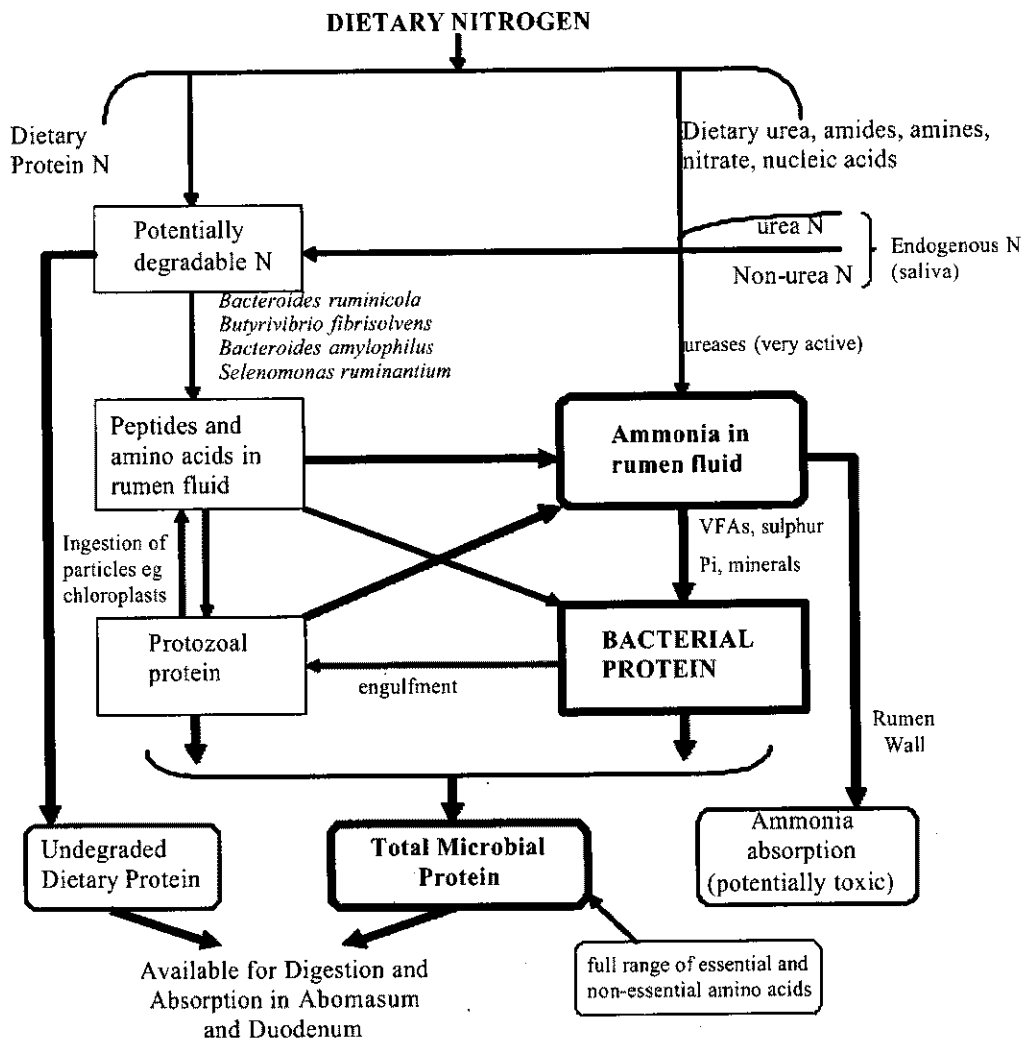
In the dairy cow, all three major volatile fatty acids (VFAs) play an important role in the production of milk volume, protein and fat. Acetate and butyrate are utilized by the mammary cells for energy and the production of milk fat, particularly triglycerides. Propionate, converted to glucose, is utilised by the milk producing cells to give lactose, milk triglycerides and energy for cellular activity. Glucose supply appears to be a major limiting factor for maximal milk production. A decrease in the uptake of acetate and  $\beta$ -hydroxybutyrate and an increase in the uptake of propionate may lead to a decrease in milk fat production and an increase in the deposition of fatty acids in adipose tissue (Rook 1976).

## 1.5 Nitrogen metabolism in the rumen

### 1.5.1 *Proteolysis and deamination of dietary protein*

The primary nitrogen containing compounds in ruminant diets are proteins, amides, amines, amino acids, nitrates, nucleic acids and urea. Proteins make up 60 to 80% of total plant nitrogen and can be divided into two categories; soluble and insoluble protein (Van Soest 1994). Urea, amides, amines, nitrate and nucleic acids are considered non-protein nitrogen (NPN). Figure 1.5 summarises the major nitrogen transactions and pools within the rumen and the important flows into and out of these pools and the rumen (Leng and Nolan 1984).

**Figure 1.5.** Nitrogen metabolism in the rumen (adapted from Leng and Nolan 1984)



Protein degradation is dependant both on factors inherent to the feed in question (susceptibility to microbial attack, solubilisation, enzymatic degradation) and the rate of flow of feed through the rumen (Lean 1987). For example, hays, silage and ryegrass and barley have a protein degradability of 71 to 90% in the rumen. Legumes, maize and cooked soyabean meal protein degradability is 51 to 70% in the rumen and lucerne chaff and cottonseed meal protein is 31 to 50% degradable (Webster 1980). Soluble dietary protein is extensively broken down in the rumen. Initially, protein chains are degraded to peptides and amino acids by proteolysis. Proteolysis is the hydrolytic degradation of the peptide bonds holding the peptides and amino acids in chains (Shirley 1986). Common bacteria possessing proteolytic activity through the use of proteases include *P. ruminicola*, *B. fibrisolvens*, *B. amylophilus* and *S. ruminantium* (Hungate 1966). Peptides and amino acids play the key role in nitrogen metabolism in the rumen, with between 20 and 50% of the total nitrogen in bacteria probably originating from this source (Nolan 1975). Peptides are often incorporated into the bacterial cell on hydrolysis of proteins with amino acids being released into the rumen fluid (Baldwin and Allison 1983).

Amino acids are then either incorporated into bacterial cells as bacterial protein, or degraded to branched chain volatile fatty acids, ammonia, carbon dioxide, methane and some heat of fermentation in deamination (Baldwin and Allison 1983; Shirley 1986). Annison (1956) found the concentration of  $\alpha$ -amino nitrogen (nitrogen arising from amino acids) in sheep does not normally rise above approximately 0.1mg/ml. Annison (1956) also showed that rapid increases in the  $\alpha$ -amino nitrogen and ammonia concentrations above resting concentration are followed by a sharp fall in the  $\alpha$ -amino nitrogen concentration 2 to 3 hours after cessation of feeding. These results indicate the rapid utilization or degradation of  $\alpha$ -amino nitrogen in the rumen. No evidence was found for the absorption of  $\alpha$ -amino nitrogen from the rumen to the portal system, indicating utilisation in the rumen. The optimal pH for both proteolysis and deamination is between 6 and 7 (Lewis and Emery 1962). Under most nutritional conditions in the rumen, the pH will allow extensive breakdown of dietary protein. However, studies have shown lower rumen ammonia concentrations are associated with higher rates of non-

structural carbohydrates fed (Lana *et al.* 1998; MacGregor *et al.* 1983). Lana *et al.* (1998) found that when concentrate percentage was increased in the diet, deamination rates decreased. A decrease in rumen pH also reduced deamination *in vitro* in forage fed cattle. However, cattle fed 90% concentrate were not affected by low rumen pH, demonstrating differences in microbial populations on the two diets (Lana *et al.* 1998). The addition of monensin (350 mg/cow/day) has also been shown to decrease ruminal ammonia production by more than 30% by inhibiting amino acid-fermenting bacteria (Yang and Russell 1993).

The rumen ammonia pool is of great importance in the metabolism of nitrogen. Approximately 80% of rumen isolates can grow with ammonia as their sole nitrogen source (Bryant and Robinson 1963), leading to the assumption that most of the nitrogen in amino acids passes through the ammonia pool prior to incorporation into microbial protein. Notwithstanding this, significantly more than 20% of amino acids were directly converted into microbial protein, resulting in immediate benefit in microbial growth (Maeng and Baldwin 1976). The most important ammonia-producing bacteria appear to be *P. ruminicola*, *S. ruminantium*, *M. elsdenii* and some strains of *B. fibrisolvens* (Allison 1970). Sources of ammonia in the rumen ammonia pool include; degradation of dietary protein and hydrolysis of dietary non-protein nitrogen, hydrolysis of urea recycled to the rumen and degradation of microbial protoplasm (Owens and Bergen 1983). Fates of ruminal ammonia include; incorporation into microbial cells, absorption through the rumen wall and passing into the omasum. The ammonia concentration in the rumen is relatively low and turns over rapidly, though concentrations can vary over a wide range, for example from 0.01 to 8 mM in sheep with no toxic effects being observed (Annison *et al.* 1954).

Most ruminal ammonia is incorporated into microbial protein. Many cellulolytic bacteria require ammonia for protein synthesis and growth (National Research Council 2001). The requirement for ammonia is directly related to substrate availability, fermentation rate, microbial mass and yield (National Research Council 2001). Ammonia concentrations of 11.8 to 12.9 mmol ammonial nitrogen/ litre of rumen fluid are needed

in the rumen to maximise the rate of barley dry matter fermentation (Mehrez *et al.* 1977). Lower concentrations of ammonial nitrogen will maximise fermentation rates on highly fibrous diets *in vitro* (Orskov 1982; Satter and Slyter 1974).

The second fate of ammonia from the rumen ammonia pool is absorption across the rumen wall into the blood stream, with the rate being dependant on the un-ionised concentration in rumen fluid. Absorption occurs to a greater extent in diets high in nitrogen and is optimal at rumen pH 7. When rumen pH is < 6, absorption is relatively slow (Lean 1987). The mechanism of absorption is a two-way diffusion, the direction and extent of which depends on the quantity of ammonia already present in the rumen. When there is a low concentration of ammonia in the rumen, for example 0.01 mM, the normal process of incorporation of ammonia into microbial cells is saturated and the urea present in the blood flows across the rumen wall to the rumen (Preston and Leng 1987). Excess ammonia diffuses across the rumino-reticulo-wall into the blood (Preston and Leng 1987). In a 40 kg sheep receiving 800 g of hay and 50 g of casein per day, rumen ammonia can average approximately 40 mM with a portal ammonia concentration of around 0.38 mM (Lewis *et al.* 1957). Ammonia, which is toxic, under normal circumstances, does not remain in the portal blood for long before the liver converts it to urea, a safer and less toxic compound. Moreover, ammonia is usually absent from the peripheral blood of animals receiving diets giving rise to appreciable amounts of ammonia, indicating the efficiency of the liver in removing ammonia from the portal blood (Lewis *et al.* 1957). In fact, ammonia appears in the peripheral blood only when the rumen concentration exceeds 55 to 60 mM (Lewis *et al.* 1957). At these ruminal ammonia concentrations, the urea cycle, the pathway converting ammonia to urea, is saturated. If too much ammonia is absorbed across the rumen wall at any point in time, the animal may experience symptoms associated with ammonia toxicity, often resulting in death if left untreated. Toxic signs appear when the concentration of ammonia in the peripheral blood exceeds 0.6 to 0.9 mM (Lewis *et al.* 1957), ruminal ammonia concentrations are above 59 mM and ruminal pH is above 8 (Owens and Bergen 1983). Ammonia toxicity is treated by acidification, or removal of the rumen contents (Owens and Bergen 1983).

The third fate of ammonia is to become trapped in rumen digesta and pass out of the rumen to the small intestine. In this case, ammonia is not absorbed in the small intestine, but passes out of the body via the faeces and is lost as a nitrogen source (Preston and Leng 1987).

Apart from the nitrogen present in the rumen in the peptide and amino acid and ammonia pools, significant amounts are also present in bacterial and protozoal biomass. Protozoa engulf large numbers of bacteria (Kurihara *et al.* 1967) and ammonia is excreted as a by-product. Rumen ammonia concentrations have been shown to be higher in sheep with rumen protozoa than without on the same diet (Klopfenstein *et al.* 1966). Only a small percentage of the total protozoal population appears to pass from the rumen to the abomasum and small intestine to be degraded and used as protein (Leng and Nolan 1984).

The combination of bacterial and protozoal protein passing out of the rumen makes up the total microbial protein pool in the rumen available for digestion and absorption in the abomasum and duodenum. The other dietary protein also available is the undegraded dietary protein passing through the rumen.

### **1.5.2 Undegradable dietary protein (bypass protein)**

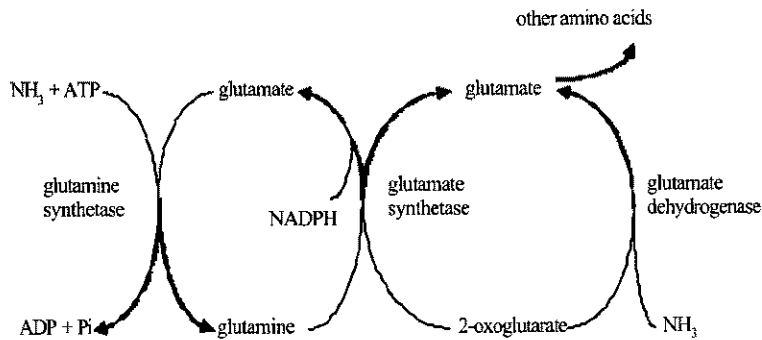
Undegradable (insoluble or 'bypass' protein) dietary protein (UDP) passes through the rumen and becomes available in the abomasums and duodenum for digestion and absorption. UDP normally constitutes 20 to 30% of the dietary crude protein (CP), although some feeds contain more UDP than others. For example, fish meal contains 55% UDP (Hussein *et al.* 1991) whereas protein from barley or wheat is 80 to 90% rumen degraded. Cottonseed meal (41% CP) is between 50 and 60% degradable (National Research Council 2001). The ability to feed UDP is an important production tool, however it needs to be fed in relation to energy intake. If cows are fed additional bypass protein in the diet when in a state of negative energy balance, further weight loss will occur (Lean 1987).

### 1.5.3 Non-protein nitrogen metabolism

A wide variety of non-protein nitrogen (NPN) substances are present in plants, including amino acids, peptides, amides, nitrate, purines, choline and betaine (Allison 1970). The importance of nitrates in impairing production and health of cattle is unknown. With increasing feed costs, the importance of NPN sources as a cheap source of nitrogen is becoming more important. Non-protein nitrogen sources are converted in the rumen to ammonia. Urea, probably the most important source of NPN, is hydrolysed to ammonia through the enzyme urease, excreted by some microbes (Allison 1970). Hydrolysis of urea to ammonia is rapid, often faster than utilization of ammonia, leading to potentially toxic ammonia concentrations in the rumen. It is, therefore, important to consider feeding rapidly fermentable carbohydrate sources with urea to encourage increased microbial growth.

The main disadvantage with NPN such as urea is the lack of amino acids. Rumen bacteria however, have the ability to synthesise their own essential amino acids from both non-essential amino acids and non-protein nitrogen sources, an important advantage. This being the case, rumen bacteria can survive on NPN sources such as urea while still getting their full complement of amino acids (Appendix, Table 1). Loosli *et al.* (1949) fed sheep a purified diet containing urea as the sole source of nitrogen and showed that the microbial biomass contained all the essential amino acids needed by non-ruminants. However, a continuous supply of fermentable carbohydrate, ammonia, peptides, amino acids and other nutrients is needed to promote efficient utilization of ATP for microbial protein yield (Baldwin and Allison 1983). Amino acids can be synthesised by two processes. At high ammonia concentrations, nitrogen assimilation occurs via a process involving glutamate dehydrogenase. When ammonia concentrations are low, ammonia is fixed in a two-step process involving glutamine synthetase and glutamate synthase. Figure 1.6 shows the synthesis of glutamate and the other essential amino acids from ammonia.

**Figure 1.6.** Synthesis of amino acids in the rumen from ammonia (Leng and Nolan 1984)



#### 1.5.4 Nitrogen recycling and the urea cycle

Nitrogen recycling to the rumen is a characteristic unique to ruminant animals that serves to augment diets low in nitrogen (Owens and Bergen 1983). Movement of urea from blood into the rumen and conservation of urea in the body by reduced excretion of urea in the urine are both mechanisms of recycling nitrogen for use by microbes on low nitrogen diets (Preston and Leng 1987). Between 23 and 92% of plasma urea is recycled to various portions of the digestive tract (Kennedy and Milligan 1980). While plasma urea appears to diffuse readily into the rumen when the concentration of ammonia in the rumen is low, it does so less readily as the concentration increases and ceases when there is about 12 mmol of ammonial nitrogen/L of rumen fluid (Kennedy and Milligan 1980). A maximum of 6 g nitrogen/day is recycled to the rumen in sheep and 24 g/day in cattle (Houpt 1970). The quantity of nitrogen recycled to the rumen can be negatively related to the ruminal ammonia concentration and positively related to plasma urea concentration and to organic matter digestion in the rumen (Owens and Bergen 1983). In studies with sheep on low quality roughage diets, amounts of urea from blood plasma entering the rumen were between 0.5 and 2.3 g nitrogen/day (Nolan 1975). Kennedy (1980) found that the rates of transfer of urea to the rumen were 11 to 14 g nitrogen/day for Hereford steers fed low quality hay. Upon supplementation of this diet with sucrose, the transfer of urea across the rumen wall increased to 23 g nitrogen/day. There has been conflicting evidence as to how much of the nitrogen is transferred via the salivary route in comparison to through the rumen wall. The transfer of ammonia across the rumen wall is thought to be an attenuated process, being controlled by a regulatory gene expression

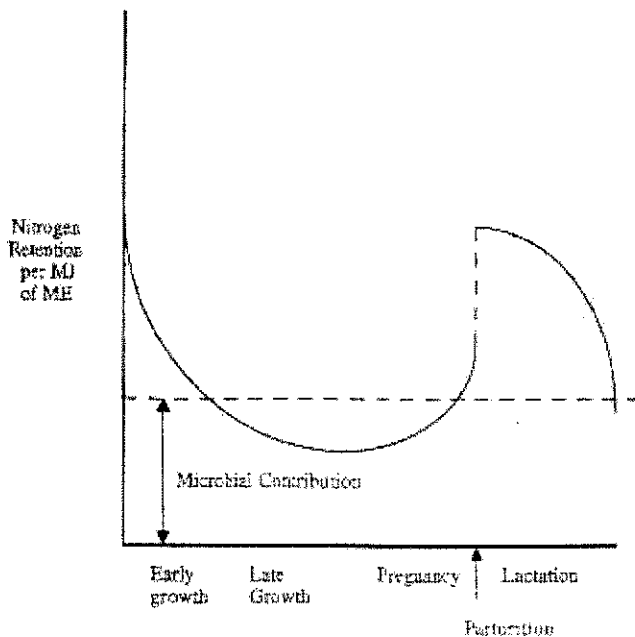
mechanism (Houpt 1970). Liberated ammonia rapidly diffuses through the ruminal epithelium and becomes trapped by conversion to ammonium at normal ruminal pH. Recycling is reduced at high ammonia concentrations by inhibiting urease in the rumen wall or decreasing the ammonia diffusion gradient. As a consequence, although the influx of nitrogen may vary markedly as a result of diet, this system stabilises the supply to microorganisms and in effect saves some nitrogen for when the supply through the diet is low.

It is still not clear whether urea recycling is controlled to the animal's advantage. Although it appears that entry of urea to the rumen from the plasma and saliva is subject to some control (Allen and Miller 1976), microbial growth and feed digestibility can still be limited by ammonia deficiency (Campling *et al.* 1962).

#### ***1.5.5 Protein requirements in the dairy cow***

Microbial CP provides a constant source of protein to a ruminant and can easily meet the requirements for maintenance. However, in a dairy cow, the demands for protein are variable and depend on the physiological status of the animal, as well as on genetic, age and nutritional differences. A lactating cow, for example, has greater requirements for protein than an animal at maintenance (Figure 1.7). Lactating dairy cows fed diets with 13.1 to 13.5% total CP had significantly lower milk yields than those fed diets with 17% CP in the total diet ( $P = 0.001$ ) (Frank and Swensson 2002). At parturition and during lactation, the flow of microbial protein cannot supply sufficient total protein to meet all requirements (Figure 1.7). Microbial contribution to available protein is at a maximum when total crude protein in the diet is approximately 14%. The only way to overcome this protein deficit is to increase the intake of undegraded dietary protein (Orskov 1982) (discussed in 1.5.2). When cows grazing highly productive rye-clover pasture were supplemented with 1 kg of UDP, milk production increased by 13% (Sharp 1983).

**Figure 1.7.** Protein requirements of ruminants at various stages of production (adapted from Orskov *et al.* 1981).



## 1.6 Fibre definition and requirements

### 1.6.1 Fibre definition

Fibre can be defined nutritionally as the slowly digestible or indigestible fraction of feeds that occupies space in the gastro-intestinal tract of animals (Mertens 1997). These consist of the lignin, cellulose and hemicellulose fractions of the plant. There have been numerous attempts in the past to classify nutritional components of feed, thereby establishing quantities in various feeds and requirements by ruminants for those various components. The system most recently embraced for describing the energy content of plant material (neutral detergent fibre (NDF) method) was initially developed by Van Soest and Wine (1967). The NDF method used sodium sulphite to remove contaminating proteins from NDF, however it was found that this method did not adequately remove starch from grains and corn silage (National Research Council 2001). Modifications

corrected this problem, but sulfite was removed from the procedure because of concern with possible lignin and phenolic compounds being lost (Van Soest *et al.* 1991). The National Research Council (2001) currently use the modified NDF model, with the addition sodium sulphite step when defining fibre requirements, based on evidence that sodium sulfite is critical for the removal of nitrogenous contamination from heated feeds (Hintz *et al.* 1996).

### 1.6.2 *Fibre requirements in the diet*

Lactating dairy cows require fibre in the diet to maximise production and maintain health by sustaining a stable environment in the rumen (Allen 1997; Grant 1997). An adequate intake of NDF and ADF is necessary for maintaining ruminal pH within the normal range (Jung and Allen 1995; Mertens 1997). At this stage, the minimum requirement for fibre in the diet remains poorly defined, although inadequate dietary fibre intake has been associated with low milk fat, rumen acidosis and dietary inefficiency (Van Soest 1994). Fibre must be of adequate quality and particle size to ensure maximum dry matter intake (DMI) (Journet and Remond 1976; Spahr 1977; Wangsness and Muller 1981) and optimal chewing activity (Grant *et al.* 1990a; Grant *et al.* 1990b), rumen fermentation and milk fat percentage (Van Soest 1963; Woodford *et al.* 1986). Krause *et al.* (2002b) found that decreasing forage particle size decreased ruminal pH from 6.02 to 5.85 where as Yang *et al.* (2001) reported no effect on rumen pH in cows fed a total mixed ration. However, the length of time pH remained above 6.2 each day was lower for cows fed short particle forage ( $P < 0.1$ ) (Yang *et al.* 2001). The ability of roughages to physically stimulate chewing time is particularly important due to the increased production of salivary buffers during chewing, aiding in the maintenance of rumen pH above 6.0 and preserving optimal rumen function.

The National Research Council (2001) recommends a minimum of 25% NDF in the total dietary dry matter (DM), 75% of which is supplied by coarse forage to maintain rumen function and health. This sets the limit for the use of non-forage fibre sources that are less effective in stimulating chewing than forage fibre. Diets contributing less NDF from forage than 75% of total NDF or if they are not fed as a total mixed ration, require higher

minimum concentrations of NDF (National Research Council 2001). However, because the effectiveness of fibre within by-product feeds and forages is variable, due to rumen fermentation being variable, chemical and physical properties alone should not be used in isolation. Measurements of NDF, being based purely on chemical analysis, are therefore not sufficient to define effective fibre.

Recent research has aimed at defining fibre requirements using a combination of both chemical and physical factors. Two factors have been suggested in this equation; physically effective NDF (peNDF) and effective NDF (eNDF). Physically effective NDF is related to the physical characteristics of fibre that influence chewing activity (Mertens 1997) and would be expressed as a product of NDF concentration and a physical effectiveness factor determined by total chewing response (Grant 1997). Physical effectiveness would be determined by attributes such as chewing time and the biphasic nature of ruminal contents (floating mat of large particles on a pool of liquid and small particles) (Armentano and Pereira 1997; Mertens 1997). Chewing time is strongly related to forage content ( $P < 0.05$ ) (Woodford *et al.* 1986) and particle size (Grant *et al.* 1990b) and is a reasonably practical variable to measure. Mat consistency, although a good outcome is difficult to measure (Armentano and Pereira 1997). Feed characteristics such as fibrousness, roughage value, physical structure and particle size of the feed also determine peNDF.

Effective NDF is related to the total ability of a feed to replace forage or roughage in a ration, so that the percentage of milk fat produced by cows eating the ration is effectively maintained (Mertens 1997). The animal response that is associated with eNDF, therefore, is milk fat percentage, or the extent of milk fat depression associated with a feed. Effectiveness factors for NDF can vary between 0 when a feed has no ability to maintain milk fat percentage to  $> 1$  when a feed maintains milk fat percentage more effectively than maintaining chewing activity (Mertens 1997).

Mertens (1997) found that a minimum peNDF intake of 22.3% of the ration DM was required to maintain a ruminal pH of 6.0. Kolver (1998) proposed that high quality

pasture had an eNDF between 40 and 50% of total NDF. Currently, there is no accurate estimate of eNDF and peNDF for perennial ryegrass or white clover pastures, however the eNDF of similar Australian pastures has been estimated to be 65 to 75% of total NDF (Porter *et al.* 2001). A study incorporating results from 14 data sets from cows grazing pure pasture indicated that 41.1% NDF or 11.5% effective NDF (of DM%) was required to maintain rumen pH at 5.8 to 6.0, corresponding to 29% eNDF of total NDF% (Kolver and de Veth 2002). Under some conditions the diet of pasture-fed dairy cows may contain less than 20% eNDF and cows could be at greater risk of ruminal acidosis. It is important to note that even limited supplementation of low NDF pastures with grain-based concentrates will produce diets that have extremely low NDF concentrations.

Measures of fibre effectiveness in the rumen such as eNDF and peNDF should still be viewed as preliminary and with caution, particularly as an indicator of the incidence of sub-clinical acidosis in dairy cows fed diets that are highly digestible in the rumen. Not all studies agree on the effect that physical characteristics of feeds have on rumen pH, with one study reporting no effect of increasing the forage: concentrate ratio or particle size, thereby increasing the intake of eNDF or peNDF in the diet on rumen pH (Yang *et al.* 2001). This particular study indicated that the rumen fermentability of starch had a larger effect on rumen pH than the physical characteristics of feeds.

Many pasture-based diets, especially those supplemented with grain, will be near the lower limit of effective fibre required in the diet. Stating a single requirement for eNDF does not ensure that this will be sufficient to meet the needs of cattle because of the difficulty in determining the true eNDF of pastures. Estimates of diet eNDF should be matched with observations from the field of cud-chewing, faecal formation, milk fat and protein content and rumen pH to ensure that cows are receiving a diet that will keep rumen function optimal and safe.

### ***1.6.3 Physiology of indicators of adequate fibre in the field***

Effective NDF accounts for factors that affect peNDF; factors that affect ruminal acid production and affect milk fat production. A change in ruminal acid production affects

ruminal pH, affecting the buffering capacity of the rumen and therefore the quantities and ratios of volatile fatty acids produced, directly impacting on milk fat content (Mertens 1997).

Maintaining milk fat content has been the focus of much of the recent research and field application of effective fibre (Armentano and Pereira 1997; Beauchemin 1991a; Beauchemin and Buchanan-Smith 1989; Grant *et al.* 1990a; Grant *et al.* 1990b; Krause *et al.* 2002a); hence the interest in measuring eNDF. Milk fat content can have significant economic impacts, is easy to measure and reflects animal performance. It is however, a reasonably insensitive measure of effective fibre with some studies documenting no change in milk fat content associated with improved rumen function when roughage in the diet was increased (Beauchemin and Buchanan-Smith 1989). This indicates that other factors such as chewing time, ruminal pH, and saliva production measured in collaboration with milk fat content may be useful in assessing effective fibre more accurately. Certainly, the *trans* 10 conjugated fat content in the rumen and amounts of precursors, for example linoleic acid and linolenic acid, in the diet may also have profound effects on milk fat content (National Research Council 2001). Thomson *et al.* (2001) reported that high quality pasture contains 4 to 5% total lipid, 60% of which was linolenic acid and 13% linoleic acid. Changes in rumen biohydrogenation increase the molar proportions of *trans* fatty acids that inhibit milk fat synthesis (Kolver and de Veth 2002). To enable these changes to occur, it has also been proposed that rumen pH must be low (National Research Council 2001). Low fibre concentrations in the diet may be one contributing cause to this low pH. However, recent research has examined the effect of adding rumen protected conjugated linoleic acid to the diet or infusing it post-ruminally to monitor impact on milk *trans* fatty acids and milk fat depression. Bell and Kennelly (2003) found that when synthetic conjugated linoleic acid was infused into the abomasum, the milk fat concentration of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers of conjugated linoleic acid was significantly increased. However the increase in *trans*-10 isomers was associated with milk fat depression. Similarly, when rumen-protected conjugated linoleic acid isomers were added to early lactating dairy cow diets, overall milk fat content and milk fat yield decreased in a dose dependant pattern (Moore *et al.*

2004). Monensin is also known to increase *trans* fatty acid concentrations in cultures of mixed ruminal microorganisms. Jenkins et al (2003) found that the combination of monensin with soybean oil (a source of unsaturated plant oil) caused an interaction giving higher *trans*- 10 C18:1 fatty acid concentrations in ruminal contents than would be expected simply by adding their individual effects with a 70% dry matter barley diet. Given the known association of *trans*- 10 C18:1 fatty acids with milk fat depression, the potential for more severe milk fat depression with the addition of monensin to the diet indicates that care should be taken when the two products are combined in dairy cow diets. With the potential human health benefits associated with the presence of conjugated linoleic acid in bovine milk, the feeding of polyunsaturated fatty acids and possible associated milk fat depression is likely to increase in the industry. This may not necessarily be associated with a low rumen pH and therefore should be examined with other indicators of acidosis in dairy cattle.

Optimal chewing time per kg DM has been estimated at between 24 minutes chewing activity (Woodford and Murphy 1988) to maintain milk fat percentage at 2.9% and 30 minutes to maintain milk fat percentage and optimal rumen function (Mertens 1997). Mertens (1997) analysed data from 36 studies to establish chewing requirements to maintain milk fat percentage (Table 1.2).

**Table 1.2.** Summary of chewing requirements in dairy cattle (adapted from Mertens 1997)

Requirement	Chewing time/ day (min)	Chewing time/ kg DM (min)
3.4% milk fat	589	27.7
3.6% milk fat	744	36.1

Saliva production, associated with chewing time, may also be an important physical measurement for effective fibre (Allen 1997; Balch 1971), although because of the difficulty in measuring saliva flow, contention remains as to how different salivary flow is on different diets. Saliva acts to buffer acids produced during ruminal fermentation of feeds, thereby promoting fibre digestion. The rate of eating is important in determining

the buffering of the feed-saliva mixture (Van Soest 1994). High-concentrate and pelleted forage diets have been characterised by less net saliva flow than diets higher in roughage (Van Soest 1994) because chewing time is decreased. Eating and ruminating stimulate the flow of saliva, although some flow continues constantly. Saliva secretion is estimated to be 1.5 to 2 times higher during chewing compared with resting (Cassida and Stokes 1986). Increasing proportion of NDF in the diet and increasing length of particle size of forage increases chewing time (Beauchemin 1991b). Beauchemin et al (1989) estimated that total saliva production for cows increased by 7 L/day or 2.6% when corn silage diets were supplemented with hay producing 1 hour increased chewing time. These 7 L would provide an increase in  $\text{HCO}_3^-$  of approximately 875meq and  $\text{HPO}_4^{2-}$  of 175meq (Lean 1987). However, one study found that increased chewing time did not increase total daily saliva production, and therefore buffering capacity of the rumen (Maekawa *et al.* 2002). Instead, in that study, cows offered silage and concentrate separately ate more concentrate than silage (F:C = 43:57), with a mean rumen pH of 5.77 compared to the TMR diet (F:C 50:50) with a higher mean rumen pH of 5.99 ( $P = 0.09$ ). The authors concluded that feeding separate ingredients compared with TMR increased the risk of acidosis, because cows ate a higher proportion of concentrate.

Faecal examination in the field may also give an indication of the effective fibre intake in the diet. Although there are numerous potentially confounding factors such as worm burden, pasture intake and disease status in the herd to consider, a herd with a large percentage of dung heaps that are soft and un-formed combined with evidence of scouring around the tail may indicate lack of effective fibre in the diet. Again this evidence needs to be supported by other indicators such as feed type and quality, low milk fat content and reduced chewing times in the herd (Mertens 1997).

## **1.7 Rumen pH and fermentation**

### ***1.7.1 Importance of pH in rumen function***

Rumen pH plays an important role in health and rumen function. The optimal pH for cellulolysis, proteolysis and deamination is between 6 and 7 (Lewis and Emery 1962;

Mould *et al.* 1983). A transitory, 4 hour decrease in rumen pH to 5.4 *in vitro* decreased the flow of microbial N from 0.39 g/day to 0.37 g/day, signifying that cellulolytic bacteria may have limited access to substrate (de Veth and Kolver 2001b). Dry matter digestibility decreases with decreasing pH (Tilley *et al.* 1964). Induction of sub-acute ruminal acidosis over a 5 day period in one study reduced daily mean rumen pH from 6.36 to 5.72 and increased daily mean time of rumen pH below 6 from 2.58 to 15.62 hours (Krajcarski-Hunt *et al.* 2002). This resulted in significant reduction of the 24 and 48 hour NDF digestibility percentage of corn silage (44.0 to 37.2% and 56.1 to 44.8% respectively) and legume hay (35.3 to 26.3% and 49.0 to 35.8% respectively) ( $P < 0.05$ ) (Krajcarski-Hunt *et al.* 2002). Kolver and de Veth (2002) reviewed 23 international studies examining rumen pH on pasture-based diets. Results across studies indicated a lower ruminal pH was correlated with higher microbial nitrogen flow from the rumen, total and individual volatile fatty acid concentrations, milk and milk component yields and dry matter intake with lower concentrations of milk fat and protein percentage and fat to protein ratio (Table 1.3).

**Table 1.3.** Prediction of ruminal pH from animal and dietary variables for lactating cows fed a pasture-based diet across studies (adapted from Kolver and de Veth 2002)

	R <sup>2</sup>	P	Number of treatment means from literature
Microbial N flow (g N/day)	0.41	<0.001	26
Total VFA (mM)	0.3	<0.001	86
Propionate (mM)	0.15	<0.001	75
Acetate (mM)	0.14	<0.001	75
Butyrate (mM)	0.14	0.001	75
Milk yield (kg/day)	0.21	<0.001	90
Milk fat yield (kg/day)	0.15	<0.001	88
Milk protein yield (kg/day)	0.18	<0.001	88
DMI (kg DM/day)	0.04	0.042	115
Milk fat (%)	0.15	<0.001	90
Milk protein (%)	0.13	<0.001	90
Fat: protein	0.09	0.008	77

Kolver and de Veth (2002) also reported in the review of lactating cows fed a diet containing a mean of 80% pasture, a mean daily ruminal pH of 6.16 and range of 5.6 to 6.7. However, these results were based in cows grazing a number of pasture species and direct extrapolation to Australasian conditions, where herds graze predominantly ryegrass and clover pastures, may not be appropriate. Few studies have been conducted on this pasture combination, with one study reporting a mean daily rumen pH of 5.89 when cows ate an estimated 15.6 kg DM of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) per day (Wales *et al.* 2001).

Rumen pH is known to fluctuate throughout the day depending on diet, time of feeding of concentrates and supplementation of fibre sources such as hay following milking in many Australasian systems. Variation due to biphasic feeding patterns induced by twice daily feeding at or after milking may produce a substantial, but relatively short-lived depression of ruminal pH. Daily mean ruminal pH may, therefore, not adequately represent the highly variable characteristics of ruminal pH. While mean daily ruminal pH may remain relatively high, nadir of ruminal pH may be less than 5.8 and be considered 'acidotic' at some, but not all parts of the day. Notwithstanding this, the threshold of ruminal pH at which Australasian pasture fed cows may be considered 'acidotic' has not yet been defined and will probably need consideration in collaboration with other indicators of acidosis and with measures of outcome including milk and milk solids production, risk of disease or more subtly, changes in ruminal fibre digestion and microbial protein production.

Physical form of the diet, for example the reduction of forage particle size or processing of grain decreases ruminal pH (Krause *et al.* 2002b; Lean 1987). This is due to more rapid breakdown of carbohydrates in the rumen and possible decreased saliva production. The amount of rumen fermentable carbohydrates present in the rumen or digestibility of starch in the rumen is also negatively correlated to rumen pH (Krause *et al.* 2002b; Yang *et al.* 2001). Feeds high in pre-formed acids such as some silages will also reduce rumen pH (Lean 1987).

Rumen pH starts to decline immediately after feeding of concentrates or silage. Concentrates cause a more rapid decline in rumen pH than silages. The nadir of rumen pH for the feeding of concentrates separate to forages has been found to be between 2 and 4 hours post feeding. Krause *et al.* (2002b) found that nadir of rumen pH in cows given coarse silage was 5 hours post feeding, with those given finely chopped silage having a much less pronounced nadir at 9 hours post feeding. Where concentrates or grains are being fed separately from forages, samples should be taken 2 to 4 hours following feeding of the concentrate (Nordlund 2000). For grass only or total mixed ration herds, samples should be collected 4 to 8 hours after feeding (Nordlund 2000). To provide an overview of herd status, samples of rumen fluid should be collected from a minimum of 6 to 12 cows (Garrett *et al.* 1999; Olson 1997). A portable pH meter capable of measuring pH from a very small volume of rumen fluid yields very similar results to a standard meter with probe (Garrett *et al.* 1999). Consequently, portable pH meters provide a valuable tool for practitioners wishing to measure rumen pH.

The repeated measurement of ruminal pH remains the only definitive way to quantify the balance between acid production, acid removal and buffering capacity of the rumen. A ruminal pH of 6.4 to 6.8 is considered desirable for optimum cow health and performance (Erdman 1988).

The measurement of ruminal pH from a sub-sample of a herd will assist with the diagnosis of ruminal acidosis in practice. There is a high likelihood of ruminal acidosis in a herd when more than 30% of sampled cows have ruminal pH of 5.5 or less (Garrett *et al.* 1999; Olson 1997). A ruminal pH of 5.6 to 5.8 suggests a marginal or developing problem of ruminal acidosis, while a pH of greater than 5.9 was considered 'normal' (Olson 1997).

### ***1.7.2 Physiological buffering capacity of the rumen***

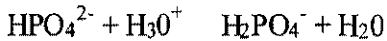
Rumen pH varies with diet, time after feeding and location of sampling within the rumen. Notwithstanding this, rumen pH normally fluctuates between 6 and 7. It is critical for optimal rumen function to maintain rumen pH as constant and within this range as

possible. The physiological buffering capacity of the rumen allows rumen pH to be maintained between 6 and 7 on most diets in most conditions. However, when highly fermentable carbohydrates are fed to animals, particularly without prior exposure, rapid fermentation occurs in the rumen resulting in a decrease in rumen pH from between 6 and 7 to 5.5 or lower. The amount of decrease in pH after an increase in fermentation rate depends on the buffering capacity of the rumen (Counotte *et al.* 1979). Unfortunately with a continual increase in concentrate feeding, carbohydrate challenges to the rumen often result in the buffering capacity being overwhelmed and acidosis occurring. It is now common practice to use dietary buffers, modifiers and antibiotics to work in conjunction with the physiological buffering capacity of the rumen (sections 1.12.3 and 1.12.4).

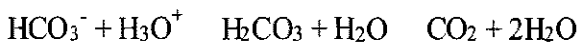
Buffering capacity is defined as the number of moles of  $H^+$  per litre required to cause a given change in pH (Counotte *et al.* 1979). The best substances for buffering in a given pH range are those whose pK values are such that  $pK \pm 1$  lies within this range. The primary rumen buffers are bicarbonate from saliva production and volatile fatty acids present in the rumen (Counotte *et al.* 1979). Secondary buffers include phosphorus buffers (sometimes classified as neutralising agents) (Counotte *et al.* 1979) and carbonate buffers, also sourced from saliva (Lean 1987). The pK of bicarbonate and dihydrogen phosphate are within the range 6 to 7 (Czerkawski 1986). In the normal pH range of the rumen, the most important buffers are those originating from saliva with the weak volatile fatty acids more important in lower pH ranges. Saliva in cattle is produced from a number of glands; parotid, mandibular, sublingual, labial, ventral, medial and dorsal buccal and pharyngeal (Kay 1960). Of these, the parotid, buccal and pharyngeal glands are responsible for the production of most  $HCO_3$  and  $HPO_4$  with the parotid responsible for 40 to 50% of total saliva produced. Saliva production in cattle is usually between 70 and 180 L/day with concentrations of  $HCO_3$  and  $HPO_4$  being 125 and 25 meq/L respectively (Lean 1987).

Buffering capacity is normally influenced by factors altering the amount or quality of saliva production, concentration of ruminal acids and  $CO_2$  and rate of absorption or passage of digesta through the rumen (Lean 1987). The average pH of saliva is about 8.0

(Counotte *et al.* 1979). At this pH, 86% of the phosphate is in the  $\text{HPO}_4^{2-}$  form. Upon entering the rumen, the following reaction occurs:



This reaction occurs until 10% is in the form of  $\text{HPO}_4^{2-}$  and 90% as  $\text{H}_2\text{PO}_4^-$  and raises the rumen pH but reduces the buffering capacity of the phosphate system (Counotte *et al.* 1979). Bicarbonate acts similarly but is more important when the rumen pH is 6.25 or lower because the pH value of the equilibrium reaction is 6.25 (Counotte *et al.* 1979).



When rumen pH is less than 6.0, the loss of water from the rumen and decrease in bicarbonate concentration and therefore buffering capacity poses a potential acidosis risk, especially when additional concentrates may be offered before buffering capacity is able to recover. Below pH 5.5, lactate fermenting bacteria are unable to grow, partly because of their need for bicarbonate, which is not present in these conditions, allowing lactic acid to build up in the rumen further depressing pH.

## 1.8 Techniques for rumen fluid collection

### 1.8.1 Rumen cannula

Collection of ruminal contents from cattle with an indwelling ruminal cannula remains the preferred method for the determination of ruminal pH. However, practical limitations, including the need to regularly clean and monitor rumen cannula sites, limits use in commercial dairy situations. Some large herds have rumen cannulated cows to provide a ready monitor of rumen function, although this is not common practice in Australia.

### 1.8.2 Rumencentesis

The collection of ruminal fluid using percutaneous needle aspiration is a more practical method for the determination of ruminal pH. Rumen fluid is collected using a 14 gauge 1 ½ inch disposable needle attached to a 30 ml eccentric syringe. A 2 cm by 2 cm area is shaved on the left paralumbar fossa approximately one hand length ventral to the lumbar spinal transverse processes and one hand width caudal to the last rib, avoiding the major muscle masses. This area is surgically prepared, local anaesthetic injected, and the

sampling needle is inserted firmly through into the rumen. A minimum of 4 ml should normally be obtained from most animals using this procedure. This method may not be completely benign in healthy cattle, because milk yields were shown to be reduced for 24 to 48 hours post sampling (Opatpatanakit et al, unpublished data) in one trial. Garrett *et al.* (1999) found that pH of rumen fluid collected via rumenocentesis was 0.28 units lower than that of fluid collected through a ruminal cannula. Filtration or aspiration of the rumen fluid had no effect on pH.

### **1.8.3 Rumen stomach tube**

A stomach tube suited to collecting ruminal fluid can be constructed using a 3 ½ metre long, 19 mm diameter plastic reinforced garden hose attached to an equine stomach pump. The other end should be riveted to a 10 to 15 cm length of copper pipe with multiple holes drilled along all sides. This reduces risk of blockages with ingesta. Samples of ruminal fluid taken by stomach tube have a higher pH than those obtained through a rumen cannula (Erdman 1988), either as a result of salivary contamination or sampling in the fibre mat of the rumen. Therefore ruminal pH samples collected by stomach tube should be interpreted with caution. This method will be more specific, but less sensitive for the detection of acidosis. The rumen fluid collected from the stomach tube should be examined for evidence of saliva contamination before measuring pH and samples discarded if contaminated. Duffield *et al.* (2000) compared samples obtained from the cranial ventral rumen, with rumenocentesis and stomach tube samples. Rumenocentesis samples were sensitive for detecting pH of the cranial ventral rumen, while stomach tube samples were poorly sensitive. Both tests had similar specificities. The most accurate field test for ruminal pH was rumenocentesis (81%).

## **1.9 Rumen lactic acidosis in cattle**

### **1.9.1 Definition and clinical presentation of ruminal acidosis**

Acidosis by definition is a pathological condition resulting from accumulation of acid or depletion of the alkaline reserve (bicarbonate content) in the blood and body tissues and characterised by increase in hydrogen ion concentration (Blood and Studdert 1988).

Rumen acidosis is a decrease in pH in the rumen of cattle from an inciting cause. Rumen lactic acidosis (grain overload, grain poisoning, acute indigestion) develops in sheep and cattle that have ingested large amounts of unaccustomed feeds rich in ruminally fermentable carbohydrates (Crichlow and Chaplin 1985; Nocek 1997). The resulting production of large quantities of volatile fatty acids and lactic acid decreases rumen pH to non-physiological levels, simultaneously weakening the buffering capacity of the rumen. Low rumen pH can result in ruminitis, metabolic acidosis, lameness, hepatic abscessation, pneumonia and death (Lean *et al.* 2001). Acidosis can be divided into two categories; clinical and sub-clinical.

Clinical acidosis presents as a mild to severe form depending on the type and amount of feed ingested. Cattle with mild clinical acidosis may show anorexia, decreased milk production and scouring (Underwood 1992). Signs progress in the severe form to include severe metabolic acidosis, depression, dehydration, toxemia, and 'downer cow' syndrome (Bolton and Pass 1988). Peracute acidosis may result in recumbancy, coma and death in 8 to 10 hours (Underwood 1992). Clinical signs in the acute form develop within 8 hours and precede onset of metabolic clinical acidosis which peak in 36 hours (Underwood 1992). Clinical acidosis generally affects one to a few cows in a herd caused by unusual sudden dietary changes. Additional problems occurring as a sequel to acute ruminal acidosis include hypocalcaemia resulting from calcium malabsorption, laminitis from the release of histamine and endotoxins into the circulation, polioencephalomalacia from an induced thiamine deficiency and ruminitis leading to liver abscessation (Bolton and Pass 1988). The two most common bacteria thought to be responsible for liver abscessation are *Fusobacterium necrophorum* and *Corynebacterium spp.*, able to reach the liver through the portal circulation via damaged ruminal epithelium (Bolton and Pass 1988).

Sub-clinical acidosis is usually of greater economic importance impacting all or a significant proportion of the herd. Symptoms associated with sub-clinical acidosis include decreased milk fat content, feed conversion efficiency, feed intake and rate and extent of roughage degradation (Lean *et al.* 2000), laminitis causing lameness (Nocek

1997; Owens *et al.* 1998), liver abscessation (Owens *et al.* 1998), scouring (Nocek 1997) and a higher incidence of left and right displacements of the abomasum (Shaver 1997). Sub-clinical acidosis often goes unrecognised and undiagnosed until a significant proportion of the herd are involved and obvious clinical signs become evident. At this stage, large financial losses and long-term health issues such as whole herd lameness problems may be inevitable. The cut-off pH for which sub-clinical acidosis is diagnosed is controversial with some authors suggesting 5.5 by rumenocentesis (Garrett *et al.* 1999). However *in vitro* fibre digestibility is reduced when pH drops below 6.2 (Calsamiglia *et al.* 1999; Grant 1994; Grant and Mertens 1992).

### **1.9.2 Proposed aetiology of ruminal acidosis in cattle**

The aetiology, effects and prevention of ruminal acidosis have been extensively researched and reviewed (Dirksen 1970; Huber 1976; Hungate *et al.* 1952; Nocek 1997; Owens *et al.* 1998; Slyter 1976; Underwood 1992). Both *in vitro* and *in vivo* trials have been conducted, however much of this research has concentrated on feeding large amounts of starch-based concentrate as the cause of acidosis (Crichlow and Chaplin 1985). Ruminal pH in experimental cases often was  $< 5$  and caused severe acute clinical disease (Nocek 1997). This pH range appears to be lower than that of cattle solely fed on pasture where mean daily pH has been reported to be between 5.6 and 6.4 (Carruthers *et al.* 1996; Kolver *et al.* 1998; O'Mara *et al.* 1997; Stockdale 1994; Van Vuuren *et al.* 1992), with optimal ruminal pH  $> 5.8$  (de Veth and Kolver 2001a) to 6.0 (Van Soest 1994) for fibre digestion.

There has been little research on the aetiology of acidosis induced primarily by pasture feeding (Wales *et al.* 2001), but more on feeding of starch-based concentrates to cows on pasture (Clayton *et al.* 1999; Lean *et al.* 2001; Opatpatanakit *et al.* 1994; Wales *et al.* 2001). Traditionally in Australia and parts of New Zealand, starch-rich concentrates are fed twice daily at milking without adequate buffering.

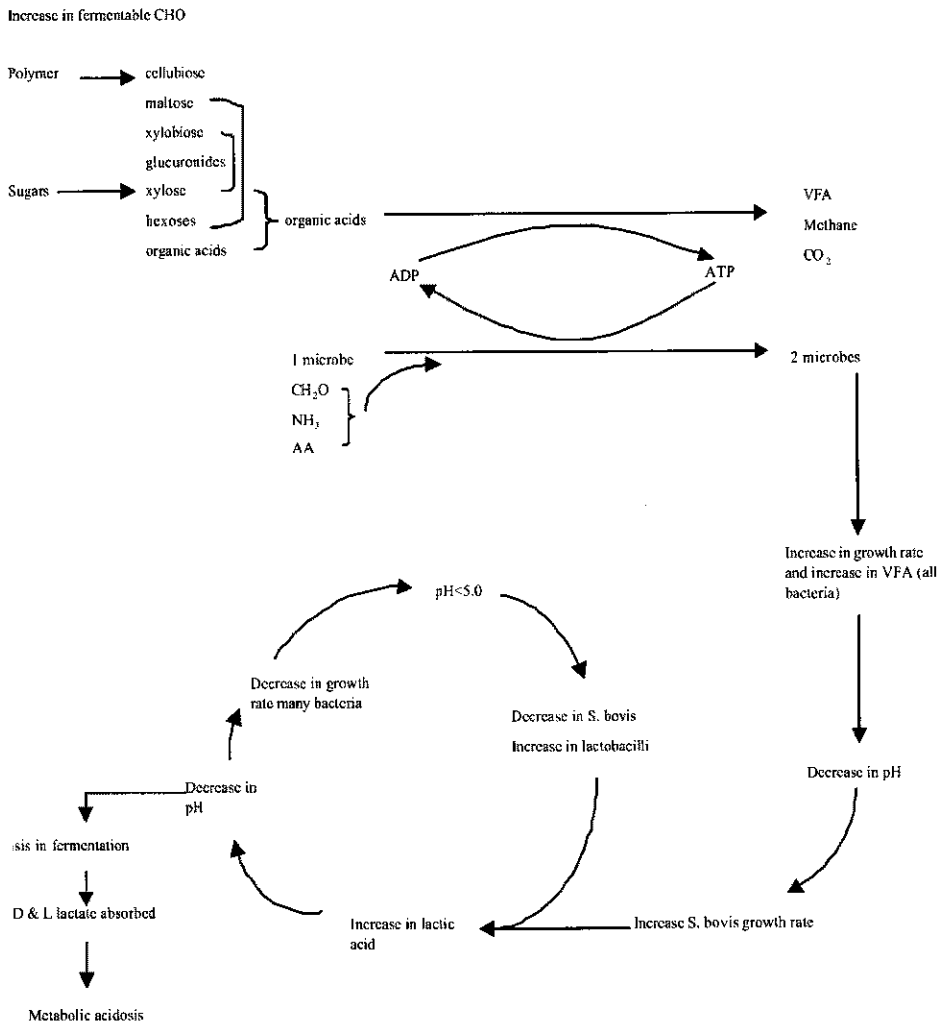
Lean *et al.* (2000) proposed the following mechanisms that can lower ruminal pH; access to preformed acids in feeds; a failure to produce buffering with endogenously derived

buffers such as salivary bicarbonate; production of large amounts of the weak volatile fatty acids acetic acid, butyric acid and propionic acid; and production of lactic acid in the rumen. Pastures low in NDF and/or high in non-structural carbohydrates and supplementation of pastures with silages and grain, expose cattle that are predominantly pasture-fed to the risk of acidosis.

#### *1.9.2.1 The aetiology of ruminal acidosis on starch rich diets*

Figure 1.8 indicates the proposed sequence of events involved in the aetiology of acute ruminal acidosis induced by overload of starch, when, for example, fed grain.

**Figure 1.8.** Proposed sequence of events associated with the induction of acute ruminal acidosis. CHO = carbohydrate (adapted from (Baldwin and Allison 1983; Nocek 1997).



Initially, this metabolic insult results in an increase in the growth rate of all bacteria in the rumen, resulting in an increase in total volatile fatty acid production and a decrease in ruminal pH. When large amounts of starch are added to the diet, growth of *S. bovis* is no longer restricted by a lack of this energy source and this population grows faster than other species of rumen bacteria (Russell and Hino 1985). *S. bovis* produces lactic acid, an acid ten times stronger than acetic, propionic or butyric acid, the accumulation of which eventually exceeds the buffering capacity of rumen fluid. Glucose produced from the breakdown of starch is converted to fructose 1,6-diphosphate. Russell and Hino (1985) found that fructose 1,6-diphosphate has a positive feedback on the conversion of pyruvate to lactate by activating lactate dehydrogenase. Fructose 1,6-diphosphate is also converted to triose phosphate in increasing concentrations. Triose phosphate acts to inhibit pyruvate formate lyase. The net effect of these changes is a switch from predominantly acetate and formate production to lactate production (Russell and Hino 1985).

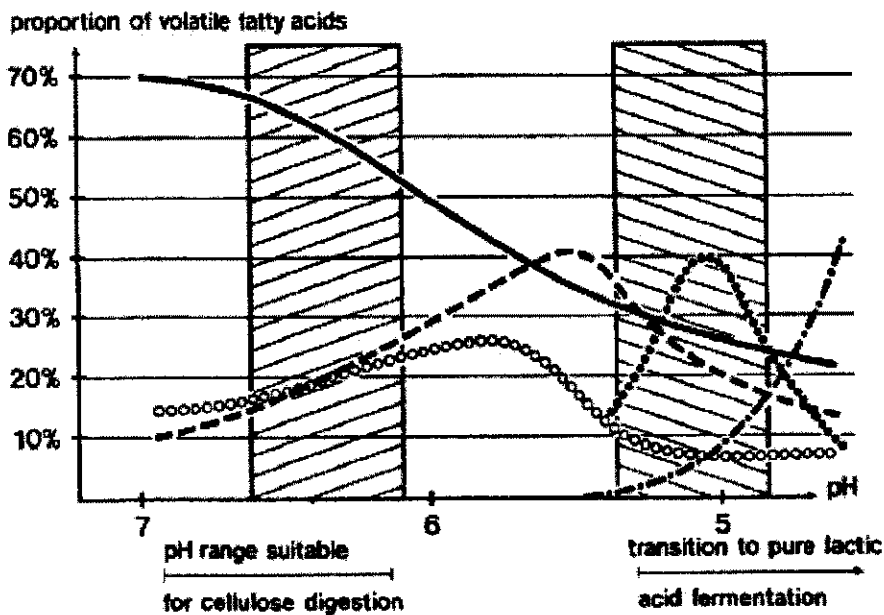
The increase in VFA concentrations may also initially decrease reticulo-ruminal motility by acting on receptors in the rumen wall. These receptors, in sheep, are activated when non-dissociated VFA concentrations exceed 3.0 mM (Crichlow and Chaplin 1985). Crichlow and Chaplin (1985) found that the decrease in ruminal motility was independent of a decrease in rumen or systemic pH and occurred when venous blood concentrations were normal. A decrease in motility results in a decrease in rumination and less production of saliva. Saliva contains high concentrations of bicarbonate ions and is an important buffering mechanism for the rumen. Saliva is produced through stimulation of the rumen and mastication.

A decrease in bicarbonate and increase in lactic acid concentrations in the rumen further decreases ruminal pH. When ruminal pH is maintained above 5.5, an equilibrium exists between producers and utilisers of lactic acid, such that lactic acid does not accumulate in the rumen (Nocek 1997). When pH is less than 5.5, no cellulolytic and relatively few saccharolytic bacteria, including *P. ruminicola*, a significant producer of VFAs, survive. *S. bovis* multiplies until ruminal pH is less than 5.0, a pH that allows an increase in

*Lactobacillus* growth. Both of these bacterial species produce D and L-lactic acid (Figure 1.8). D and L-lactate are absorbed across the rumen wall and depress blood pH. As L-lactate is metabolised more rapidly than D-lactate, the metabolic acidosis is due in large part to accumulation of the latter (Bolton and Pass 1988). Chemical damage of the surface epithelium of the rumen mucosa occurs and results in the adherence of debris and penetration by particulate matter from the rumen. Bacterial and mycotic organisms begin to invade the rumen wall causing a ruminitis to occur. Rumen papillae are damaged and can slough from the rumen wall (Ahrens 1967; Lee *et al.* 1982). Histamine is produced from histidine in the rumen by *Allisonella histaminiformans* and absorption of histamine into the blood is increased by low rumen pH (Russell and Garner 2003). As lactate passes from the abomasum into the intestinal tract, further absorption occurs, promoting an osmotic gradient. The resultant increase in fluid in the lumen is responsible for the profuse diarrhoea and subsequent dehydration seen in clinical acidosis.

**Figure 1.9.** Proportions of acids produced in the rumen when pH falls from 7.0 (after Kaufmann & Ruhr, 1967 reprinted from: Rosenberger, Clinical Examination of Cattle, 1979, Paul Parey Scientific Publishers, Berlin and Hamburg)

— = acetic acid; --- = butyric acid;      = propionic acid; ••• = propionic acid derived from breakdown of lactic acid;    · = lactic acid



In sub-clinical acidosis, often the pH will fluctuate in the rumen during the 24 hour period, with a nadir recorded 2 to 4 hours after feeding concentrate in the bail (Harrison *et al.* 1989). Krajcarski-Hunt *et al.* (2002) found that induction of sub-clinical ruminal acidosis significantly reduced mean daily rumen pH from 6.36 to 5.72, increased daily time below pH 6.0 from 2.58 to 15.62 hours/day and increased daily time below pH 5.6 from 0.26 to 9.91 hours/day. Risk of acidosis is potentially increased if cattle are fed forages high in non structural carbohydrates (sugars and starches) with low effective fibre content, such as clovers and young lucerne (alfalfa) and possibly ryegrass that may not stimulate adequate rumination and salivation in combination with concentrate feeding.

#### 1.9.2.2 Pasture-based acidosis

The Australian dairy industry is still predominantly pasture-based with concentrates being offered twice daily in the milking parlour. Clinical and sub-clinical rumen acidosis is currently poorly defined under this type of feeding system. However, when the amount of concentrate or carbohydrate percentage is increased in the diet and NDF% decreased, the concentration of total volatile fatty acids increases (Lana *et al.* 1998; Russell 1998). Rumen pH will often fluctuate during a 24-hour period. Nadirs in pH are recorded 2 to 4 hours after feeding concentrates to cows in the milking bails (Carruthers and Neil 1997; Harrison *et al.* 1989). Short periods of exposure to pH of 5.4 *in vitro* had a transitory negative effect on rumen microbial populations as represented by microbial nitrogen flow from the rumen (g/day). Microbial populations were clearly able to recover during intervals when pH was optimal (pH = 6.3) (de Veth and Kolver 2001b).

The risk of acidosis may be increased when cattle are fed forages high in NFC such as sugars and starches, or low in effective fibre, such as clovers and young lucerne (alfalfa), and possibly ryegrass. These carbohydrate sources may not stimulate adequate rumination and salivation. In this situation, rumen pH may remain depressed for longer periods of the day. Kolver and de Veth (2002) collated animal and dietary variables from 23 pasture-based studies and reported that the mean daily ruminal pH ranged from 5.6 to 6.7 across studies. The diets collated in that study contained at least 47% pasture with an average content of 81% across all treatments.

Highly digestible pasture, when combined with concentrate feeding, increases bacterial growth rates and production of acetate, propionate and butyrate. Rumen pH subsequently decreases and is negatively associated with total VFA concentrations ( $P < 0.001$ ;  $R^2 = 0.3$ ) (Kolver and de Veth 2002). During the transitory period of low pH (4 to 8 hours), de Veth and Kolver (2001b) found that the acetate: propionate ratio decreased *in vitro*, primarily because of a decrease in acetate concentrations. Other pasture-based *in vitro* studies reported no significant change in ratio, concluding that the individual VFA concentrations are determined by substrate preferences rather than rumen pH (de Veth and Kolver 2001a). This finding is challenged in an analysis of *in vivo* studies where rumen pH was strongly negatively related to propionate concentration (mM) ( $P < 0.001$ ) both within and across 75 studies (Kolver and de Veth 2002). In the same study, the ratio of acetate to propionate did not significantly predict rumen pH. Lactate concentrations did not change with prolonged exposure to sub-optimal pH (5.4), remaining between 0.05 and 0.13 mM (de Veth and Kolver 2001b). Concentrations of lactate recorded in concentrate-induced acidosis have been significantly higher (18.8 to 48.0 mM, 2 to 4 hours following ingestion) (Crichlow and Chaplin 1985), suggesting that either the switch from acetate and formate to lactate production by *S. bovis* and the growth of *Lactobacilli* may not occur in pasture-based acidosis or that clearance of lactate from the rumen is more efficient. A transitory, 4 hour decrease in rumen pH to 5.4 *in vitro* decreased the flow of microbial N from 0.39 g/day to 0.37 g/day, signifying that cellulolytic bacteria may not die, rather have limited access to substrate (de Veth and Kolver 2001b). Under the same conditions, ammonia nitrogen flow (g/day) did not greatly change, possibly because both deamination of protein and utilisation of ammonia were reduced by both low pH and limited availability of suitable energy sources.

Some intensive studies in Australia have examined the effects of feeding of starch-based concentrates to cows on pasture (Clayton *et al.* 1999; Lean *et al.* 2000; Opatpatanakit *et al.* 1994; Wales *et al.* 2001). However, no extensive surveys of the industry have been documented.

### ***1.9.3 Indirect indicators of ruminal acidosis***

#### *1.9.3.1 Low milk fat concentration*

Ruminal pH is positively associated with milk fat concentration (Kolver and de Veth 2002). Therefore, repeated assessment of bulk milk vat recordings of fat percentage as an indicator of acidosis in a herd may be useful. However, the relationship between ruminal pH and milk fat concentration is not absolute, being confounded by stage of lactation, dietary fat content, and body fat mobilization (Westwood and Lean 2001). A reduction in concentration of milk fat should be considered to indicate the possibility of low ruminal pH, and as a signalment for investigations using other means of evaluating rumen function.

#### *1.9.3.2 Chewing time*

Chewing time can indicate the presence or lack of adequate fibre in the diet and therefore is also an indication of sub-clinical acidosis in a herd. Practical guidelines for the assessment of the proportion of cows chewing within a herd have been proposed (Lesch and Sawyer 1981). When cattle are not actively grazing or walking, 50% or more of the herd should be ruminating and/ or actively chewing. Where fewer than 50% of cattle are identified with chewing activity, the potential for sub-optimal ruminal pH and rumen function should be considered. Chewing activity when assessed together with other animal and feed measures can be a useful non-invasive measure of rumen function in a dairy herd.

#### *1.9.3.3 Faecal changes*

The faeces of cows can provide indirect evidence of clinical and sub-clinical acidosis. Typically, the faeces are more liquid, contain undigested fibre and grain, are often lighter in colour and may contain gas bubbles. The smell of the faeces may be bitter - sweet rather than the typical herbaceous character found from cows fed on grass. Faecal moisture content is increased as a result of a lactate-based osmotic overloading of the large intestine, the lactate coming from the rumen (Bolton and Pass 1988). Lactate also causes sequestration of large quantities of water in the rumen inducing dehydration. Increased proportions of undigested feeds resulting from reduced microbial cellulolytic

activity at a lower ruminal pH also contribute to diarrhoea (Bolton and Pass 1988). Notwithstanding this, faecal pH is poorly correlated with ruminal pH due to fermentation and buffering in the hind gut (Clayton *et al.* 1999) and should be interpreted with caution. Faeces can be scored on a 1 to 5 scale ranging from dry with excessive amounts of fibre from cows on a predominant hay diet (score 1) to scouring with no form and a large constituent of water from cows on a diet of minimal fibre (score 5).

#### 1.9.3.4 Laminitis/lameness

Lameness of cows is common in the Australian dairy industry, with a reported incidence of 7.5% during lactation in Australia (Harris *et al.* 1988). Many factors have been implicated in the onset of foot lameness. Environmental factors including mud and moisture, lane and yard design and temperature may combine with nutrition, animal management and with individual animal characteristics to cause lameness (Chesterton *et al.* 1989). In most cases, a high prevalence of foot lameness will reflect the combined effects of several predisposing factors.

Laminitis (*pododermatitis aseptica diffusa*) is regarded internationally as an important predisposing factor to lesions of the claw, yet the role of laminitis in the pathogenesis of lameness in the Australian dairy industry is largely unknown. Acidosis has been implicated as a common cause of laminitis in dairy herds. Lesions associated with laminitis include solar haemorrhage and abscessation (Bergsten 2000; Dewes 1978; Tranter and Morris 1991); bruising of the solar corium (Dewes 1978; Harris *et al.* 1988); under-run sole (Bergsten 2000; Tranter and Morris 1991); white line lesions (Bergsten 2000; Tranter and Morris 1991). Claw lesions consistent with laminitic changes have been described for Australian dairy cattle (Jubb and Malmø 1991; Vermunt 1992). Many studies have identified nutritional factors that increase the risk of laminitis, including the feeding of diets high in rapidly fermentable carbohydrates (Nocek 1997; Shaver 1997). Factors responsible for laminitic episodes on pasture based diets remain unidentified. Laminitis can be defined as acute, sub-acute, chronic and sub-clinical depending on the severity and duration of the condition.

The aetiology of laminitis has been extensively researched and reviewed in the past (Nocek 1997; Ossent and Lischer 1998; Vermunt 1992). Acute laminitis occurs after a metabolic insult such as excessive feeding of a highly fermentable carbohydrate source results in decreased ruminal and subsequently systemic pH. However, ruminal pH may have returned to normal when laminitic lesions are first diagnosed as a result of the 4 to 8 week delay between nutritional insult and the onset of laminitis.

The reduction of systemic pH activates a vasoactive mechanism increasing digital pulse and total blood flow (Nocek 1997). Engorgement of digital veins and dilation of arterio-venous anastomoses of the lower limb result, diverting blood from the peripheral tissues (Ossent and Lischer 1998). Vasoactive substances may include histamine, lactic acid, serotonin or endotoxins (Westwood and Lean 2001), creating vascular constriction and dilation. Increased blood pressure results in damage to the blood vessels and oedema. Ischaemia and hyperaemia of the corium, causing tissue anoxia may become evident clinically 4 to 8 weeks after initial insult occurs (Westwood and Lean 2001). Signs include sole haemorrhages, discolouration of the horn from white to yellow and softening of the horn texture. Sub-clinical and chronic laminitis may also develop following a similar aetiology, but are not associated with systemic symptoms of illness. Claw lesions are associated with the production of inferior quality hoof horn, changing conformation of the hoof. The dorsal wall surface may appear concave with horizontal (growth arrest) lines extending around the circumference of the wall (Westwood and Lean 2001). The sole may appear flattened with overgrowth of the abaxial wall. Chronic laminitis probably occurs following repeated nutritional insults to the claw.

A practical assessment of lameness in a herd was developed by (Sprecher *et al.* 1997) using a simple scoring system. A number of individual cows from the herd are scored for locomotion on a scale of 1 to 5 where 1 = clinically normal, 3 = moderately lame with the cow standing and walking with an arched back and 5 = severely lame, showing pronounced arching of back and refusing to bear weight on one limb.

### 1.9.3.5 Feed characteristics

Assessment of the chemical and physical characteristics of diet is an important diagnostic tool when assessing nutritional problems in the herd. The examination of chemical properties such as percent dry matter, digestibility, energy, NSC content, CP, NDF and ADF will provide an overview of feed quality. This combined with physical properties such as quality, particle size and stem to leaf ratios of forages may indicate the possibility of sub-optimal rumen function leading to diseases such as acidosis. Risk of ruminal acidosis will be greater when pasture is immature, leafy, with a high leaf to stem ratio and growing rapidly (Westwood and Lean 2001).

The low pH of silages (pH less than 4.0), resulting from pre-formed lactic acid can contribute to lower ruminal pH and therefore acidosis. This is particularly relevant when considering maize and whole crop cereal silages. For example, if corn silage, which contains 4.6% lactic acid is fed at 6 kg dry matter/ cow/ day, an extra 276 g lactic acid will enter the rumen/ cow/ day. With a molar weight of 90.08, this is equivalent to 3 M. Normally, on a forage based diet, in a 240 litre rumen, concentrations of lactic acid are less than 1 mM. Therefore, on forage based diets, the rumen would typically contain 240mM. If an extra 3 M of lactic acid was added to the rumen in a day (10 times the normal concentration), it would undoubtedly have an impact on rumen pH. Notwithstanding this, rumen flux has not been considered and may impact on the results. Chop length of forages used for silage or hay will further influence ruminal pH, with short chop silage and hay reducing chewing and rumination time and therefore saliva production (de Boever *et al.* 1993; Grant *et al.* 1990a; Grant *et al.* 1990b; Sudweeks *et al.* 1975). Particle size is negatively correlated with chewing activity/ kg DM (Allen 1997; Mertens 1997) and positively with ruminal pH (Allen 1997). For practical purposes, greater than 25% of the forage component of the diet should contain forage of 10 cm length or more. Particle size and processing also applies to concentrates being fed. Starchy cereal grains, particularly wheat, can be rapidly rumen fermentable, produce lactic acid and increase risk of acidosis (Opatpatanakit *et al.* 1994).

## 1.10 Composition and qualities of southern and sub-tropical Australian pastures

### 1.10.1 Overview of pasture composition

Pasture composition varies greatly with area due to species differences and climatic conditions. Australasian pastures have typically been poorly defined in the past with limited studies examining composition of pastures over a period of time (Fulkerson *et al.* 1998; Jacobs *et al.* 1999; Stockdale 1999a; Stockdale 1999b; Stockdale *et al.* 2001). A summary of results from these trials is presented in Table 1.4 for the three areas represented in this study.

**Table 1.4.** Range of dietary variables for three dairying areas in Australia; Gippsland/Western Districts Victoria, Irrigation area of Southern NSW/ Northern Victoria and Sub-tropical area on central coast of NSW (adapted from Fulkerson *et al.* 1998; Jacobs *et al.* 1999; Stockdale 1999a; Stockdale *et al.* 2001).

Factor	Western Districts/ Gippsland <sup>a</sup>	Irrigation area <sup>b</sup>	Subtropical			Optimum for early lactation dairy cow
			Biennial ryegrass	Perennial rye-white clover	Kikuyu	
DM %	-	-	-	-	-	50-60
CP(%DM)	10-25	11.6-21.3	26.6	21-25	16-24	18
NDF (%DM)	43.6-69.2	43-63.6	37-48	38-50	45-60	30
ADF (%DM)	-	27.2-36	17-27	<21-24	>21-30	21
NSC (%DM)	-	-	14.5	10.5	9	36
ME (MJ/kg)	8.4-11	8.9-11.6	<10-11.9	<9-11	8.5-9.5	11.9

a. composition of pasture predominantly ryegrass, <10% clover

b. composition pasture: 52% white clover/ 40% perennial ryegrass (spring), predominantly perennial ryegrass/ paspallum/ weeds (autumn)

### 1.10.2 Carbohydrate degradation, fibre, moisture content and protein in pasture

Pasture fibre varies greatly depending on area, season and composition of pasture. An adequate intake of neutral detergent fibre (NDF) is required in the diet to maintain rumen pH within the normal range (Mertens 1997) with recommendations of a minimum of 30%

NDF in the diet of an early lactating dairy cow (National Research Council 2001). The NDF concentrations described for Australian pastures (Table 1.4) indicate that pastures generally should meet adequate NDF requirements for dairy cows. However, problems with this data set include the length of time of collection in some studies and the limited number of farms sampled. Unpublished data and personnel observations (IJ Lean et al) in all three of these areas suggest that NDF concentrations in pastures grazed can be significantly lower in certain seasons than the minimum concentrations recorded above (Table 1.4). This may increase the risk of acidosis in cattle grazing the pastures, particularly if supplemented with highly fermentable carbohydrates.

New Zealand data indicates that spring and autumn temperate pastures range from 25 to 48% NDF, concentrations significantly lower than current Australian data even though environmental conditions may well be similar in certain seasons and areas.

Australasian temperate pastures particularly during winter and spring are often characterized by high moisture content. The ingestion of high moisture feeds results in associated reduction in chewing and ruminating time and therefore saliva production (Van Soest 1994). This places cattle at increased risk of sub-clinical acidosis.

### ***1.10.3 Nitrogenous fertiliser and pasture growth***

Nitrogen fertilisation of temperate pastures has been found to increase productivity. Input of nitrogen increases plant growth rate leading to higher forage (dry matter) yield for a given age of re-growth (Peyraud and Astigarraga 1998). Other effects include reduction in water soluble carbohydrates (Valk *et al.* 1996), possible reduction in palatability, although dry matter intake is not affected (Peyraud and Astigarraga 1998) and an increase in nitrogen content (Zhang *et al.* 1995). Many temperate dairy pastures in Australasia are predominantly ryegrass at one or more seasons of the year. Ryegrass pastures are currently limiting milk production because of low non-structural carbohydrate concentrations, higher than optimal levels of structural carbohydrates and high concentrations of highly degradable protein (Porter *et al.* 2001). Porter *et al.* (2001) found that under Australian winter conditions, nitrogen fertilisation of Concord, Maverick,

Quartet and Aires ryegrasses increased dry matter yields at all stages of growth ( $P = 0.001$ ), concurring with other studies (Jacobs *et al.* 2001). The annual/ biannual cultivars produced more dry matter than the perennial cultivars and grew more in the third leaf stage. Metabolisable energy and crude protein content of ryegrass was significantly increased and concentration of water-soluble and non-structural carbohydrates decreased with additional nitrogen fertiliser (McKenzie *et al.* 1999; Porter *et al.* 2001), supporting previous research (Valk *et al.* 1996). Importantly, a significant increase in the structural carbohydrates (21% ADF and 37% NDF) ( $P = 0.001$ ) with nitrogen fertilisation was also noted. Interestingly, perennial ryegrass- white clover dairy pastures in western Victoria showed a decrease in pasture NDF with nitrogen fertilisation during autumn to early winter (McKenzie *et al.* 1999). The apparently contradictory results reflect the challenge in practice; whether or not to graze nitrogen fertilised grass according to date or on mass. While fertilisation increases the growth, grazing earlier may result in grazing a pasture of lower fibre content.

Current recommendations for dairy pasture used as a sole feed are 32% NDF and 22% ADF with concentrations found by Porter *et al.* (2001) barely meeting requirements. Therefore, although nitrogen fertilisation increases fibre content in ryegrass, problems associated with low dietary fibre, particularly when pasture is supplemented with low fibre concentrates may still arise.

## **1.11 Prevention of ruminal acidosis**

### ***1.11.1 Adequate fibre***

Fibre requirements and indicators of adequate fibre in the diet, including milk fat content, chewing time and faecal examination have been discussed. Controversy currently exists as to the most appropriate way to define fibre requirements in the diet and the recommended minimum concentrations of fibre. The current system of definition using NDF may not be adequate. Notwithstanding this, there is agreement that a combination of chemical and physical properties of fibre in the diet should be examined. Chewing time is particularly important in the prevention of ruminal acidosis, stimulating the natural flow

of saliva, which buffers the rumen in pH range 6 to 7. Chewing time is increased when long stem forages are fed and reduced when particle size is decreased, either by reducing forage particle size (Krause *et al.* 2002b) or increasing processing of concentrates. Fibre remains a generally cheap option to aid in the prevention of acidosis pending supply in the area. Many dairy farmers choose to feed a poor quality fibre source including low quality hay or straw. There are two potential problems with this approach. Firstly, cattle may eat good quality lush pastures and concentrate substances in preference to poor quality fibre sources offered. Secondly, where animals do eat poor quality fibre sources with low CP% and ME (MJ/kg DM), the need increases for higher quality forages or concentrate supplements to meet energy and protein requirements.

Shepherd (2003) showed using simulation modeling that many cows within large herds will have limited access to grazed forage. This arises under circumstances where cows that are milked early in the milking process have access to pasture before those milked later. These cattle will consequently have inadequate intakes of fibre.

It is recommended that a good quality fibre source such as good quality cereal, pasture or legume hay is offered as a prevention of acidosis. Distribution of the hay should be such that all cattle get access. Amounts of forage in the diet should be calculated to provide more than 32% NDF, with greater than 80% from the forage (National Research Council, 2001).

### ***1.11.2 Gradual adaption to starch rich feeds***

The majority of dairy cows in Australasia are fed some concentrate supplements during lactation. In Australia this is frequently offered twice daily at milking. The potential problems arising from this system depend on the amount and type of concentrate fed. In addition, cows are often fed poorer quality pastures during the dry period with higher NDF concentrations in the diet than during lactation and no concentrate supplement. A sudden dietary shift from high fibre diets to lower fibre/ higher concentrate diets coupled with a decrease in feed intake by approximately 30% during the week prior to calving (Grant and Albright 1996) disrupts rumen function and places cattle at increased risk

from metabolic disorders including acidosis. Therefore, it is becoming increasingly common in the Australian dairy industry to feed a transition diet for several weeks prior to calving. During the transition period, fibre levels in the diet are ideally progressively reduced to allow the introduction of lactating cow concentrates (Lean *et al.* 1998). Transition diets often include smaller amounts of concentrates than are fed after calving to allow rumen micro flora to adjust to a change in diet. Some dairy feeding systems also allow for individual cow identification in the lactating herd and adjustments in concentrate proportions and amount can be made depending on the number of days in milk. Both strategies help minimize the disruption in rumen function, therefore reducing the risk of acidosis. The NSC content of the transition diet should not routinely exceed 38%.

Transition diets are also often used to introduce other dietary controls for ruminal acidosis used frequently in lactating cow rations, including buffers, neutralizing agents, rumen modifiers and antibiotics.

### **1.11.3 Rumen buffers and neutralising agents**

#### *1.11.3.1 Definitions of dietary buffers*

Dietary buffers have been commonly used in dairy cattle diets for a number of years for their perceived positive effect on production and prevention of acidosis. For a compound to act as a buffer in the rumen, it must be water soluble, a weak acid, base or salt thereof and have a pKa near the physiological pH of the rumen. A true buffer should lessen the decrease in pH without causing a pH increase compared with a neutralising agent that elevates pH (Staples and Lough 1989). Buffers used in the dairy industry include sodium bicarbonate, sodium sesquicarbonate, potassium bicarbonate, magnesium carbonate and calcium carbonate, of which sodium bicarbonate is the most common (Erdman 1988). Neutralising agents include sodium carbonate, potassium carbonate, magnesium oxide, sodium hydroxide and calcium hydride (Staples and Lough 1989). Sodium bentonite is used as a buffer but does not have properties consistent with a buffer.

### 1.11.3.2 *Sodium bicarbonate*

The effects of sodium bicarbonate as a buffer have been well researched with the product being available on the market for the last 40 years. Staples and Lough (1989) reviewed 40 studies with typical responses including an increase in milk production and milk fat content. Fat content was probably increased because of an associated decrease in the production of trans-C18:1 fatty acids through ruminal biohydrogenation (Kalscheur *et al.* 1997; Kennelly *et al.* 1999). Staples and Lough (1989) also found an increase in acid detergent fibre digestion in nine of twelve studies reviewed with some articles also reporting increases in ruminal liquid dilution rates. Other responses include increased rumen pH particularly on high concentrate diets in some studies (Kalscheur *et al.* 1997), increased total volatile fatty acid production and increased acetate to propionate ratios (Kennelly *et al.* 1999).

Dietary sodium bicarbonate acts as a buffer in the same way as endogenous sodium bicarbonate found in saliva. Sodium bicarbonate works in an optimal pH range of 6.2 to 6.5 with a pKa value of 6.25. Not all studies have shown an increase in rumen pH in sodium bicarbonate treatment groups. There may be two reasons for this finding. Firstly, if the initial rumen pH was < 6.0, the buffering capacity of sodium bicarbonate would decrease. Secondly, studies with diets containing greater than 30% DM from forage show less pronounced effects by dietary buffers on rumen pH and milk fat percentage (Erdman 1988). Those studies also averaged 6 to 7% more ADF in the diet, possibly contributing to higher endogenous bicarbonate buffering in both control and treatment groups. Good quality ryegrass/ sub-clover pastures in Australia low in NDF and high in soluble sugars have the potential to induce sub-clinical acidosis (Lean *et al.* 2000), particularly when supplemented with concentrates in the bail. The supplementation of sodium bicarbonate in this situation needs further research. Clayton *et al.* (1999) found that the feeding of 200 g/day of sodium bicarbonate has limited effects on measures of gastro-intestinal tract acidity including rumen pH. This may be associated with the high solubility and short period of effectiveness of sodium bicarbonate in the rumen. The use of buffers should therefore not replace the requirement for effective dietary fibre.

#### 1.11.3.3 *Magnesium oxide (causmag)*

Magnesium oxide is classified as a slow-releasing neutralising agent because of its undefined pKa and relative insolubility in water (Erdman 1988). However, a review of a number of articles by Erdman (1988) indicated the effectiveness of magnesium oxide in raising rumen pH and milk fat percentage. Staples and Lough (1989) reviewed responses to magnesium oxide on diets containing corn silage with increases in either or both milk yield (0.4 to 3.1 kg/cow/day) and milk fat (0.03 to 0.7%) recorded when magnesium oxide was included at 0.4 to 0.8% of DMI. The acid consuming capacity of magnesium oxide is between 41.9 and 49 meq/day, significantly higher than other buffers and neutralizing agents such as sodium bicarbonate with 11.9 meq/day (Schaefer *et al.* 1982). Water solubility however is variable and dependant on the particle size of the magnesium oxide product (Erdman 1988). Processing to a minimum size of 425  $\mu$ m appears to enhance the effect (Staples and Lough 1989). Kalscheur *et al.* (1997) found the addition of 0.5% magnesium oxide and 1.5% sodium bicarbonate to both low and high forage diets increased rumen pH. In the same study, buffering had a negative effect on the presence of *trans*-C<sub>18:1</sub> fatty acids in the milk on low forage/ high concentrate diets, increasing milk fat percentage.

#### 1.11.3.4 *Sodium bentonite*

Sodium bentonite is a colloidal, hydrated, aluminium silicate clay, consisting principally of montmorillonite. It is not a buffer but has a high ion exchange and moisture absorbing capacity (Bringe and Schultz 1969). A number of Northern American studies with feed lot dairy cattle indicate bentonite increased milk fat concentrate and yield on high concentrate, low fibre diets (Bringe and Schultz 1969). Australian studies in cows grazing pasture supplemented with up to 10 kg/head/day of grain based concentrates have shown no such effects on milk yield or composition (Ehrlich and Davison 1997; Hamilton *et al.* 1988). However, Ehrlich and Davison (1997) found that feeding 4% bentonite did increase rumen pH, lower rumen ammonia, increase faecal protein and decrease faecal starch. The lack of consistent evidence of effect coupled with necessary high feeding rates (0.5 to 1 kg/head/day) suggests a limited role for bentonite at this stage in the Australian dairy industry.

#### 1.11.3.5 *Calcium carbonate (limestone)*

Limestone has limited buffering capacity in the rumen at physiological pH because of low solubility despite the high potential acid-consuming capacity (Erdman 1988).

Haaland and Tyrrell (1982) found that limestone had greatest effect on buffering capacity between pH 5.0 and 4.5. However, it may regulate pH in the intestines (Kellaway and Porta 1993), increasing faecal pH when added at 1.8 to 2.5% of the diet (Haaland and Tyrrell 1982; Haaland *et al.* 1982; Russell *et al.* 1980). Rumen ammonia and total VFA concentrations do not appear affected with the addition of limestone to the diet (Haaland and Tyrrell 1982; Haaland *et al.* 1982).

### 1.11.4 *Rumen modifiers*

#### 1.11.4.1 *Monensin*

Rumen modifiers and feed antibiotics play an important and significant role in the Australian dairy industry with the positive effects of supplementation increasingly well recognised. Sodium monensin (Rumensin™), an ionophore antibiotic produced by *Streptomyces cinnamonensis* selectively modifies the rumen flora and improves the digestive efficiency of cattle (Lean *et al.* 2000). Modes of action of monensin include modification of rumen total VFAs and increasing propionate percentage (Burrin and Britton 1986), reduction of feed intake (Abe *et al.* 1994; Fox *et al.* 1988; Thonney *et al.* 1981) and changes to rumen gas production including reduced methane output (Stanier and Davies 1981). Monensin also increases the efficiency of feed utilisation (Potter *et al.* 1976; Raun *et al.* 1976), reduces rumen liquid and solid turnover rates (Lemenager *et al.* 1978), decreases ruminal degradation of forage protein (Horn *et al.* 1981), increases retention of zinc and selenium and increases milk production (Lean *et al.* 2000). Milk fat percentage is significantly lower in cows treated with monensin (Abe *et al.* 1994) causing some concern regarding its use in the industry. Importantly, monensin has been found to reduce risk of acidosis *in vitro* (Nagaraja *et al.* 1987) and prevent clinical acidosis in cattle experimentally induced to exhibit acidosis (Avery *et al.* 1980; Nagaraja *et al.* 1981) with rumen pH increasing in treated animals (Horn *et al.* 1981; Nagaraja *et al.* 1981). Monensin acts by inhibiting the growth of *S. bovis*, as well as other major lactate-

producing rumen bacteria such as *Lactobacillus*, *Butyrivibrio* and *Lachnospira* *in vitro* (Dennis *et al.* 1981). D- and L-lactate concentrations in the rumen were lower, rumen pH higher and blood D- and L-lactate, pH and pCO<sub>2</sub> did not change in cattle treated with monensin when acidosis was experimentally induced with finely ground corn or glucose (Avery *et al.* 1980; Nagaraja *et al.* 1982). Importantly, ionophore antibiotics must be present for at least 2 days prior to carbohydrate challenge in order for effective control of acidosis (Nagaraja *et al.* 1981). Table 1.5 summarises the effects of the ionophore antibiotics compared with non-ionophore antibiotics on the inhibition of lactic acid production *in vitro*. Although monensin on its own only has a maximum inhibition of lactic acid production of 76% compared with the control, the combination of monensin and tylosin can achieve 93% *in vitro* equaling that of virginiamycin.

**Table 1.5.** *In vitro* lactic acid inhibition by antimicrobial compounds (adapted from Nagaraja *et al.* (1987))

Compound	Total lactic acid		L(+) lactic acid		D(-) lactic acid	
	<sup>1</sup> IC <sub>50</sub> (µg/mL)	<sup>2</sup> I <sub>max</sub> (%)	<sup>1</sup> IC <sub>50</sub> (µg/mL)	<sup>2</sup> I <sub>max</sub> (%)	<sup>1</sup> IC <sub>50</sub> (µg/mL)	<sup>2</sup> I <sub>max</sub> (%)
Monensin	0.54	76	0.26	80	>24.0	49
Lasalocid	0.57	83	0.30	89	9.5	64
Virginiamycin	0.13	93	0.13	96	0.64	83
Tylosin	0.45	92	0.35	97	1.64	69
Monensin + Tylosin <sup>3</sup>	0.39	93	0.30	98	1.8	69

<sup>1</sup> Concentration of antimicrobial concentration providing 50% inhibition of lactic acid concentration compared with control fermentation

<sup>2</sup> Maximum inhibition of lactic acid production compared with control

<sup>3</sup> Monensin and tylosin mixed at a ratio of 3:1 (w:w)

Milk production responses indicate that the combined benefits of this product fed at 250 to 300 mg/head/day, are reflected in increased milk production (Beckett *et al.* 1998). Given, that monensin has an effect in controlling acidosis, especially at higher dose rates.

(450 mg/head/day), use of the product is recommended to control disease and increase milk production.

#### 1.11.4.2 Lasalocid

Lasalocid (Bovatec™) is also an ionophore with similar activity to monensin although probably not used as commonly as monensin as a feed additive in the Australian dairy industry. Lasalocid treatments of cattle with experimentally induced acidosis have higher rumen pH and lower L- and D-lactate concentrations than control cattle or cattle treated with monensin (Nagaraja *et al.* 1982; Nagaraja *et al.* 1985). Table 1.5 indicates maximum inhibition of lactic acid production *in vitro* compared with the control is approximately 83% in comparison to monensin of 76% (Nagaraja *et al.* 1987). Dennis *et al.* (1981) found that lasalocid delayed the growth of *S. ruminantium* *in vitro* at concentrations of 6 to 12 µg/ml for 19 to 25 hours respectively. Monensin also delayed growth at these concentrations, but only for 10 hours. Increased total VFA concentrations in combination with increased molar concentrations of propionate were also found in lasalocid treated cattle compared with controls when experimentally induced with acidosis, a similar finding to monensin (Nagaraja *et al.* 1981). Thonney *et al.* (1981) found that lasalocid treated cattle cows had higher dry matter intakes ( $P < 0.01$ ) and greater weight gains ( $P < 0.005$ ) than monensin treated cows.

It does appear likely that this product will have efficacy in modifying the risk of acidosis. There is less information available on responses to lasalocid in pasture fed cattle than on monensin. Lasalocid is an alternative ionophore to monensin and use can be recommended under similar circumstances.

#### 1.11.4.3 Virginiamycin

Virginiamycin (Eskalin™) is an antibiotic with primarily gram positive activity. It is effective in reducing lactic acid production *in vitro* (Nagaraja *et al.* 1987) by removing *S. bovis* (Hedde *et al.* 1980), the organism primarily responsible for lactic acid production (Lean *et al.* 2000) on starch-based diets. Al Jassim and Rowe (1999) found *S. bovis*, which produced only L-lactate, to be very sensitive to virginiamycin *in vitro* at

concentrations of 2 µg/ml. Effects seen on addition of virginiamycin include higher rumen pH, lower *Lactobacillus* and *S. bovis* counts causing lower L-lactate concentrations (Clayton *et al.* 1999; Coe *et al.* 1999), higher faecal pH (Clayton *et al.* 1999) and higher propionate as a percentage of total VFAs on a 22% concentrate diet (Hedde *et al.* 1980). Other lactic acid producing bacteria include *L. vitulinus* and *S. ruminantium*. Sensitivity of *L. vitulinus* isolates, which produced D- and L-lactate, to virginiamycin varied from being highly sensitive to resistant at 8 µg/ml virginiamycin *in vitro* (Al Jassim and Rowe 1999). *S. ruminantium*, a gram negative bacterium, also produced D- and L-lactate, and was very resistant to virginiamycin even at 8µg/ml (Al Jassim and Rowe 1999). The combination of monensin and tylosin as outlined in Table 1.5 needs further investigation on pasture-based diets as an alternative to virginiamycin. Other effects of supplementing with virginiamycin include increased average daily weight gain and/ or feed conversion efficiency, reduction in incidence and severity of liver abscesses (Rogers *et al.* 1995), and reduced *in vitro* digestibility depressing feed intake in some trials (Thomiley *et al.* 1996).

#### 1.11.4.4 Tylosin

Tylosin (Tylan™), a macrolide antibiotic was registered for use in 1997 as a feed additive in the Australian dairy industry. Tylosin, like virginiamycin has been shown to be effective in reducing lactic acid production *in vitro* (Nagaraja *et al.* 1987). Tylosin reduces total VFAs, and D- and L-lactate concentrations in the rumen, however milk production and milk fat content is not affected (Lean *et al.* 2000). In addition, the feeding of tylosin reduces liver abscesses, a potential sequel to acidosis, by 40 to 70% (Nagaraja and Chengappa 1998). The combined response of tylosin and monensin has also been examined (Coe *et al.* 1999; Lean *et al.* 2000). This combination produced significantly lower rumen pH, higher volatile fatty acid and blood urea nitrogen concentrations and lower mean glucose concentrations than animals treated with virginiamycin or tylosin (Lean *et al.* 2000). The lower pH was probably due to the higher concentrations of total VFAs in the rumen rather than lactic acid, which was lower in the plasma than other treatments not containing tylosin. When tylosin is mixed with monensin at a rate of 1:3, the maximum inhibition of lactic acid production *in vitro*

compared with the control is 93%, a rate equaling that of virginiamycin (Table 1.5). The tylosin-monensin combination also sustained a higher level of milk production, greater weight gain over the trial period than other treatments in combination with significant changes in rumen and blood metabolites (Lean *et al.* 2000).

## 1.12 Conclusion, aims and hypotheses

Subclinical acidosis is likely to be an important nutritional problem in dairy herds in Australasia, both economically and as a health issue. American studies have outlined the importance of this condition in herds fed high levels of concentrates and various diagnostic tools which may be employed to confirm the presence of acidosis. Notwithstanding this, acidosis under Australasian conditions remains poorly defined. Higher quality pastures containing increasing concentrations of water-soluble carbohydrates and less effective fibre, particularly in the winter/ spring season are being offered to dairy cows. This good quality pasture combined with the supplementation of increasing amounts of highly fermentable concentrates, used as a tool to boost production, predisposes the herd to acidosis. Previous studies have primarily used rumen pH as the sole indicator of determining acidosis in cows. Rumen pH is known to fluctuate greatly in response to diet and therefore may not be a good sensitive or specific test for diagnosis of acidosis. Extensive research on the effects of rumen buffers and modifiers has been conducted. However the role of rumen buffers and modifiers in the control of acidosis in dairy cows in pasture-based systems needs further definition.

The primary aim of this project was to define a case of acidosis in Southern Australian dairy herds, thereby determining the prevalence of acidosis in this area. A case of acidosis would be defined both by rumen biochemistry indicating rumen function and possible outcomes of acidosis.

Secondarily, after defining acidosis, the aim was to determine risk factors associated with acidosis in dairy herds. This included evaluating the effectiveness of rumen buffers,

modifiers and antibiotics on acidosis and quantifying the quality of pastures within regions at different seasons.

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## Chapter 2.

# MATERIALS AND METHODS

## 2.1 Summary

*This chapter details the biochemical, epidemiological and statistical methods used in the randomised cross-sectional survey examining the effects of acidosis on cattle health and production. One hundred dairy herds were randomly selected in five areas of NSW and Victoria; 34 herds in Finley, 17 in Camden, 9 in Taree, 21 in the Western Districts and 19 in Gippsland. An orally presented survey of diet, feed and pasture management and cattle health and production data was conducted on each farm. Lactating and transition herds (cows 3 weeks prior to calving) were scored for body condition, rumen fill and faecal consistency. Lactating herds were also scored for lameness. A fresh pasture sample was taken from the day and night milking paddock for a feed analysis. Eight fresh cows (< 100 days in milk) were randomly selected and samples of faeces, blood and rumen fluid (rumenocentesis and stomach tube) were taken. Stomach tube and selected rumenocentesis rumen fluid samples were assayed for volatile fatty acids, ammonia and D-lactate. Selected samples were also assayed for L-lactate. Blood and faecal samples were stored for future use.*

## 2.2 Introduction

The general materials and methods outlined in this chapter describe the herds and cows studied, the consumables and equipment used in data collection and the collection and preparation of samples for analysis. The specific scoring systems used in the study and the techniques used in the biochemical analysis of samples are detailed in the specific materials and methods section. It also describes epidemiological and statistical approaches to the data. This research project was granted approval from the University of Sydney ethics committee for the period of two years from July 2001 to July 2003. All animal sampling took place during this time.

## 2.3 General materials and methods

### 2.3.1 *Study design*

#### 2.3.1.1 *Study type*

A cross-sectional survey was conducted to define 'acidosis' in Southern Australian dairy herds and to determine risk factors associated with 'acidosis' in these herds.

#### 2.3.1.2 *Sample size*

An initial estimate of 120 herds was made based on a number of anticipated risks for hypotheses of interest. However, the number of farms sampled was limited to 100 due to cost, time and labour constraints, representing 1.2% of Victorian and New South Wales dairy farms (Dairy Australia 2003). The 100 herds provide for determining a difference in the odds of acidosis being present for a univariate association with a statistical power of 0.6, with  $\alpha = 0.05$  for a prevalence of 30% in one group and 40% in the group of interest.

### 2.3.2 *Collection of herds for inclusion in the study*

#### 2.3.2.1 *Herd selection*

Units of interest in this study included the herd, cows within herd and samples obtained from these cows. The target population was dairy cows in herds in New South Wales and Victoria (Figure 2.1). One hundred dairy herds were selected from lists supplied by local veterinary practices in five areas of NSW and Victoria; 34 herds in Finley region (July-August 2001), 17 in Camden region (November-March 2001-2), nine in Taree (April 2002), 21 in the Western Districts, Victoria (September-October 2001) and 19 in Gippsland (May 2002). Sampling dates for each area are summarized in Table 2.1. The Finley region encompassed the Northern Victoria irrigation area centred around Numerkah and the Southern Riverina centred around Finley. The Camden region encompassed farms situated in close proximity to Camden, Bowral and Jamberoo on the South Coast of NSW. Herds sampled in the Western Districts of Victoria were within driving distance to Timboon and Heywood whilst herds sampled at Gippsland were within driving distance to Foster. To be eligible for selection herds had to be within

approximately a 1.5 hour drive of the veterinary clinic to allow sample processing to occur and to have cows in the first 100 days of lactation present. Herds were selected at time of enrolment using a random number chart by counting down the list by the number (0 to 9) arising from the random numbers table and counting sequentially using the list as continuous until a pre-requisite number of herds in each area was filled. Numbers enrolled for each area reflected numbers of dairy herds in the region that each site represented. Farmers were contacted to ensure their willingness to participate in the study. The promise of feedback of results from the study was used as an inducement to encourage participation of farmers in the study. Replacements for farmers who were not willing to participate were either the next herd identified by the random numbers process or, ultimately, volunteer herds from the area.

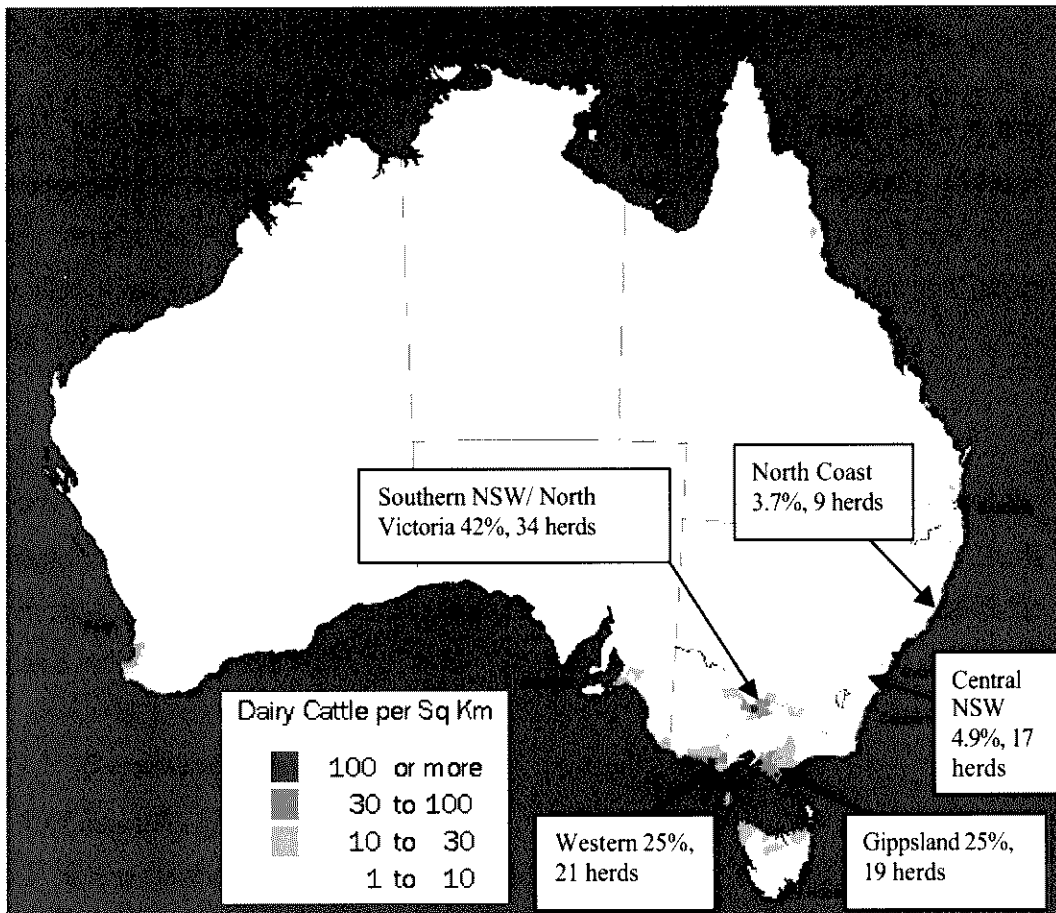
**Table 2.1.** Sampling dates for each region

<b>Region</b>	<b>Number of herds sampled in region</b>	<b>Sampling period</b>
Finley <sup>A</sup>	34	19 July – 5 September 2001
Western District of Victoria	21	2 – 31 October 2001
Camden <sup>B</sup>	17	12 December 2001 – 28 March 2002
Taree	9	3 – 12 April 2002
Gippsland	19	29 April – 21 May 2002

<sup>A</sup>Herd F033 was sampled on 4 November 2001 and herd F034 sampled on 15 August 2002

<sup>B</sup>Herd C072 sampled on 13 June 2002

**Figure 2.1.** Areas where herds were sampled in relation to major dairy regions in Australia. Number of herds sampled from each of the districts approximated the percentage of milk produced by the regions in 2001 (ABARE, 2004).



Herds selected within each region, apart from Camden, were sampled over approximately a one month period and measures were therefore clustered within district, herd and time of sampling. Criteria for eligibility limited the number of herds available in the Western Districts, central New South Wales and sub-tropical New South Wales and Gippsland. Rejection rates were less than 10% of farmers approached in all regions, except Western Districts, where rejection rates approached 20%. Number of herds sampled from each of the districts approximated the percentage of milk produced by the regions in 2001; Southern NSW and Northern Victoria 42%, Gippsland 25%, Western Districts 25.2%, Central NSW 4.9% and North Coast 3.7%, of total milk respectively (ABARE 2004). Two of the five areas sampled, Camden and Taree, were difficult to obtain random

samples from due to the sample processing and cost constraints and Camden herds were over-represented compared to milk production from the central region of NSW. Herds in the Taree region were selected by the local veterinary clinic because there were relatively few herds within driving distance to get samples back to the laboratory for processing.

Each herd participating in the survey was identified with a letter and number corresponding to area and number of herd from the total number, for example, 34 herds were sampled in Finley and 21 herds in the Western Districts. Herd 1 in Finley to be sampled was identified as 'F001'. The first herd to be sampled in the Western Districts was 'WD035'.

#### *2.3.2.2 Exclusion criterion*

Herds were excluded from the study if they were not willing to have eight cows of the samplers choice from the lactating herd sampled for rumen fluid, blood and faeces. Herds were also sampled if there were not sufficient cows in the lactating herd that satisfied the criteria outlined in section 2.3.2.

### **2.3.3 Questionnaire development**

#### *2.3.3.1 Selection of risk factors investigated*

Risk factors investigated were selected predominantly from those factors which have previously been identified or suggested in the literature as being associated with the acidosis syndrome. Farmer demographics were also included in the survey to build confidence with the respondent and give an indication of the type of farms surveyed.

#### *2.3.3.2 Questionnaire design*

The survey contained questions on farm management considered to be pertinent to describing farm demographics and to acidosis, based on an evaluation of literature published on factors leading to acidosis in ruminants and experience of the condition in the field. Open-ended, partially closed and closed questions were used in the questionnaire. Questions relating to possible confounding factors were also included. Scoring sheets for assessment of lactating cow and transition cow herds were also

determined. A draft questionnaire was used on two farms in the Camden region to assess the appropriateness of the questions and to refine interview technique and farm and herd evaluation techniques before commencement of the survey. Amendments were made to the survey design as required and a final questionnaire completed.

#### **2.3.4 Assessment**

The interview and sampling were conducted by the investigator over a 10 month period, with each visit taking between three to four hours. The visit to each property took place over two days. The formal interview, farm walk, pasture assessment and herd assessment was conducted before afternoon milking on day 1. The sampling of the eight randomly selected lactating cows was conducted after morning milking on day 2. Each part took approximately two hours.

##### **2.3.4.1 Blinding**

The interviewer was blind to farming practices or previous health problems relating to the herd and individual cows selected prior to selection of the herds and cows. A single person administered all surveys and was not aware of the results of rumen samplings from the cows until after the survey was conducted. When diets were later entered into CPM (Cornell\*Penn\*Miner, 2003) (version 3.0.4a), the data entry person was blinded to the rumen biochemistry results from each herd. The assessors of pasture texture were also blinded to other results pertaining to the herds from which the pasture samples came.

##### **2.3.4.2 Recall period**

The recall period for the study was up to 12 months prior to the interview. All measures reported in the thesis are determined by farmer recall except assessment conducted by the interviewer on the day and biochemical results reported from analysed rumen fluid. The weight of concentrate fed on farm was also checked on the day and concentrate specifications checked with feed companies.

##### **2.3.4.3 Feedback**

Individual feedback was given as an incentive and benefit to those dairy producers who

participated in the survey. Participants were sent a letter of appreciation, a summary of general conclusions from the study, an individual herd and cow assessment, a copy of the pasture analysis from their property and a summary of how their feeding practices related to feeding practices in their area.

### **2.3.5 Questionnaire presentation**

The questionnaire (Appendix, form 1) was answered in a face-to-face interview with the farmer on the afternoon before sampling. Questions were asked and recorded on the sheet provided for each farm. The following list defines individual questions that could have been interpreted differently with headings relating to the survey headings.

#### Part 2

1. Total size of the home farm was defined as the number of acres/ hectares immediately accessible to the milking yard, i.e. the cows are able to walk to the dairy. This included the house block with the dairy and any adjacent blocks. Out-blocks where dry cow, heifers or other stock were kept were not included.

2. Total size of the milking area includes the area defined in question 1 minus any land the cows cannot graze, for example bush.

3. Improved pasture was defined as pasture the cows could graze that was not fodder crops or native grass. Improved pasture was any pasture that had been planted, for example ryegrass, clover, kikuyu or fescue and included irrigated pasture.

Irrigated pasture would normally be a subset of improved pasture.

Fodder crops were not included in improved pasture and included crops such as lucerne, sorghum, and turnips.

6. The total amount of milk that the herd produced the day before the questionnaire was presented (excluding calf milk) was confirmed with data provided by the milk factory.

7. The somatic cell count was checked using the two most recent milk factory test sheets. This figure represented the mean of the two figures from the day preceding and on the day of visit where applicable. In herds that did not get daily reports, the most recent factory test result was recorded.

8. The milk fat and protein percentage was checked using the most recent factory test sheet.

### Part 3

4. Percentage farm renovated was defined as the percentage of farm ploughed and planted and did not include that portion of farm only over-sown.
8. New pasture was defined as pasture planted with ryegrass or fescues or other grasses in the last 12 months. This included pasture areas that were over-sown and renovated.
9. P,N,K,S = phosphorus, nitrogen, potassium, sulphur applied to pastures

### **2.3.6 Collection on farm and clinical assessment**

#### *2.3.6.1 Collection on farm*

A significant amount of information about the farm and herd was collected following the questionnaire by walking around the farm and through the dairy and in discussions with the farmer. This information was recorded on the “collection on farm” form used for each farm (Appendix, form 2). The following explanations refer to the “collection on farm” form.

#### Lactating cows

The amount fed to each cow in the bail (question 1) was checked by weighing two samples. If feed compositions were unknown, a sample of the feed was bagged and kept in a sealable plastic bag, labeled with date and herd ID.

The total amount fed outside (question 2) included hay, straw, silage and concentrates either in a total mixed ration or fed separately.

#### Lactating cows – concentrates

The mix (question 1(ii)) included a pre-purchased mix of the grains and the amount of mix fed was specified as either /cow/day or total/day. If the percentage breakdown or individual amounts were available, lines a) to e) were used and a mix specified.

Corn gluten feed (question 1(iii)) was also known as milk richer (distributed by Castlegate Holdings). Corn gluten meal is different from corn gluten feed and was recorded under ‘other’. Other byproducts included as ‘other’ included, for example, citrus pulp.

### Lactating cows – other feeds

For each of the silage, hay and straw questions, quality was described by qualities such as fresh versus musty, moldy, old, amount of leaf versus stalk content and weed content. Total amount fed was given in (kg) and converted from bales fed. Amount wasted was estimated from a visual estimate of wastage on ground on the sampling day.

Samples of silage and hays fed were kept when necessary to analyse and placed in sealed plastic bags.

### Lactating cows – pasture

Pasture height grazed (question 5) was the average height pasture to which the pasture was grazed (not including areas left because of previous or recent cow dung contamination).

Pasture sample taken (question 10) referred to using the 0.1m<sup>2</sup> square to cut five random pasture squares to the average height the pasture was grazed. This was done by standing in the middle of the paddock or grazing strip and throwing the square five times around the paddock. Areas that had not been grazed due to previous dung patches were not sampled, as these were considered to be less available to the cows.

### Transition cows

All definitions for the lactating cow section above applied to transition cows.

A pasture sample was not taken from a paddock containing transition cows.

### Lameness examination

Cows that are allowed to walk at their own pace (question 5) will be able to visualize the track in front of them in order to avoid stepping on sharp stones or in holes. If the farmer is pushing the herd too quickly, the cows at the back will start pushing forward consistently and may weave from side to side at the back of the herd. The cows where possible were assessed for gait abnormalities on the day of visit when these were walking

to the dairy.

#### *2.3.6.2 Identification of cattle from the herd for clinical assessment*

In addition to the eight cows selected for rumen, blood and faecal sampling, 15 cows were randomly selected from the lactating herd for clinical assessment. Cattle were identified using freeze brand/ ear tags for scoring of body condition, rumen fill and locomotion to ensure that one cow was not scored twice. The cattle that were identified in the lactating herd were scored for body condition, rumen fill and locomotion. Fifteen cows were also randomly selected for clinical assessment from the transition herd (cows 3 weeks prior to calving) if the number of cows in the transition herd totaled more than 15. Cows in the transition herd were scored for body condition and rumen fill in a similar way to the lactating herd. Fifteen animals were selected randomly from the lactating and transition herd (where applicable) using the following criteria;

- Where herd size < 20, the first 15 cows in the paddock were scored.
- Where herd size 21 - 50, 15 even identification (ID) numbered cows were scored.
- Where herd size > 50, 15 cows with ID numbers divisible by five, then numbers divisible by two, if necessary, were scored.
- Where animals identified by name only, score cows as randomly as possible throughout the paddock.

The ID number, body condition score, rumen score and locomotion score were documented on the scoring sheet provided (Appendix, form 3).

Twenty faecal samples were scored from both the lactating and transition herds. The transition herd was only scored if the number of cows was greater than 15. The selection of faecal samples was random and no faecal sample was scored twice. The faecal samples were randomly selected by starting at one corner of the paddock and walking diagonally towards the opposite corner, scoring any faecal sample en route.

### 2.3.7 Cow selection

Eight cows were sampled from each herd for rumen fluid, blood and faeces. These animals were randomly selected from the herd using a random number chart after satisfying the following criteria;

1. They had to be lactating cows, in the first 100 days of lactation.
2. The total sample consisted of three primiparus and five multiparus animals.

Herd records were examined prior to the sampling day and a total of 16 animals were selected (six heifers and 10 mature cows) where possible using a random numbers table. Of these, three heifers and five cows were selected on the day. Some herds did not have extra animals fitting the criteria. Cows were sampled 2 to 4 hours after morning milking of individual cows when cows were not fed either a TMR or PMR immediately after milking. Cows fed a TMR or PMR immediately after milking were sampled 4 to 6 hours after milking. The identified cows were milked and then held in the milking yard or in the adjacent yard until sampling time. Access to water was given where possible. Data that were collected for each animal on the sampling day is included in Table 2.2. All data were recorded on the day on the provided herd collection sheet. Some herds sampled did not herd test individual animals, therefore these data were not available.

**Table 2.2.** Data collected from individual cows on sampling day

Variable	Units
Cow ID (freeze brand/ ear tag/ name)	Numbers 1-10,000/ name
Parity	Lactation number 1 – 10
Days in milk	Days 1 – 100
Last herd test	Date
Somatic cell count at last herd test	5,000 – 1,000,000
Milk produced per day at last herd test	Litres
Percent fat last herd test	Percent
Percent protein last herd test	Percent
Health history in last 4 months	History of health incidences, farmer treatment, veterinary treatment
pH of rumen fluid (via stomach tube and rumenocentesis)	2.0 – 8.0

When cows were randomly selected from the herd list and satisfied the criteria but had a debilitating illness that could affect the results of the trial on the day of sampling these were removed. Examples of such illnesses included, but were not limited to mastitis, metritis and keratoconjunctivitis. If the cow was suffering from an illness that would not affect the results of the trial and risk to the cow was not increased by sampling on the day, then the cow was not excluded from the trial. Cows that could not be caught to enable samples to be collected on the day were also excluded from the trial. Of the cows held after milking, the first eight eligible cows to be restrained were sampled. In some cases it was not possible to hold 16 animals, for example, when there were only three heifers in the herd that were < 100 DIM.

### **2.3.8 Sampling procedures**

#### *2.3.8.1 Blood collection*

Blood samples (10mls and 5mls per tube) from the eight selected cows were collected by venipuncture of the coccygeal vein or artery using an 18-gauge needle and vacuum collected into one lithium heparin and one fluoride oxalate vacutainer-tube, respectively. The site for venipuncture was prepared prior to sampling with an alcohol swab. All tubes were labelled with the respective animal identification number from the trial immediately after collection. Samples were kept on ice in an esky until centrifuged.

#### *2.3.8.2 Faecal collection*

Faecal samples from the eight cows were taken manually using an examination glove per rectum. The glove was lubricated with clean, non-sterile water prior to collection. The faecal sample (approximately 50 g) was placed in a 50 g sterile plastic specimen jar and labeled with appropriate animal identification. Faecal samples were kept on ice in an esky and transported to the laboratory.

#### *2.3.8.3 Rumen fluid collection*

Rumen samples from the eight cows were obtained by rumenocentesis and stomach tube. Both methods were used for several reasons. Firstly, a number of analyses were

conducted on the rumen fluid including volatile fatty acid analysis, ammonia and D- and L-lactate concentration. Rumen fluid was also collected for quantification of rumen bacteria using PCR techniques and rumen protozoa (these were not presented in the thesis results due to cost and time factors). Twelve ml of rumen fluid was stored for these analyses. Previous studies have indicated that a minimum of 3 ml is able to be obtained using rumenocentesis (Garrett *et al.* 1999), therefore it was decided to use stomach tube rumen fluid samples for all biochemical analyses except rumen pH in order to ensure enough sample to use. Rumen pH was measured in both the rumenocentesis and stomach tube sample from each cow, however previous studies have shown rumen fluid from stomach tube samples to have a lower sensitivity for rumen pH than that obtained from rumenocentesis (Duffield *et al.* 2000). Samples of ruminal fluid taken by stomach tube have a higher pH than those obtained through a rumen cannula (Erdman 1988), either as a result of salivary contamination or sampling in the fibre mat of the rumen. Therefore, in this study it was decided that rumen pH measured using rumenocentesis may be a more appropriate measure than rumen pH measured using stomach tube. The study provided a good opportunity in a large field study to compare rumen pH measured by rumenocentesis and stomach tube (Chapter 4).

#### 2.3.8.3.1 Rumenocentesis

A 4 cm<sup>2</sup> area on the left side of each cow was clipped and surgically prepared with alcohol and betadine spray. This area was one hand-width (10 cm) caudal to the last rib and two hand-lengths (20 cm) ventral from the transverse processes of the lumbar vertebrae. Ten ml of 2% lignocaine (20 mg/mL) was injected subcutaneous to the clipped area. A 16 gauge, 1½ inch monoject needle attached to a 30 ml syringe was inserted into the rumen. Five to 20 ml of rumen fluid was withdrawn from the rumen and placed into a plastic beaker. A new needle was used for every sampling. Cetrimide™ spray was used on the area post-sampling. Previous studies have used 13 cm long needles (Garrett *et al.* 1999). Longer needles were tested on a small number of cows (n=5) prior to the start of the study but it was found that the needles clogged, bent and did not remain sharp with the multiple use for which they were designed. On occasions where no rumen fluid was able to be obtained when the plunger was withdrawn on the first sampling, the

bevel of the needle was moved at a slightly different angle. If a sample was still not obtained, or a small sample was obtained only, and it appeared that the needle was blocked, the syringe was removed from the needle, 5 ml of air drawn into the syringe and the syringe then attached back on the needle. The air was injected into the rumen in an attempt to dislodge the blockage. If a sample was still not obtained, or if at any time the rumen fluid obtained was contaminated with blood, the sample was discarded and a rumen fluid sample was collected by stomach tube only. On two farms (one at Finley and one at Taree), there were no facilities to collect rumen fluid via rumenocentesis, so the samples were not taken due to occupational health and safety reasons.

#### 2.3.8.3.2 Stomach tubing

A stomach tube was constructed from a 3.5 metre long, 19mm diameter reinforced clear plastic garden hose with an aluminum, multi-holed probe inserted on one end to act as a filter. The aluminum end was attached to the garden hose with rivets. An equine stomach pump was attached to the other end once it was inserted into the rumen. A left-hand, drink-water gag was inserted between the top and bottom left molars of each cow to keep the mouth open during the procedure. The tube was then inserted down the oesophagus into the rumen of each cow. The stomach pump was attached and rumen fluid pumped into the tube until the rumen fluid was able to be seen and examined through the tube by the person holding the tube in the cows mouth. In cases where no rumen fluid filled the tube, the tube was moved slightly back or forward until fluid was found. The colour and consistency of the rumen fluid was able to be assessed in a preliminary fashion prior to removal of the tube from the rumen to assess for saliva contamination. When the tube contained a minimum of 100 ml rumen fluid (enough to be seen), the tube was withdrawn and the gag removed. In 90 % of samplings, significantly more fluid was obtained. The last portion of rumen fluid to be drawn into the tube was placed in a plastic beaker and tested for the presence of saliva contamination in the sample by placing a finger into the sample and withdrawing it. If there was a 'stringing' effect, the sample was discarded and a further sample taken. If three samples were taken and there was still saliva contamination, then the sample was marked accordingly and was not used in further analyses.

#### *2.3.8.4 Pasture sampling*

Pasture samples were collected from the pasture grazed by the cattle on the day before sampling. The pasture sampled was taken from either the next strip to be offered, if the farm was being strip grazed, or another paddock that the farmer deemed to be of the same pasture type, height and density to that which the cows were grazing that day. If the night paddock that was going to be grazed prior to the sampling day was different to the day pasture, it was also sampled. One 0.1 m<sup>2</sup> plastic quadrant was randomly placed on the area/s selected, replicated 4 times (total =5), with the pasture within the quadrant clipped using electric hedge clippers to the expected grazing height. The sample quadrants were obtained by standing in the middle of the paddock to be sampled and throwing the quadrant around at 5 points of the circle. Samples were placed into labeled sealable plastic bags and transported on ice in an esky to the field laboratory.

#### *2.3.9 Labeling of samples*

Samples were labeled using a standard system to ensure ease of processing and identification at a later time. Samples were labeled with date of sampling and a letter/number ID corresponding to district taken, number of animal sampled in the study and type of sample in that order. For example, a rumen fluid sample collected for ammonia analysis from cow 1 sampled from herd 1 in Finley was labeled F001A, where F = Finley, 1 = cow 1 sampled in the study and A – sample for ammonia analysis. All rumen samples were coded according to what they were processed for and blood samples according to what additive was in the collection tube. These are outlined in Table 2.3.

**Table 2.3.** District codes for labeling of samples

<b>Sample/ district</b>	<b>Code</b>
Finley	F
Western Districts	WD
Gippsland	G
Camden	C
Taree	T
Rumen protozoa	P
Rumen VFA	V
Rumen ammonia	A
Rumen fluid spare (X2)	S
Plasma – lithium heparin	L
Plasma – fluoride oxalate	F

### **2.3.10 Preparation of samples for analysis**

#### **2.3.10.1 Blood samples**

All sample tubes were centrifuged at the ambient temperature at 3,000 rpm for 15 mins in a MSE coolspin centrifuge after sampling each day. The plasma was decanted into labelled 5 ml polypropylene tubes then frozen at  $-20^{\circ}\text{C}$  for later analysis. However, due to funding restrictions, these samples were not analysed. The polypropylene tubes were labelled with standard animal identification.

#### **2.3.10.2 Faeces samples**

The faecal samples were placed in individual specimen containers, labeled with standard animal identification and frozen at  $-20^{\circ}\text{C}$  for later analysis. Due to funding restrictions, these samples were not analysed.

#### **2.3.10.3 Rumen fluid samples**

The raw rumen fluid stomach tube samples were placed in a 100 ml plastic container and the pH measured using a pH meter (model ECpHScan-WP3). Two mls of the raw sample in the plastic beaker was aliquoted into each of two, 5 ml polypropylene tubes using a Soccorex 5 ml pipette. The 2 ml sample was aliquoted into the polypropylene tubes with

2 ml of 0.9% formalin-saline solution to obtain a dilution ratio of 1:1. This occurred in the field. These samples were to be used for counting protozoa. The tubes were weighed three times; before the formalin-saline solution was put into the tubes; after the formalin-saline solution was placed into the tubes; and after the rumen fluid was placed into the tubes. The weights were recorded in a book so that accurate calculations of the ratio of rumen fluid to formalin-saline could be established. All weighing of tubes was done at the field laboratory. The rumen fluid samples with formalin-saline were not used for this thesis.

The remaining portion of the raw filtered sample of rumen fluid was placed in two 12 ml centrifuge tubes in the field and kept on ice until centrifuged. This was done as soon as possible at 3,000 rpm for 15 mins using an MSE coolspin centrifuge at the ambient temperature. Ten ml of the supernatant was removed, divided into two aliquots of 5 ml each and stored in polypropylene tubes until analysed for rumen ammonia and rumen volatile fatty acids. The remaining supernatant was aliquoted equally into two 5 ml polypropylene tubes as spare rumen fluid. The samples were labeled with standard animal identification, and frozen at  $-20^{\circ}\text{C}$  until analysis. Data collection is defined in Table 2.4.

**Table 2.4.** Data collection summary for samples

<b>Variable</b>	<b>Sample preservation</b>	<b>Units</b>	<b>Place of analysis</b>
Rumen pH	Raw rumen fluid	2.0 – 8.0	In field
Rumen Protozoa	Raw rumen fluid + 1% formalin-saline Room temperature	$10^5$ /mL	In storage at present
Rumen bacteria	Supernatant – frozen (use spare fluid)		Murdoch University
Rumen ammonia	Supernatant – frozen	mmol/L	Agriculture WA
Rumen VFA	Supernatant – frozen	mmol/L	Agriculture WA
Rumen D-lactate	Supernatant - frozen	mmol/L	Agriculture WA
Rumen L-lactate	Supernatant - frozen	mmol/L	Agriculture WA
Spare rumen fluid x2	Supernatant – frozen		

#### 2.3.10.4 Pasture samples

Pasture samples were weighed to give a pre-drying wet weight, frozen as quickly as possible, then transferred to labeled paper bags and freeze-dried at the MC Franklin laboratory, University of Sydney. The samples were then reweighed to determine dry matter (DM) % and submitted for NIR modeling to Dairy One forage testing laboratory, 730 Warren Rd, Ithaca, New York, 14850. Each sample was analysed for DM, NDF, crude protein (CP), degradable protein, crude fat, ash, acid detergent fibre (ADF), lignin, non-fibrous carbohydrate (NFC), non-structural carbohydrate (NSC), starch, sugar, calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K) and sulphur (S) content.

## 2.4 Detailed Materials and Methods

### 2.4.1 Clinical assessment of the herd

#### 2.4.1.1 Body Condition Score

Body condition scoring is a method of evaluating the tissue reserves of dairy cattle that is relatively unaffected by gut-fill, pregnancy or skeletal size. Body condition score was assessed using the five-point scale (Edmondson *et al.* 1989), where score one is emaciation and five is obesity (Appendix, Table 2). Animals were scored to 0.25 points on the scale. Body condition scoring was performed by assigning a score to each body part of the cow that most closely matched those shown on the chart. A single overall body condition score was assigned. Fifteen cows from both the lactating herd and transition herd (where applicable) were condition scored at random in the paddock.

#### 2.4.1.2 Rumen Fill

Rumen scoring of cattle is a method of evaluating rumen fill and fibre ingestion. Cows that have ingested large amounts of fibrous feeds such as hay may have a greater rumen fill than animals that have ingested feeds with little fibre. Rumen fill was scored using a five-point scale where 1 = flat and 5 = fully distended rumen. Fifteen cows from both the lactating herd and transition herd (where applicable) were condition scored at random in the paddock. The rumen fill was scored by examining each cow from the back, and comparing the left para-lumbar fossa (rumen fill) to the right para-lumbar fossa.

#### 2.4.1.3 Locomotion Score

Cows in the lactating cow herd were examined for lameness. There are a number of reasons for lameness including laminitis, a condition directly related to acidotic episodes. Cows were scored using a five-point scale (adapted from (Sprecher *et al.* 1997), Appendix, Table 3). A locomotion score is based on position of the back when walking and standing, position of the feet and favoring of the feet when walking. Results were recorded on the survey form.

History of foot lameness and treatment by either the herdsman or veterinarian in the last four months was recorded for each of the eight cows sampled.

#### 2.4.1.4 Faecal score in paddock

Faecal scoring is a method of evaluating the passage of digesta through the cow. Explosive scours may be linked to acidotic episodes. Faecal scoring was performed by applying a score to a dung sample in the paddock that most closely matched the description on the scoring sheet. Dung samples were scored between one and five using the following criteria;

- 1- Firm cowpat, well formed, no evidence of excessive liquid component.
- 2- Less formed than (1) but still holding shape, may contain whole grain.
- 3- Softer, less formed and evidence of more liquid, may contain grain.
- 4- Minimal formation of cowpat on ground, may contain grain.
- 5- No formation of cowpat on ground, scouring on ground as cow walks, may contain grain.

Twenty faecal samples were scored from both the lactating and transition herds. The transition herds were only scored if the number of cows was greater than 15. The selection of faecal samples was random and no faecal sample was scored twice. The faecal samples were randomly selected by starting at one corner of the paddock and walking diagonally towards the opposite corner, scoring any faecal sample en route.

#### *2.4.1.5 Faecal staining score on animal*

Faecal staining was scored in the lactating herd as part of the collection on farm. A score for overall faecal staining of the herd was given to each lactating herd. This was ascertained by estimating the percentage of cows with evidence of scouring around the perineum using the following five-point scale;

- 1- All cows clean around tail
- 2- Mild staining of switch/ tail 15 - 20%
- 3- Marked faecal staining, some explosive scours, < 50% affected
- 4- High prevalence of severe scours
- 5- > 80% showing evidence of scouring

#### *2.4.2 Measurement of pH in rumen fluid*

A standard pH meter (model ECpHScan-WP3) was used to measure rumen pH. The pH meter was calibrated with buffers of pH 4, 7 and 10 before each sampling period. The probe was cleaned with distilled water between samples. The probe was inserted into the rumen fluid and the pH recorded on the sheet provided immediately after collection.

#### *2.4.3 Biochemical analyses*

##### *2.4.3.1 Rumen volatile fatty acids*

Ruminal fluid was analysed for volatile fatty acids by the Department of Agriculture, WA using a gas chromatograph (GC). The raw rumen samples were thawed at room temperature, mixed, and allowed to stand until settled. A half ml of the supernatant of each sample was placed into separate microcentrifuge tubes and centrifuged at 13,000 rpm for 10 mins. For each sample, 100 µl of the supernatant together with 1ml of pre-made internal standard solution was placed into a GC vial. One hundred samples were run at once. With each run, a standard and blank were also run. The standard contained 1 ml internal standard solution and 100 µl working standard solution (pre-made). The blank contained 1 ml internal standard solution and 100 µl distilled water. The GC vials were then mixed following placement of lids and auto-analysed using the HP Capillary Gas Chromatograph. Seven identical samples were analysed every 100 samples to evaluate

accuracy of the results. Concentrations (mM) of propionic, acetic, isobutyric, butyric, isovaleric, valeric and caproic acid were reported for each sample. The coefficients of variation for assay of propionic, acetic, isobutyric, butyric, isovaleric, valeric and caproic acids were 3.8%, 5.1%, 3.2%, 4.0%, 3.4%, 3.7% and 3.2% respectively.

#### 2.4.3.2 *Rumen ammonia*

Rumen fluid was analysed for ammonia concentration at the Department of Agriculture, WA. The assay of ammonia is an enzymatic assay linked through glutamate dehydrogenase to the oxidation of NADPH. This oxidation of NADPH was monitored at 340 nm in a spectrophotometer. The basis of the assay is a Boehringer Mannheim/ R-Biopharm (Cat. No. 1 112 732). The coefficient of variation (CV) for ammonia assay depended on the concentration of ammonia; with a CV of 3.7% for concentrations up to 30 mg/L and 1.1% for concentrations greater than 30 mg/L up to approximately 50 mg/L.

#### 2.4.3.3 *Rumen D and L-lactate*

Rumen fluid was analysed for D-lactate concentration at Agriculture WA. The oxidation of D-lactate is an enzymatic assay linked through D-lactate dehydrogenase by NAD<sup>+</sup>. The increase in NADH was monitored at 340 nm in a spectrophotometer. The basis of this assay is a Boehringer Mannheim/ R-Biopharm kit (Cat. No. 1 112 821). The coefficient of variation (CV) for assay of D-lactate also depended on the concentration of D-lactate; with a CV of 22.7% for concentrations up to 1 mM and 2% for concentrations greater than 1 mM.

Selected rumen fluid samples were also analysed for L-lactate at Agriculture WA using the same kit used for D-lactate. The oxidation of L-lactic acid requires the presence of the enzyme L-lactate dehydrogenase with the increase in NADPH monitored at 340 nm in a spectrophotometer. The assay was completed using a Boehringer Mannheim/ R-Biopharm kit (Cat. No. 1 112 821). The coefficient of variation (CV) for assay of L-lactate also depended on the concentration of L-lactate; with a CV of 7.9% for concentrations up to 1 mM and 1.4% for concentrations greater than 1 mM.

## 2.4.4 Diet analysis

### 2.4.4.1 Forage testing

The pasture samples, TMR samples and some conserved forages or unknown grain mixes were submitted to Dairy One Forage Laboratory for testing by near infrared reflectance spectroscopy, a technique using mathematical models to relate composition of active chemical groups in specific feed constituents to energy absorption in the near infrared region of the spectrum (Leeson *et al.* 2000). Feeds were analysed using spectral properties of the feed with multiple nutrients determined simultaneously. The technique is calibration dependant and uses the LOCAL calibration software. Results for each feed sample included percent DM, NDF, CP, degradable protein, crude fat, ash, ADF, lignin, NFC, NSC, starch and sugar. Percent calcium, phosphorus, magnesium, potassium and sulphur were also included in analysis but not included in the thesis results. Analysis of TMR, conserved forage and grain mix samples included DM, CP, soluble protein, ADF, NDF, fat, ash and NFC percentage. Grains were not routinely tested for feed values; these were estimated using values previously obtained for Australian grains. Only two grain-mixes were analysed and 8 total mixed ration samples which were used to validate the results from the combination of the individual feeds. Many of the pasture samples, although often dominant in one pasture type, contained a mixture of grasses. It is acknowledged that this may have affected the accuracy of the reported results because NIR testing depends on calibration sets from similar feed ingredients. However, wet chemistry, although a better option, in a study of this magnitude, was cost prohibitive.

### 2.4.4.2 Validation of diets using CPM dairy

Diets from all herds were verified using the ration formulation program CPM dairy (Cornell\*Penn\*Miner, 2003) (version 3.0.4a). Pasture analysis for each farm where applicable from Dairy Forage One was entered as a feed into CPM dairy. Cow, environmental and management factors were entered for each herd. The complete diet as recorded on the questionnaire, including pasture estimates, was entered. The predicted model based on dietary intake using markers such as milk production, DMI and peNDF cap was compared with the requirements needed to satisfy cow, environmental and management factors. The peNDF cap was the maximum amount of peNDF the ration

could contain (kg/day) before limiting intake. Several rules were followed for data input:

1. When the cows were permitted to back graze and the ME and DMI was under estimated in the model, the pasture intake was increased 50%. Where backgrazing was noted on the questionnaire or *ad libitum* or extensive grazing conditions occurred on the day of sampling, the peNDF cap was used to determine appetite of the cow by constraining DMI of the pasture.
2. Where backgrazing was noted on the questionnaire or *ad libitum* or extensive grazing conditions occurred on the day of sampling, the peNDF cap was used to determine appetite of the cow by constraining dry matter intake of the pasture.
3. Where silage, hay or straw was fed with obvious wastage noted on the questionnaire, the assumed wastage was 20% when fed in the paddock on the ground and 30% when fed in rings.
4. When pastures (n = 8) were not sampled but described on the questionnaire, values were estimated using pastures with a similar structure from the same area and time of year.
5. Dry matters were measured on the pastures from Camden, Taree and Gippsland. The dry matters from pastures in Western Districts and Finley were estimated from previous samples analysed in the area at similar times of the year. Dry matters were measured by recording the pre- and post- freeze drying weights of the pasture samples and calculating a percentage dry matter.
6. Herds 4 (Finley), 30 (Finley) and 94 (Gippsland) were included in tables that reported whether feeds were used or not but not in tables reporting quantities used. These herds could not be validated using CPM dairy due to quantities reported by the farmer fed on the day.

#### 2.4.4.3 Pasture texture score

Each pasture sample was scored on a 1 to 5 scale for texture, after freeze-drying, by two people blinded from the history of each farm. On this scale, grasses scored 1 = very soft grasses such as clovers and legumes with minimal effective fibre, 2 = very soft ryegrass, 3 = firmer ryegrass/ lush kikuyu/ fresh oat/ fescue, 4 = mature oat/ mature fescue/ phalaris/ kikuyu and those scored 5 included coarse forages such as maize and forage

sorghum, high in effective fibre. A mean was taken of the individual scores.

#### ***2.4.5 Data Validation and Screening***

Data were entered onto Microsoft Excel for Windows (version 9.0.0.2719) spreadsheets. All herd and cow data were checked manually against original questionnaire recording sheets. Biochemical and pasture analysis data were checked against original computer printouts from the laboratory. Diets from all herds were verified using the ration formulation program CPM dairy (Cornell\*Penn\*Miner, 2003) (version 3.0.4a) before exporting data back into an excel spreadsheet and transferring to a Statistix (Version 7) file.

The data were screened using Statistix (Version 7) and SPSS (Version 11.0). The screening process included examining histograms and box and whisker plots of each variable to determine the presence of outliers and assess normality of the data. Where data were assessed as not following a normal distribution, non-parametric tests were used in analysis.

Data were examined on both a cow and herd level. Production data relating to individual herd tests were examined on a cow level and random effect of herd was included in the model (Chapter 4). The random effect of herd was also included in the model for outcome variables such as scour and locomotion score discussed in Chapter 5. Dietary variables were assessed on a herd level (Chapter 6) and as such random effect of herd was not included in the analysis of these data. Specific statistical analyses and epidemiological approaches to data analysis are outlined in the relevant chapters.

Computer programs used for statistical analyses include Statistix (Version 7, NIH Software), SPSS (Version 11.0 for Windows), Egret (Version 2.03.1, Cytel Statistical Software), Stat Xact (Version 4.0.1, Cytel Software Corporation, 1999), Genstat (Version v.7.1.0.198) and Winbugs (Version 11.4).

### *2.4.5.1 Grouping of herds into categories*

Data collected in the study were both on a cow and herd level. It was important to compare both sets of data, therefore herds were grouped into categories based on individual cow classifications determined by cluster analysis. Herds containing three (37.5%) or more cows allocated to the same category were grouped. Where 3/8 cows from category 1 were found in a herd, the herd was classified as 'ACID', 3/8 cows from category 2 in a herd classified the herd as suboptimal rumen function 'SO' and 3/8 cows from category 3 in a herd classified the herd as normal function 'NA'. The number of herds used in categories ACID, SO and NA were 10, 35 and 69 herds respectively. Twenty herds met the criteria of 2 categories and were used twice in analysis; one herd was in categories 1 and 2, two herds were in categories 1 and 3 and 17 herds were in categories 2 and 3.

### *2.4.6 Statistical analyses*

#### *2.4.6.1 K-means cluster analysis*

This analysis is used to identify relatively homogenous groups of cases based on selected characteristics, using an algorithm that can handle large numbers of cases. The use of the algorithm requires the number of clusters to be specified. F-statistics can be computed for each variable used in the analysis, providing information about the contribution of each variable to the separation of the groups. In this study, predictive variables used in the cluster analysis were converted to standardised variates (z-score) before clusters were assessed. This was done because the size of some variables (for example acetate concentrations) were 100 fold that of others (for example d-lactate concentrations), causing difficulties with the analysis. Initially, after manually specifying the number of clusters, the analysis constructs cluster centres by selecting  $k$  well-spaced observations. Cases are then assigned to clusters based on distance from the cluster centres. The cluster centres are then updated based on the mean values of cases in each cluster. In this study, the biochemical data from rumen fluid analysis of each cow sampled were used as the variables. These included concentrations of acetate, propionate, butyrate, isobutyrate, isovalerate, valerate, caproate, d-lactate and ammonia, and rumen pH. Differing numbers of clusters were explored, however it was found that when using four or more clusters,

the numbers of cases allocated to one cluster were too few ( $n < 10$ ) to sensibly interpret. The use of only two clusters did not capture all the important qualities seen when three clusters were used.

#### *2.4.6.2 Discriminant analysis using step-wise method*

Discriminant analysis is useful in situations where cases cannot be classified using K-means cluster analysis because of missing predictor variable data. The procedure generates discriminant functions based on linear combinations of the predictor variables that provide the best discrimination between the groups. The functions are generated from a sample of cases for which group membership is known and applied to new cases with measurements for some of the predictor variables but unknown group membership. This procedure was particularly useful in this study where 114 cases were unclassified using K-means cluster analysis because of missing data. The unclassified cases were reclassified with results presented in Table 4.3. The method was also used to examine the clusters provided from cluster analysis; group allocation was nearly identical to that provided by cluster analysis.

#### *2.4.6.3 Random effects modeling*

Random effects modeling was used in this thesis in various analyses to investigate whether or not certain unmeasured factors were influencing the analysis results. The two factors included as random effects included herd, when some individual cow data were analysed (Chapter 4) and district, when herd data were analysed (Chapter 5). Our rationale for including herd as a random effect term when cow data were analysed for example, was to investigate whether or not unmeasured, herd-level factors (such as environmental conditions or genetic merit) were influencing the dependant variable over and above that of the modelled fixed effects.

#### *2.4.6.4 Bayesian statistics*

In Chapter 4, a logistic regression model was developed to quantify the influence of factors that influenced the risk of a cow being classified as acidotic at the time of sampling. To do this, a Bayesian approach was used which allowed us to account for the

hierarchical nature of the data. We parameterised the relationship between the logit of the outcome variable (acidosis present or absent) as a function of the explanatory variables that were included in the fixed effects model. Prior distributions were assigned to the unknown quantities, which in this case were the regression coefficients for each of the parameterised fixed effects and the variability of herd, which was included as a random effect. Bayes' theorem was applied using a Markov chain Monte Carlo algorithm implemented in WinBUGS 1.4 (Spiegelhalter *et al.* 1999) which allowed the data that was observed to inform the revision of the assigned prior distributions into posterior distributions which were then used for inference.

Prior distributions centred at zero and with small precision (inverse variance) were used for each of the fixed effects. A gamma hyperprior was used for the precision of the herd-level random effect. For the Bayesian analyses we ran the Markov chain Monte Carlo sampler for 40,000 iterations and discarded the first 1,000 'burn-in' samples. Convergence was visually assessed by plotting cumulative path plots for each of the monitored parameters (Yu 1994, Yu and Mykland 1998, Robert and Casella 1999) and quantified using the Raftery and Lewis convergence diagnostic (Raftery and Lewis 1992a, Raftery and Lewis 1992b). Parallel chains were run using diverse initial values to ensure that convergence was achieved to the same distribution (Gelman 1996). Posterior sample sizes were determined by running sufficient iterations to ensure that the Monte Carlo standard error of the mean was at least one order of magnitude smaller than the posterior standard deviation for each parameter of interest.

## 2.5 References

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### Chapter 3.

## FEEDING PRACTICES ON ONE HUNDRED DAIRY FARMS IN NEW SOUTH WALES AND VICTORIA

### 3.1 Summary

*The feeding practices of 100 Australian dairy herds were obtained from 34 herds in Finley, 17 in Camden, 9 in Taree in NSW, 21 in Western Districts, and 19 in Gippsland in Victoria. This data set is part of a wider study examining the prevalence and effects of acidosis on Australian dairy cattle health and production. A questionnaire designed to gather information about feeding practices on the day of sampling was completed and a pasture sample collected where applicable for analysis. Diets were evaluated for nutritional adequacy using CPM Dairy (Cornell\*Penn\*Miner v3.04) computer program.*

*The mean ( $\pm$  SD) age of farmers or herdspersons sampled was  $42 \pm 10.3$  years, and herds milked  $342 \pm 233$  cows. Mean ( $\pm$  SD) milk production on the day of sampling was  $22.8 \pm 4.98$  L/cow/day. The most prevalent feeding system (54%) in all areas was a combination of pasture with grain offered in the bail during milking. The combination of pelleted grain/ by-products in the bail and pasture grazing was fed on 25% of farms. Only one herd in the study fed pasture solely.*

*The percent of concentrate used in the diet ranged from a mean of 25.1% in the Western Districts to 44.1% in Taree. Wheat, the most prevalent grain used in all areas except Gippsland, was fed at up to 9.8 kg DM/cow/day. Supplemental protein sources were used in all areas, with the most common being canola meal, cottonseed meal and lupins. The estimated mean dietary CP% in all regions was adequate, however, some individual farms were feeding less than recommended concentrations (12.0% CP) in the diet. By-product feeding was prevalent, particularly in the Camden area with the most commonly used, brewers grain and wheat millrun fed at amounts of  $2.8 \pm 1.37$  kg DM/cow/day and  $1.6 \pm 1.26$  kg DM/cow/day, respectively.*

*Most farms incorporated at least one mineral in the ration and sodium bicarbonate (38%), limestone (67%) and magnesium oxide (43%) were most frequently fed. Monensin and virginiamycin were used in all areas; 60.6% of farms fed monensin in Finley at a mean rate of  $218 \pm 90.9$  mg DM/cow/day compared with 23.5% of farms in the same area that fed virginiamycin at a mean rate of  $157 \pm 74.4$  mg DM/cow/day. This chapter gives a unique insight into the feeding practices of 100 Australian dairy herds and provides evidence that a wide variety of systems are used to feed dairy cows in Australia.*

## **3.2 Introduction**

The Australian dairy industry is in a period of change. External factors contributing to this change include deregulation of the domestic market for fluid milk, changing feed costs, declining milk prices, the development of new and improved pasture species, and in some areas, a fluctuation in availability of irrigation water (Dairy Australia 2003). The industry has moved from being mostly pasture-based to feeding increasing amounts of concentrates, in an effort to increase milk production and boost income (Dairy Australia 2003). The feeding of by-products is also a practice that may have increased, however, there are no comprehensive surveys to confirm this. Previous descriptions of pastures have often been based on small numbers of farms in specific areas (Fulkerson *et al.* 1998; Jacobs *et al.* 1999; Stockdale 1999a; Stockdale 1999b; Stockdale *et al.* 2001).

In this chapter, the feeding practices of dairy herds in Southern Australia, including the forage to concentrate ratio, the quantities and qualities of pastures and forages and by-products and agents used to control fermentation are described.

## **3.3 Materials and Methods**

### **3.3.1 Herd selection**

One hundred herds were selected from five areas in NSW and Victoria, representing major areas where dairying is concentrated. Refer to section 2.3.1.

### 3.3.2 *Dates of sampling*

Sampling dates for each region are summarised in section 2.3.2.1.

### 3.3.3 *Questionnaire*

A questionnaire was completed for each herd involved in the study in a face-to-face interview with the farmer. Refer to section 2.3.3.

### 3.3.4 *Lactating cow diet*

The amount of concentrate fed to cows in the milking bails was checked by weighing two samples of grain dispensed. The quality of conserved forages fed was described in terms of freshness, proportion of leaf and stem, weed content and smell. Amount of feed wasted was estimated visually from residues left on the ground on the day of sampling. Feed samples whose chemical analysis was unknown were analysed at Dairy One Forage Testing Laboratory using the NIR model. Where samples were not analysed, analysis of similar feeds were used to estimate the feed value of the sample.

Pasture samples were taken the day before rumen sampling from either the next strip to be offered if the farm was being strip grazed or another paddock that the farmer deemed to be of the same pasture type and mass. The pasture to be offered the night prior to rumen sampling, if different, was also sampled. Five by 0.1m<sup>2</sup> quadrants were randomly placed on the area/s selected, with the pasture in the quadrants clipped using electric hedge clippers to the estimated grazing height. Areas around dung pats were not sampled, as these would have been less available to the cows. Samples were placed into plastic bags, sealed and transported on ice to the field laboratory. The weight of the samples was then determined and the sample frozen as quickly as possible before freeze-drying at the University of Sydney. The samples were then re-weighed to determine DM content. Pasture intake was calculated using the weight of pasture cut from the pasture quadrants and the size of paddock grazed. The total estimated amount of wet pasture available from the paddock was divided by the number of lactating cows in the paddock to give the amount of pasture eaten per cow per day. This amount was converted to dry matter after DM % of the pasture was established.

### **3.3.5 Forage testing**

The pasture samples, total mixed rations (TMR) and some conserved forages or unknown grain mixes were submitted to Dairy One Forage Laboratory for testing by near infrared reflectance spectroscopy (Leeson *et al.* 2000). Refer to section 2.4.4.1.

### **3.3.6 Evaluation of diets using CPM dairy**

Diets from all herds were evaluated using the ration formulation program CPM dairy (Cornell\*Penn\*Miner, 2003) (version 3.0.4a). Refer to section 2.4.4.2. After evaluating the diets using the program, dietary amounts fed were entered into a Microsoft Excel for Windows (version 9.0.0.2719) spreadsheet on a DM basis.

### **3.3.7 Statistical analyses**

Data summarised in Tables 3.1 to 3.14 were analysed using the statistical package Statistix (Version 7 NIH Software). Summary statistics obtained included mean, standard deviation, range, and first and third quartiles. In Table 3.1, overall mean and standard deviation for each variable were recorded rather than weighted means as there was no systematic difference in the data sets. The percentage concentrate fed in the diet (see section 3.4.2.2) was analysed using One-way ANOVA after assumptions were met.

## **3.4 Results and Discussion**

### **3.4.1 Demographics of dairy farms and farmers in the study**

The mean ( $\pm$ SD) age of the farmer was  $42 \pm 10.3$  years, very similar to  $44 \pm 11.9$  years reported in a previous NSW survey (Webster 1996). The length of time the participants had been associated with dairying in this survey was relatively low ( $25 \pm 3.7$  years) compared to  $37 \pm 16.0$  years found by Webster (1996). This reflects a higher number of young herdspersons, children of the property owner and share farmers answering the survey compared to the people surveyed by Webster (1996), where over 80% of respondents were owners or managers. The length of time the participant had been on the property was also consequently less,  $17 \pm 13.6$  years as opposed to  $23 \pm 19.1$  years (Webster 1996).

The description of farm area, composition of herd and herd production by region is shown in Table 3.1.

**Table 3.1.** Mean demographics ( $\pm$ SD) by region of farms and herds surveyed in the study

Parameter	Finley (n = 34)	Western Districts (n = 21)	Camden (n = 17)	Taree (n = 9)	Gippsland (n = 19)
Total farm size (ha)	253 (198.5)	212 (82.6)	255 (383.8)	188 (87.4)	213 (172.9)
Milking area (% total farm)	77 (23.6)	90 (11.4)	56 (35.0)	67 (13.6)	90 (11.7)
Improved pasture (% milking area)	90 (12.4)	90 (21.3)	94 (18.7)	96 (12.9)	91 (26.5)
Irrigated pasture (% milking area)	95 (11.8)	0	23 (39.8)	23 (32.8)	1 (1.3)
Fodder crops (% total farm) <sup>A</sup>	7 (7.8)	2 (4.5)	6 (15.4)	4 (6.4)	6 (24.5)
Number of dairy cows <sup>B</sup>	337 (178.5)	375 (175.4)	189 (93.1)	266 (129.1)	488 (396.1)
% lactating cows	73 (13.7)	95 (11.1)	79 (7.9)	81 (5.3)	69 (23.7)
% dry cows	19 (11.0)	4 (10.5)	14 (6.9)	14 (4.4)	25 (24.3)
% transition cows	8 (5.9)	2 (3.0)	8 (4.2)	5 (2.6)	6 (5.6)
Milk production (L/cow/day)	22.4 (4.1)	27.1 (4.8)	24.0 (3.6)	18.6 (2.1)	19.4 (4.7)
Somatic cell count (cells/ml)	183,059 (77096)	174,476 (63135)	187,706 (68631)	191,889 (59139)	188,421 (53384)
% fat	3.8 (0.45)	3.6 (0.37)	3.7 (0.30)	3.9 (0.19)	4.4 (0.34)
% protein	3.4 (0.21)	3.3 (0.21)	3.1 (0.09)	3.2 (0.10)	3.4 (0.26)

<sup>A</sup> fodder crops = lucerne, sorghum

<sup>B</sup> sum of lactating, dry and transition cows; transition cows = cows in last 3 weeks before calving separated from dry herd and often lead fed, dry cows = non-lactating cows not fed transition diet

### 3.4.2 Feeding practices of 100 herds

#### 3.4.2.1 Types of feeding systems

Feeding pasture supplemented with concentrates, either grain or pellet was the most common feed management method used (Table 3.2). Only one farm in the study was feeding pasture only and that farm commenced concentrate feeding within a week of the survey. This finding can be compared with the estimated 33% of farmers in 1991 that fed pasture only (Kellaway and Porta, 1993). This may be due to a combination of reduction of grain costs on farm in real terms and an increase in pressure to boost milk production. Feeding pellets in the bail was less common than feeding grain in the Finley district. This observation may reflect a lack of close feed mills offering pellets at the time of the survey combined with good availability of cheap grain, and may therefore reflect a difference in cost between those options.

Total mixed rations were not commonly fed in any area except Camden (23.5%). This observation probably reflects the small farm areas and easier and cheaper access to commodities (by-products) used in TMR. Partial mixed rations, in which cows were also given access to pasture and/or concentrate in the bail at milking were more prevalent than TMR, particularly in Camden (29.4%) and Gippsland (31.6%).

**Table 3.2.** Farms (% of total in each region) using different feeding systems

Feeding system	Finley (n=34)	Western Districts (n=21)	Camden (n=17)	Taree (n=9)	Gippsland (n=19)
Pasture only	2.9	0	0	0	0
Pasture and grain mix in bail	73.5	61.9	17.6	44.4	47.4
Pasture and pellet only in bail	14.7	33.3	29.4	44.4	21.1
Partial mixed ration ± grain in bail ± pasture	8.8	4.8	29.4	11.1	31.6
TMR only	0	0	23.5	0	0

#### 3.4.2.2 Estimated feed intake

Dry matter intakes were estimated from the field data (see section 3.3.4) and validated using CPM dairy (see Tables 3.3 to 3.7). The mean DMI were quite consistent ranging from 17.2 to 21.4 kg DM/cow/day. The percentage of concentrate fed in the diet varied with area ( $P = 0.001$ , ANOVA). Camden and Taree had the highest concentrate

percentage in the diet (40.7 and 44.1% respectively). The most commonly fed fibrous by-product was wheat millmix/millrun, although barley maltings, brewers grain, citrus pulp, corn gluten feed, wheat millmix/millrun, almond hulls, rice hulls, rice pollard, oat pollard and lupin hulls were also fed. Wheat millmix/ millrun was generally fed as a constituent of pellets. By-products were much less commonly used in areas where pellets were not as prevalent. Fibrous by-products were also more common in herds fed TMRs.

**Table 3.3.** Mean total dry matter intake (kg DM/cow/day), concentrate and forage intake in Finley (n = 32) (values expressed as DM)

	Mean	Standard deviation	Quartile 1 – Quartile 3	Range
Total DMI (kg/cow/day)	18.1	3.75	15.3 – 20.4	11.6 – 30.2
Concentrate (% of diet)	28.3	12.57	18.8 – 38.5	0 – 51.5
Concentrate intake (kg/cow/day) (n=31)	5.2	2.74	3.6 – 7.3	0 – 10.9
Pasture intake (kg/cow/day) (n=32)	9.3	3.29	7.1 – 11.6	3.0 – 18.0
Silage intake (kg/cow/day) (n=9)	3.3	2.08	1.5 – 5.2	1.0 – 6.5
Hay intake (kg/cow/day) (n=21)	3.4	1.77	2.3 – 4.9	0.5 – 6.9
Fibrous by-product intake (kg/cow/day) (n=7)	1.6	1.91	0.4 – 1.8	0.3 – 5.8

**Table 3.4.** Mean total dry matter intake (kg DM/cow/day), concentrate and forage intake in the Western District of Victoria (n = 21) (values expressed as DM)

	Mean	Standard deviation	Quartile 1 – Quartile 3	Range
Total DMI (kg/cow/day)	21.4	2.61	19.7 – 22.5	16.6 – 27.8
Concentrate (% of diet)	25.1	11.64	14.2 – 35.5	5.4 – 46.3
Concentrate intake (kg/cow/day) (n=21)	5.5	2.81	2.9 – 8.1	0.9 – 10.5
Pasture intake (kg/cow/day) (n=21)	15.5	2.43	13.9 – 17.6	9.3 – 18.8
Silage intake (kg/cow/day) (n=0)	-	-	-	-
Hay intake (kg/cow/day) (n=6)	1.3	0.69	0.6 – 1.8	0.3 – 2.2
Fibrous by-product intake (kg/cow/day) (n=9)	0.6	0.27	0.4 – 0.8	0.3 – 1.1

**Table 3.5.** Mean total dry matter intake (kg DM/cow/day), concentrate and forage intake in Camden (n = 17) (values are expressed as DM)

	Mean	Standard deviation	Quartile 1 – Quartile 3	Range
Total DMI (kg/cow/day)	19.0	2.74	16.9 – 20.7	14.7 – 25.2
Concentrate (% of diet)	40.7	16.28	26.7 – 51.1	22.8 – 77.6
Total concentrate intake (kg/cow/day) (n=17)	7.8	3.45	4.9 – 10.3	3.7 – 14.8
Pasture intake (kg/cow/day) (n=15)	9.9	3.37	7.7 – 12.4	2.8 – 15.8
Silage intake (kg/cow/day) (n=6)	2.5	2.78	0.7 – 3.6	0.7 – 8.0
Hay intake (kg/cow/day) (n=10)	2.7	2.06	0.9 – 4.0	0.25 – 6.5
Fibrous by-product intake (kg/cow/day) (n=17)	2.9	2.39	1.4 – 4.0	0.3 – 8.6

**Table 3.6.** Mean total dry matter intake (kg DM/cow/day), concentrate and forage intake in Taree (n = 9) (values are expressed as DM)

	Mean	Standard deviation	Quartile 1 – Quartile 3	Range
Total DMI (kg/cow/day)	19.3	2.11	17.1 – 21.1	16.0 – 22.0
Concentrate (% of diet)	44.1	12.04	34.2 – 52.0	33.0 – 69.5
Concentrate intake (kg/cow/day) (n=9)	8.4	1.91	6.8 – 10.0	6.6 – 11.8
Pasture intake (kg/cow/day) (n=9)	9.7	2.31	8.6 – 11.8	5.2 – 12.7
Silage intake (kg/cow/day) (n=3)	1.5	0.61	1.1 – 2.2	1.1 – 2.21
Hay intake (kg/cow/day) (n=3)	1.9	1.53	0.5 – 3.5	0.5 – 3.5
Fibrous by-product intake (kg/cow/day) (n=5)	2.7	1.18	1.8 – 3.9	1.2 – 4.1

**Table 3.7.** Mean total dry matter intake (kg DM/cow/day), concentrate and forage intake in Gippsland (n = 18) (values are expressed as DM)

	Mean	Standard deviation	Quartile 1 – Quartile 3	Range
Total DMI (kg/cow/day)	17.2	3.60	13.9 – 19.7	11.8 – 23.8
Concentrate (% of diet)	36.7	11.94	28.2 – 43.3	17.7 – 58.7
Concentrate intake (kg/cow/day) (n=18)	6.5	2.83	3.9 – 8.4	2.7 – 11.7
Pasture intake (kg/cow/day) (n=17)	7.9	3.59	5.5 – 9.9	2.0 – 15.5
Silage intake (kg/cow/day) (n=10)	3.7	2.48	1.5 – 4.7	0.7 – 9.3
Hay intake (kg/cow/day) (n=9)	2.4	1.18	1.4 – 3.5	1 – 4.2
Fibrous by-product intake (kg/cow/day) (n=9)	3.0	2.45	0.9 – 5.0	0.2 – 7.2

### 3.4.2.3 Description of pastures

Pastures were grouped according to dominant species. Farms in the Northern Victoria/Southern NSW irrigation area based around Finley were sampled during July/ August with most of the sampling before irrigation started (see Table 3.8). Approximately 47% of farms fed subterranean clover (*Trifolium subterraneum* L.) and annual ryegrass (*Lolium* spp.) pastures during the day and permanent summer pasture, primarily white clover (*Trifolium repens*) and perennial ryegrass at night, with only 15% of farms feeding subterranean clover and annual ryegrass at night and permanent summer pasture during the day. Thirty eight percent of farms fed the same type of pasture (either subterranean clover and annual ryegrass or permanent summer pasture) day and night. All shaftal (*Trifolium resupinatum*) and clover dominant pastures described in Table 3.9 were found in the Finley district. Gippsland and Western District pastures varied markedly depending on pasture management strategies implemented on farm. Subterranean clover (*Trifolium subterraneum*) and white clover varieties were used but to a lesser extent than in other areas. This is particularly evident in Western District pastures where 100% of pastures sampled were ryegrass-dominant. The Camden region incorporating Camden, Bowral and Jamberoo, had the most varied pastures due to climatic differences between areas. Both kikuyu (*Pennisetum clandestinum*) and ryegrass dominant pastures were equally prevalent during the summer months. Dairy herds at Taree, in the Central Coast of NSW were sampled in April when 64% of the pasture sampled was kikuyu-dominant and 29% were paspalum (*Paspalum dilatatum* Poir.) dominant. Several of the herds were feeding a small amount of chickory (*Cichorium intybus* cv *Puna*) with kikuyu dominant pasture.

Mean values for nutritive characteristics of pasture types outlined in Table 3.8 were similar to previously reported data by Stockdale (1999a) who found that white clover grown in irrigation areas, had a CP content varying from 17.7 to 27.7% DM and NDF from 27.8 to 39.8% DM over the year. In Northern Victoria, sub-clover dominant (> 80% presence) pastures harvested throughout the year were characterised by NDF and ADF of 28.3 to 43.7% and 21.1 to 33.0%, respectively (Stockdale 1992). Clover dominant pastures in this study (93% were found in the Finley irrigation region) were characterised by a CP content of 27.3%, NDF content of 29.1% and ADF content of 19.8%. The limited observations on paspalum showed a CP content of 12.6% and

NDF of 59.1% indicating poor nutritive values, which were similar to those of (Stockdale 1999a) who found a CP content of 7.5 to 14.7% DM and NDF of 61.9 to 69.9% DM. In the same study, ryegrass was very similar to paspalum in CP and NDF content during the year (Stockdale 1999a). Jacobs *et al.* (1999) reported that ryegrass-dominant pasture in the Western Districts of Victoria had a CP content of 10.0 to 26.6% and NDF of 43.6 to 69.2%. Thirty eight percent of ryegrass-dominant samples in this study came from the Western Districts of Victoria with a CP content of 23.2% and NDF content of 42.1%. Samples of kikuyu pasture came from Camden (47%) and Taree (53%) with a CP content of 20.5%, NSC content of 13.5% and NDF content of 48.9%. This can be compared to previous studies which reported an NDF content of 43 to 56%, NSC content of 7.8 to 10.1% (spring data not available) (Fulkerson *et al.* 1999) and CP content of 16 to 24% (Fulkerson *et al.* 1998).

**Table 3.8.** Mean characteristics ( $\pm$ SD) of pasture analysed after grouping by dominant species

	Shaftal dominant <sup>A</sup> (n=3)	Clover dominant <sup>B</sup> (n=4)	Clover/ Shaftal: ryegrass <sup>C</sup> 50:50(n=28)	Ryegrass dominant <sup>D</sup> (n=58)	Lucerne <sup>E</sup> (n=3)	Kikuyu dominant <sup>F</sup> (n=17)	Paspalum dominant <sup>G</sup> (n=4)
CP%	28.9 (0.42)	27.3 (2.84)	26.5 (2.99)	23.3 (4.13)	24.1 (5.30)	20.5 (3.27)	12.6 (1.50)
ADICP	0.8 (0.26)	1.2 (0.13)	0.8 (0.17)	0.7 (0.19)	0.9 (0.30)	0.7 (0.18)	1.1 (0.05)
NDICP	5.2 (1.17)	6.0 (1.45)	4.1 (1.27)	3.6 (1.11)	2.8 (0.92)	4.3 (1.26)	4.6 (0.50)
NFC%	36.5 (1.50)	35.1 (1.85)	26.5 (4.89)	21.5 (4.57)	34.3 (2.11)	21.0 (2.19)	21.7 (2.88)
NSC%	18.4 (2.72)	21.3 (6.77)	21.5 (5.60)	20.2 (4.90)	15.4 (0.84)	13.5 (3.12)	12.5 (1.24)
NDF%	23.7 (2.25)	29.1 (1.86)	35.1 (5.01)	42.1 (4.27)	30.0 (8.00)	48.9 (5.60)	59.1 (2.47)
ADF%	19.3 (2.75)	19.8 (1.05)	22.3 (1.88)	23.9 (2.42)	25.8 (5.49)	28.2 (2.82)	35.5 (1.49)
Crude fat%	5.5 (0.40)	4.9 (0.41)	5.65 (0.67)	5.5 (0.88)	4.9 (0.85)	3.9 (0.88)	2.9 (0.37)
Lignin%	3.5 (1.00)	4.8 (0.68)	4.0 (0.72)	4.0 (0.75)	5.5 (1.03)	3.8 (0.89)	5.5 (1.21)
Ash %	10.6 (0.37)	9.7 (0.52)	10.5 (1.57)	11.1 (0.96)	9.6 (1.32)	10.2 (0.78)	8.4 (0.57)
Ca %	1.2 (0.14)	1.1 (0.10)	0.9 (0.20)	0.7 (0.15)	1.4 (0.17)	0.5 (0.12)	0.6 (0.21)
P %	0.4 (0.07)	0.4 (0.04)	0.4 (0.05)	0.4 (0.05)	0.3 (0.07)	0.4 (0.04)	0.3 (0.01)
Mg %	0.2 (0.03)	0.3 (0.01)	0.2 (0.02)	0.2 (0.04)	0.3 (0.04)	0.2 (0.03)	0.2 (0.02)
K %	4.2 (0.60)	3.8 (0.52)	3.5 (0.65)	3.3 (0.42)	3.0 (0.92)	3.3 (0.40)	2.0 (0.36)
S %	0.3 (0.01)	0.3 (0.008)	0.3 (0.06)	0.3 (0.04)	0.3 (0.03)	0.3 (0.04)	0.2 (0.02)

<sup>A,B</sup> 100% samples from Finley region<sup>C</sup> 93% samples from Finley region, 7% samples from Gippsland, clover was subterranean<sup>D</sup> 17% samples from Finley, 38% from Western Districts, 14% from Camden and 31% from Gippsland<sup>E</sup> 33% samples from Finley region, 33% from Camden and 33% from Taree

<sup>F</sup> 47% samples from Camden region, 53% samples from Taree

<sup>G</sup> 100% samples from Taree region

#### 3.4.2.4 Grains fed

Grains fed probably reflected the availability and cost of grains in each area (Table 3.9). Wheat (59%) was the most prevalent grain fed in the survey followed by triticale (34%) and barley (26%). Sorghum and rice, where used, were usually incorporated into pellets.

**Table 3.9.** Percentage of farms feeding one or more grain varieties (by region) and mean amounts ( $\pm$ SD) (kg DM/cow/day) of grain varieties used.

	Finley (n=34)		Western Districts (n=21)		Camden (n=17)		Taree (n=9)		Gippsland (n=19)	
	%	Mean	%	Mean	%	Mean	%	Mean	%	Mean
Barley	29.4	2.7 (2.00)	28.6	1.5 (1.39)	11.8	4.4 (0.71)	33.3	4.4 (1.70)	26.3	0.4 (0.14)
Corn	14.7	1.6 (2.04)	23.8	0.8 (0.16)	0	-	33.3	1.4 (0.68)	10.5	0.6 (0.05)
Oats	2.9	0.48	9.5	0.1 (0.03)	0	-	0	-	15.8	0.6 (0.06)
Rice (broken)	5.9	0.3 (0.04)	0	-	0	-	0	-	0	-
Rice (paddy)	5.9	2.2 (0.39)	19.0	1.0 (0.18)	0	-	0	-	5.3	0.2
Sorghum	0	-	0	-	47.1	1.2 (1.05)	44.4	2.6 (0.46)	5.3	0.5
Triticale	23.5	2.7 (1.63)	33.3	3.0 (2.28)	41.2	3.8 (1.19)	0	-	63.2	3.6 (2.12)
Wheat	70.6	3.2 (2.48)	71.4	3.3 (2.18)	52.9	1.37 (2.53)	44.4	5.3 (4.83)	52.6	2.2 (1.75)

#### 3.4.2.5 Protein supplements in diets

Lupins (34% CP) and cottonseed meal (42% CP) were the most common protein supplements used (Table 3.10). Protein supplements were generally incorporated into the diet in relatively small quantities. Many of the protein sources including canola meal, cottonseed meal, mung beans and safflower were incorporated in the pellet or purchased grain mix being fed.

**Table 3.10.** Percentage of farms feeding protein supplements in each region and mean amounts ( $\pm$ SD) (kg DM/cow/day) of protein supplements used.

	Finley (n=34)		Western Districts (n=21)		Camden (n=17)		Taree (n=9)		Gippsland (n=19)	
	%	Mean	%	Mean	%	Mean	%	Mean	%	Mean
Bean kibble	0	-	0	-	0	-	0	-	5.3	1.6
Black cake	0	-	0	-	0	-	0	-	5.3	0.3
Canola meal	32.4	0.6 (0.48)	38.1	0.8 (0.46)	0	-	0	-	26.3	0.4 (0.28)
Cottonseed meal	2.9	0.3	4.8	0.3	58.8	0.2 (0.16)	44.4	1.1 (0.53)	5.3	0.5
Faba beans	0	-	4.8	0.3	11.8	0.5 (0.02)	0	-	26.3	0.7 (0.63)
Lentils	0	-	4.8	1.0	0	-	0	-	0	-
Lupins	11.8	0.3 (0.26)	28.6	1.0 (0.34)	11.8	0.5 (0.14)	11.1	2.1	31.6	0.9 (0.74)
Meat meal	2.9	1.2	0	-	0	-	0	-	0	-
Mung beans	0	-	0	-	0	-	11.1	0.5	0	-
Safflower	0	-	0	-	11.8	0.3 (0.29)	0	-	5.3	0.2
Soya bean	0	-	0	-	11.8	1.6 (0.37)	0	-	0	-
Sunflower	2.9	0.3	4.8	0.3	11.8	0.4 (0.06)	0	-	0	-
Urea	2.9	0.1	0	-	5.9	0.09	55.6	0.05 (0.021)	15.8	0.1 (0.01)
Whole cotton seed	0	-	4.8	1.0	17.6	1.3 (0.55)	0	-	5.3	0.8

#### 3.4.2.6 Commodities in the diet

The most commonly used commodities were wheat millmix/ millrun, molasses and oil/fat (Table 3.11). These feeds were often used in pellets and commercial grain mixes. Oil was also commonly mixed with grain fed in the bail by farmers to reduce dust at milking. The other commodities were used predominantly in total and partial mixed rations where farmers had the equipment to feed them - Camden and Gippsland. The quantities fed were generally low, often being used to boost the energy or CP fraction of a ration. Citrus pulp, for example has an NFC of 66.4% while brewers grain contains 29% CP.

**Table 3.11.** Percentage of farms feeding commodities (by region) and mean amounts ( $\pm$ SD) (kg DM/cow/day) of commodities fed.

	Finley (n=34)		Western Districts (n=21)		Camden (n=17)		Taree (n=9)		Gippsland (n=19)	
	%	Mean	%	Mean	%	Mean	%	Mean	%	Mean
Almond hulls	0	-	0	-	0	-	0	-	5.3	2.0
Barley	0	-	0	-	11.8	2.3	0	-	0	-
maltings						(1.3)				
Biscuit meal	0	-	0	-	5.9	1.9	0	-	0	-
Brewers grain	2.9	0.7	0	-	29.4	2.8	11.1	4.1	31.6	2.4
						(1.4)				(1.52)
Cereal meal	0	-	4.8	1.3	23.5	0.3	0	-	0	-
						(0.3)				
Citrus pulp	5.9	1.1	4.8	0.7	11.8	0.9	0	-	0	-
		(1.07)				(0.7)				
Corn gluten feed	0	-	0	-	17.6	1.4	0	-	0	-
						(0.6)				
Corn hominy	5.9	1.3	4.8	0.3	5.9	0.3	0	-	10.5	2.0
		(1.48)								
Corn starch	0	-	0	-	0	-	0	-	5.3	0.2
Lupin hulls	2.9	0.5	0	-	0	-	0	-	0	-
ME14	2.9	4.1	0	-	0	-	0	-	0	-
(concentrated whey)										
Millrun/ mill mix	5.9	2.3	33.3	0.3	88.2	1.6	44.4	2.4	31.6	2.2
		(2.85)		(0.30)		(1.26)		(1.03)		(1.61)
Molasses	11.8	0.3	14.3	0.1	64.7	0.1	44.4	0.1	36.8	0.1
		(0.30)		(0.10)		(0.05)		(0.02)		(0.01)
Oat flour	2.9	0.04	0	-	0	-	0	-	0	-
Oat pollard	2.9	0.1	0	-	0	-	0	-	0	-
Oil/ fat	20.6	0.1	23.8	0.1	41.2	0.1	33.3	0.2	15.8	0.03
		(0.02)		(0.06)		(0.1)		(0.01)		(0.010)
Potato pulp	0	-	0	-	5.9	0.9	0	-	0	-
Rice hulls	2.9	-	4.8	0.3	0	-	0	-	0	-
Rice pollard	17.6	0.7	33.3	0.3	0	-	0	-	5.3	0.1
		(0.30)		(0.18)						

### 3.4.2.7 Description of buffers and modifiers

Limestone (67%), sodium bicarbonate (38%) and magnesium oxide (43%) were the most common minerals and buffers added to the ration (Figure 3.1).

**Figure 3.1.** Percentage of farms using buffers in each region

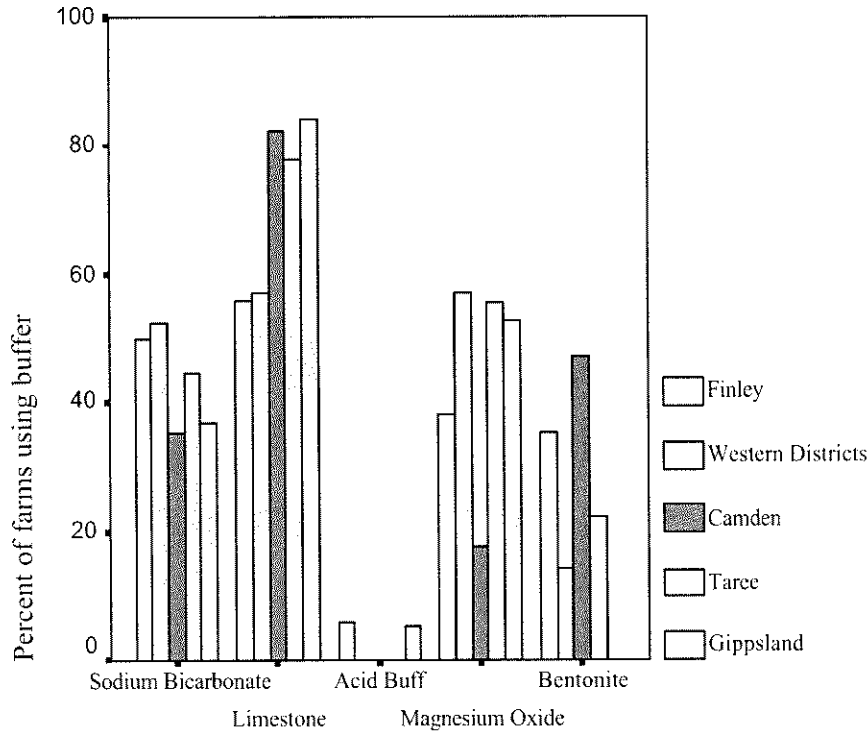


Table 3.12 summarises the amount of buffers and minerals fed per cow per day in each region. Limestone (39.4% calcium) was fed in amounts of 91 to 245 g/cow/day). Limestone has limited buffering capacity in the rumen at physiological pH because of low solubility despite the high potential acid-consuming capacity (Erdman 1988). It does alter pH in the small and large intestine and therefore may improve starch digestion (Lean 1987), however if diets contain more than 0.6 to 0.8% calcium, it may not have any effect at all (Hutjens 1991). Generally it is used as a calcium supplement with grains containing low amounts of calcium at 1% (200 g/cow/day) of the diet (Nandapi 1997). Limestone may have been added into the study herd diets either as a supplemental calcium source or as an attempt to buffer the ration. The mean concentration of sodium bicarbonate fed per cow/day ranged from 35.0 g in Finley to 182.0 g in Taree. These concentrations are also below recommended rates of 200 to 300 g/cow/day (Clayton *et al.* 1999; Kalscheur *et al.* 1997; Lean 1987). The mean concentration of magnesium oxide fed in the survey ranged between 22.3 g/cow/day

in Finley to 42.4 g/cow/day in Taree, whilst the recommended rate is 0.6 to 0.8% of DMI (120 to 160 g/cow/day) or 1.2 to 1.6% of concentrate mixture (National Research Council 2001). Dairy farmers may have been feeding buffers at a lower than recommended rate for a number of reasons. Farmers may be either unaware of the recommended feeding rate for the product when adding buffers to the ration themselves or miscalculate the correct rate per cow per day. Alternatively, there may be a lack of awareness of the pharmacological action of products. Feed mills selling premixed products may also be competing on price rather than ensuring the product contains the correct amount of buffer for the recommended feeding rate of the product.

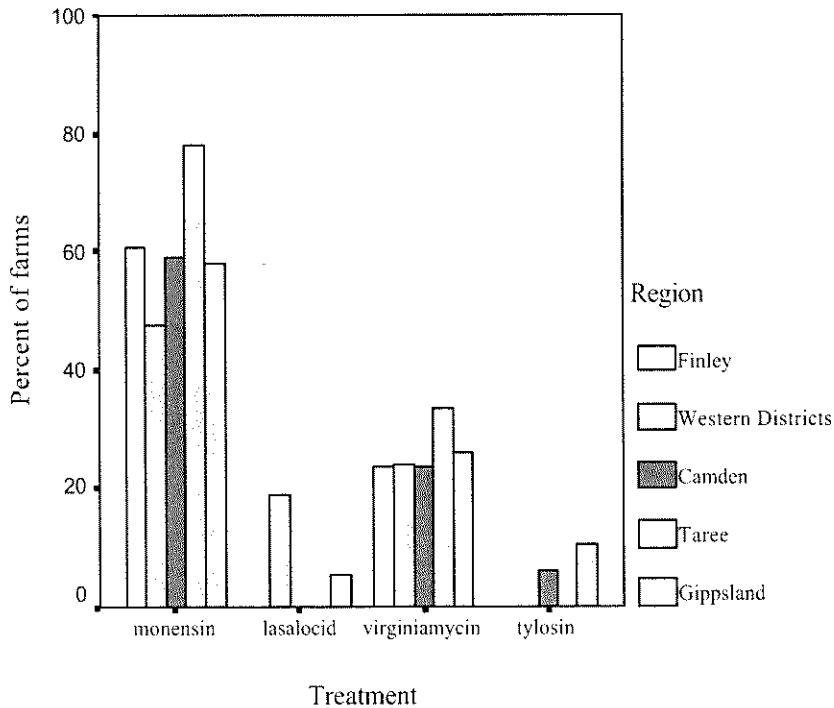
**Table 3.12.** Mean amounts ( $\pm$ SD) (g DM/cow/day) of buffers fed from those herds feeding the product in each region.

	Finley	Western Districts	Camden	Taree	Gippsland
Sodium bicarbonate	35.0 (18.84)	49.0 (22.23)	71.1 (82.51)	182.0 (85.16)	57.1 (29.77)
Limestone acid buff	90.9 (61.46)	78.2 (33.59)	190.3 (141.35)	245.2 (86.80)	100.4 (60.69)
Magnesium oxide	16.6 (16.19)	-	-	-	17.0
Bentonite	22.3 (15.31)	24.3 (10.33)	25.6 (15.09)	42.4 (4.86)	39.1 (25.34)
	81.1 (44.37)	31.3 (8.08)	59.7 (36.30)	119.2 (29.84)	-

Ionophores (monensin and lasalocid) and antibiotics (virginiamycin and tylosin) were also fed in most regions (Figure 3.2). Sodium monensin was the most commonly used rumen modifier in the survey with 60% of farms feeding the product. Lasalocid was used less frequently and only in the Western Districts and Gippsland. Milk production responses indicate that the combined benefits of monensin fed at 250 to 400 mg/cow/day are reflected in increased milk production (Beckett *et al.* 1998; Ipharraguerre and Clark 2003; Phipps *et al.* 2000; Vallimont *et al.* 2001) although some studies feeding rates as low as 80 mg/cow/day have documented positive responses in milk production (Ipharraguerre and Clark 2003). The recommended dose of monensin by the manufacturer for increased milk production and prevention of ketosis in dairy cattle is 200 to 450 mg/ head/ day. Monensin was fed at less than 250

mg/cow/day except in Camden ( $257 \pm 46.3$  mg) and Taree ( $310 \pm 88.6$  mg) (Table 3.14) where higher concentrations were fed.

**Figure 3.2.** Percentage of farms using modifiers and antibiotics in each region



The antibiotic, virginiamycin, was more commonly used (24%) than tylosin (3%) amongst surveyed herds. This was probably due to the restricted access to tylosin in Australia at the time of sampling. Both products are now available with virginiamycin only available on veterinary prescription. The recommended feeding rate for virginiamycin is 20 mg/kg of DM in complete diet (Rogers *et al.* 1995) or 0.5mg/kg BW/day (Thorniley *et al.* 1994), equivalent to 300 mg/cow/day when DMI is 15 kg/day DM. The mean amount of virginiamycin fed /cow/day in this study was less than the recommended rate in all areas (Table 3.13). Farmers feeding less than the recommended rate of virginiamycin may not be effectively controlling acidotic episodes and are at risk of increasing antibiotic resistance in target organisms. The current recommended feeding rate for tylosin is 11ppm or 200 mg/cow/day for a positive effect on milk production and body condition score (Lean *et al.* 2000) with those herds that fed tylosin in the survey also feeding less than this recommendation.

**Table 3.13.** Mean amounts ( $\pm$ SD) (mg DM/cow/day) of modifiers fed from those herds feeding the product in each region.

	Finley	Western Districts	Camden	Taree	Gippsland
Monensin	217.9 (90.86)	237.2 (70.01)	256.6 (46.32)	310.0 (88.62)	224.9 (59.17)
Lasalocid	-	261.3 (45.25)	-	-	280.0
Virginiamycin	156.9 (74.40)	243.6 (38.14)	208.0 (118.19)	159.0 (13.89)	221.2 (57.94)
Tylosin	-	-	134.0	-	112.5 (53.03)

#### 3.4.2.8 Description of total diet analysis using CPM dairy

The total diet was analysed using the CPM dairy ration formulation program. Mean analyses for each region are shown in Table 3.14.

**Table 3.14.** Mean ( $\pm$ SD) and (range) of chemical composition parameters of diets for each region.

	Finley (n=32)	Western Districts (n=21)	Camden (n=17)	Taree (n=9)	Gippsland (n=18)
CP%	20.1 $\pm$ 3.13 (12.5 – 27.2)	20.4 $\pm$ 2.88 (13.7 – 24.6)	19.0 $\pm$ 2.62 (13.1 – 23.7)	17.4 $\pm$ 2.98 (12.0 – 21.7)	20.5 $\pm$ 2.81 (16.1 – 26.6)
Undegradable protein (%CP)	34.8 $\pm$ 3.59 (29.0 – 41.3)	38.3 $\pm$ 2.78 (32.9 – 43.3)	34.9 $\pm$ 4.75 (28.7 – 45.4)	36.8 $\pm$ 5.39 (30.3 – 46.7)	31.5 $\pm$ 3.85 (25.2 – 38.9)
Soluble protein (%CP)	35.4 $\pm$ 3.6 (30.2 – 49.1)	35.2 $\pm$ 2.41 (29.1 – 38.6)	32.8 $\pm$ 3.00 (25.0 – 37.3)	33.5 $\pm$ 6.17 (26.1 – 46.1)	36.0 $\pm$ 3.37 (29.4 – 42.1)
ME (MJ/kg)	10.7 $\pm$ 0.74 (8.8 – 12.2)	10.2 $\pm$ 0.62 (9.1 – 11.3)	10.2 $\pm$ 1.06 (8.2 – 12.4)	10.1 $\pm$ 1.08 (8.8 – 11.4)	10.8 $\pm$ 0.48 (9.6 – 11.8)
NDF%	32.4 $\pm$ 5.14 (23.5 – 43.2)	36.6 $\pm$ 3.96 (26.4 – 42.6)	38.9 $\pm$ 5.54 (30.1 – 49.0)	36.2 $\pm$ 7.74 (23.5 – 46.4)	36.6 $\pm$ 3.91 (31.1 – 45.1)
peNDF%	24.1 $\pm$ 5.19 (14.9 – 34.1)	24.9 $\pm$ 3.51 (15.4 – 30.5)	25.3 $\pm$ 4.71 (16.8 – 31.9)	23.2 $\pm$ 6.13 (13.6 – 32.4)	24.4 $\pm$ 2.27 (20.6 – 28.7)
NFC%	37.2 $\pm$ 5.66 (26.3 – 48.4)	31.1 $\pm$ 5.10 (23.5 – 39.9)	31.1 $\pm$ 5.57 (22.3 – 38.9)	36.4 $\pm$ 7.30 (26.9 – 47.5)	32.0 $\pm$ 4.22 (25.9 – 38.4)
Carbohydrate B1% <sup>A</sup>	17.4 $\pm$ 6.93 (2.9 – 33.3)	15.4 $\pm$ 4.68 (7.3 – 24.6)	15.8 $\pm$ 6.43 (6.8 – 29.2)	21.0 $\pm$ 8.11 (13.5 – 35.2)	17.3 $\pm$ 5.00 (9.3 – 28.2)
Total Fat%	4.2 $\pm$ 0.67 (3.1 – 6.2)	4.7 $\pm$ 0.85 (3.2 – 5.9)	4.5 $\pm$ 1.13 (2.7 – 7.4)	3.9 $\pm$ 0.61 (2.7 – 4.9)	4.7 $\pm$ 0.58 (3.6 – 5.6)

<sup>A</sup>B1 fraction of carbohydrate or starch% + sugar% in the diet

The National Research Council (2001) reported that a cow in early lactation would require a diet with a minimum NDF of 25 to 33%, ADF of 17 to 21% and a maximum NFC of 25 to 33%. Lean *et al.* (2003) recommended a diet with the following components; 16 to 18% CP, 11.5 to 12 MJ ME/ kg DM, NFC 36% and NDF 32% (eNDF > 21%). Diets fed to cows in all regions contained adequate mean CP%, however some herds were being offered diets with CP < 16% with the lowest 12.0%. A significant number of herds were feeding diets with crude protein in excess of 20%. Previous studies have shown crude protein fed in excess of lactation requirements to negatively effect reproduction, such as lowered conception rates (McCormick *et al.*, 1999), altered uterine fluid composition (Elrod and Butler, 1993) and lowered plasma progesterone concentrations (Butler, 1998). However, on examination of 82 protein studies evaluating milk and milk protein responses, milk yield increased quadratically as diet crude protein concentrations increased (National Research Council, 2001). The maximum milk production was obtained at 23% CP (National Research Council, 2001). Only 14% of farms in this study fed more than this amount. It is unlikely that the high crude protein fed in some herds in this study therefore, had a substantial impact on milk production. Some herds in Finley and Taree were fed diets inadequate in NDF content (23.5%), possibly placing these herds at increased risk of acidosis. Low NDF contents were accompanied by high NFC contents in the same areas. The mean NFC% in the diet of cows in Finley and Taree was  $37.2 \pm 5.66\%$  and  $36.4 \pm 7.30\%$  respectively, and are higher than NRC recommendations. The National Research Council (2001) recommends that total dietary fat concentration should be less than 7% of DMI. The mean total fat concentration of the diets in all areas was less than this figure with only one herd in Camden feeding over the recommended rate.

### 3.5 Conclusions

A diverse range of feeding practices was identified across the farms and regions studied in South Eastern Australia. Farmers in those regions fed substantial amounts of concentrates to milking cows, with some herds being fed up to 14.8 kg/cow/day and regional and seasonal means varied from 5.5 to 8.4 kg/cow/day. Concentrates were most commonly fed twice daily in the milking parlour either as a grain mix or pellet. Partial or total mixed ration feeding was also prevalent. Commodities were used close to food processing units and where equipment allowed these to be incorporated into the ration. There were 19 different commodities fed. Some commodities such as corn hominy, molasses and wheat millrun were fed on a substantial proportion of farms. While most diets met recommended targets for composition, many herds fed rations too low in NDF, eNDF and CP, whilst in some herds, CP and NFC contents were too high. The recommended minimum dietary NDF% is 25 to 33 % (National Research Council, 2001), however 32 % of herds fed less than 33 % NDF in the diet. Feeding substantial amounts of concentrate at milking time and good quality pasture very low in effective fibre on some farms suggests that these farms may be at risk of acidosis. Buffers, rumen modifiers and antibiotics were extensively used in the industry and over 60% of farms in some regions fed ionophores. Notwithstanding this, of the herds feeding monensin, 25 % fed monensin at less than 200mg/ head/ day, the minimum label dose recommended by the manufacturer. The feeding rates of these products needs reviewing, as many herds fed these at rates lower than those recommended to meet nutritional requirements or efficacy.

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## Chapter 4.

# A DEFINITION OF ACIDOSIS IN DAIRY HERDS UNDER SOUTHERN AUSTRALIAN CONDITIONS

## 4.1 Summary

*This chapter details results of a cross-sectional survey examining the prevalence and effects of acidosis on rumen function of dairy cattle. One hundred dairy herds were selected from lists supplied by veterinary practices in five areas of NSW and Victoria. Eight fresh cows (< 100 DIM), consisting of three primipars and five multipars, were randomly selected from each herd. Rumen fluid was sampled from each cow by both rumenocentesis and stomach tube and samples were tested for pH. Stomach tube rumen fluid samples were analysed for volatile fatty acids (VFAs), ammonia and D-lactate concentration.*

*Results from all assays were used to group animals into categories using cluster analysis. Three distinct categories of cows were identified. The categories were further examined using logistic regression and Bayesian statistics. Importantly, despite the diversity of feeding strategies identified in Chapter 3, the random effect of herd was not a significant factor in the bayesian model and the cluster analysis results could be used to define categories. The percentages of animals in categories 1, 2 and 3 were 10.2%, 29.9% and 59.9%, respectively. Mean rumen pH for categories 1, 2 and 3 was  $5.74 \pm 0.47$ ,  $6.18 \pm 0.44$  and  $6.33 \pm 0.43$  respectively. Biochemically, categories 1, 2 and 3 were characterised respectively as follows; mean total VFA concentration (mM)  $100.74 \pm 23.22$ ,  $94.79 \pm 18.13$  and  $62.81 \pm 15.65$ , mean ammonia concentration (mM)  $2.46 \pm 2.02$ ,  $7.79 \pm 3.75$  and  $3.64 \pm 2.03$ , and mean D-lactate concentration (mM)  $0.34 \pm 0.86$ ,  $0.28 \pm 0.97$  and  $0.12 \pm 0.51$ .*

*Optimal concentrations of total volatile fatty acids and ammonia are suggested to be > 95 mM and > 2.8 mM, respectively. Fibre digestion is impaired when rumen pH is < 5.8 to 6.2. The category results suggest that biochemically, cows in category 1 may be high producing, but acidotic, and cows in category 2 lower producing due to a*

*mismatch between rumen fermentation rates of dietary energy and protein intake. Cows in category 3, although offered similar dietary inputs as category 2 cows had sub-optimal concentrations of total VFA, but were higher producing than category 2, possibly reflecting a more balanced rumen fermentation, or greater clearance of VFAs.*

## 4.2 Introduction

Acidosis is a nutritional disorder generally resulting from ingestion of large amounts of unaccustomed feeds rich in readily fermentable carbohydrates. The resulting production of large quantities of volatile fatty acids and lactic acid decreases rumen pH to non-physiological levels, simultaneously weakening the buffering capacity of the rumen. Low rumen pH can result in rumenitis, metabolic acidosis, lameness, hepatic abscessation, pneumonia and death (Lean *et al.* 2000).

### 4.2.1 Concentrate induced acidosis

The traditional model for acidosis has been extensively researched and reviewed (Dirksen 1970; Huber 1976; Hungate *et al.* 1952; Nocek 1997; Owens *et al.* 1998; Slyter 1976). However, much of this research has focused on experimentally induced acidosis using large amounts of starch-based concentrate (Crichlow and Chaplin 1985) to reduce rumen pH often to less than 5 (Nocek 1997). Initially as increased concentrations of fermentable carbohydrates are fed, there is an increase in growth rate of all bacteria, increasing total VFA production. The rumen pH subsequently falls, favouring the growth of *Streptococcus bovis* (Russell and Hino 1985). The survival rates of cellulolytic bacteria are reduced when pH drops below 6.2 (Calsamiglia *et al.* 1999). Glucose produced from the breakdown of starch by *S. bovis* is converted to fructose 1,6 biphosphate, which Russell and Hino (1985) found has a positive feedback on the conversion of pyruvate to lactate by activating lactate dehydrogenase. Fructose 1,6 biphosphate is also converted to triose phosphate. Increasing concentrations of the latter act to inhibit the activity of pyruvate formate lyase. The net effect of these changes is a switch from a fermentation predominantly favouring acetate production to one favouring lactate production. Accumulation of lactic acid, an acid 10 times stronger than either acetic, propionic or butyric acids can lower rumen pH and exceed ruminal buffering capacity. At rumen pH 4.7, growth

rate of *S. bovis* decreases and lactobacilli increase, producing greater amounts of lactic acid. The positive feedback effect results in fermentation stasis, absorption of D- and L- lactate and, in severe cases, metabolic acidosis.

The increase in VFA concentrations may also initially decrease reticulo-rumen motility by acting on receptors in the wall of the rumen. In sheep, these receptors are activated when non-dissociated VFA concentrations exceed 3.0 mM (Crichlow and Chaplin 1985). Those authors found that the decrease in rumen motility was independent of a decrease in the pH of rumen contents or blood pH. A decrease in motility results in a decrease in rumination and possibly reduced saliva production. Saliva contains high concentrations of bicarbonate ions and is an important buffering mechanism for the rumen.

#### **4.2.2 Rumen acidosis on pasture-based diets**

Australian dairy cows are still predominantly pasture-fed, but concentrates are offered twice daily in the milking parlour. Seventy nine percent of study herds were fed by this method (Chapter 3). Clinical and sub-clinical rumen acidosis is currently poorly defined under this type of feeding system. Rumen pH will often fluctuate during a 24-hour period. Nadirs in pH are recorded 2 to 4 hours after feeding concentrates to cows in the milking bails (Carruthers and Neil 1997; Harrison *et al.* 1989). Short periods of exposure to pH of 5.4 *in vitro* had a transitory negative effect on rumen microbial populations as represented by microbial nitrogen flow from the rumen (g/day). Microbial populations were clearly able to recover during intervals when pH was optimal (pH = 6.3) (de Veth and Kolver 2001b; Kolver and de Veth 2002).

The risk of acidosis may be increased when cattle are fed forages high in NFC such as sugars and starches, or low in effective fibre, such as clovers and young lucerne (alfalfa), and possibly ryegrass. These carbohydrate sources may not stimulate adequate rumination and salivation. In this situation, rumen pH may remain depressed for longer periods of the day. Kolver and de Veth (2002) collated animal and dietary variables from 23 pasture-based studies and reported that the mean daily ruminal pH ranged from 5.6 to 6.7 across studies. The diets collated in that study contained at least 47% pasture with an average content of 81% across all treatments.

Highly digestible pasture, when combined with concentrate feeding, increases bacterial growth rates and production of acetate, propionate and butyrate. Rumen pH subsequently decreases and is strongly negatively associated with total VFA concentrations ( $P < 0.001$ ;  $R^2 = 0.3$ ) (Kolver and de Veth 2002). During the transitory period of low pH (4 to 8 hours), de Veth and Kolver (2001b) found that the acetate:propionate ratio decreased *in vitro*, primarily because of a decrease in acetate concentrations. Other pasture-based *in vitro* studies reported no significant change in ratio, concluding that the individual VFA concentrations are determined by substrate preferences rather than rumen pH (de Veth and Kolver 2001a). This finding is challenged in an analysis of *in vivo* studies where rumen pH was strongly negatively related to propionate concentration (mM) ( $P < 0.001$ ) both within and across 75 studies (Kolver and de Veth 2002). In the same study, the ratio of acetate to propionate did not significantly predict rumen pH. Lactate concentrations did not change with prolonged exposure to sub-optimal pH (5.4), remaining between 0.05 and 0.13 mM (de Veth and Kolver 2001b). Concentrations of lactate recorded in concentrate-induced acidosis have been significantly higher (18.8 to 48.0 mM, 2 to 4 hours following ingestion) (Crichlow and Chaplin 1985), suggesting that either the switch from acetate and formate to lactate production by *S. bovis* and the growth of *Lactobacilli* may not occur in pasture-based acidosis or that clearance of lactate from the rumen is more efficient. A transitory, four hour decrease in rumen pH to 5.4 *in vitro* decreased the flow of microbial N from 0.39 g/day to 0.37 g/day, signifying that cellulolytic bacteria may not die, but rather have limited access to substrate (de Veth and Kolver 2001b). Under the same conditions, ammonia N flow (g/day) did not greatly change, possibly because both deamination of protein and utilisation of ammonia were reduced by both low pH and limited availability of suitable energy sources or carbon chain precursors.

Some intensive studies in Australia have examined the effects of feeding of starch-based concentrates to cows on pasture (Clayton *et al.* 1999; Lean *et al.* 2000; Opatpatanakit *et al.* 1994; Wales *et al.* 2001). However, no extensive surveys of the industry have been documented. Observations of Australasian dairy herds (IJ Lean, personal communication) suggest that up to 40% of animals may be affected with acidosis, clinical or sub-clinical, at any one time. Brown *et al.* (2000) used discriminate analysis to define cows with acute and subacute acidosis in a randomised

trial (n = 20) where acidosis was induced by concentrate feeding after initially examining rumen pH cut-points. Brown *et al.* (2000) found that no single variable measured displayed a consistent response across time that could be used to identify acute or subacute ruminal acidosis. The survey reported here was conducted to more accurately define rumen conditions present based on rumen parameters in Southern Australian dairy herds where herds were predominantly pasture based and adapted to concentrate fed primarily at milking. Because of the fluctuation of rumen pH during the day, diagnosis of acidosis based solely on rumen pH can be difficult. Cluster analysis was used to identify relatively homogenous groups of cases based on rumen biochemistry, including rumen pH, individual volatile fatty acid concentrations, ammonia and D-lactate concentrations in order to relate rumen conditions with diets offered.

### **4.3 Material and Methods**

#### **4.3.1 Herd and cow selection**

One hundred dairy herds were selected from lists supplied by local veterinary practices in five areas of NSW and Victoria. Refer to section 2.3.1. Eight animals were sampled for rumen fluid from each herd. Refer to section 2.3.2.

#### **4.3.2 Sampling and measurements**

Rumen samples were obtained by rumenocentesis and stomach tube generally after morning milking (refer to section 2.3.2). For rumenocentesis and stomach tubing technique, refer to section 2.3.5.3. Rumen fluid was prepared and stored as outlined in section 2.3.7.3.

#### **4.3.3 Analytical Procedures**

Rumen pH was measured immediately in samples of the raw rumen fluid obtained by both rumenocentesis and stomach tube (refer to section 2.4.2). Rumen fluid taken from stomach tube samples was analysed for volatile fatty acid concentration, ammonia concentration and D-lactate concentration. Two hundred selected rumen samples were also analysed for L-lactate concentration. This was a function of cost and samples were selected by selecting herds. Herds were selected based on mean rumen pH results with low, middle and high mean rumen pH herds selected. L-lactate

results were only used in rumen pH prediction as a comparison with D-lactate results. The selection process for samples was therefore not significant. Refer to section 2.4.3 for more detailed analytical procedures.

Dietary inputs presented in Table 4.5 were summarised for each cluster from the diets of each herd containing three (37.5%) or more cows allocated to the same cluster excluding missing cases. The complete diet was entered into the ration formulation program CPM dairy (Cornell\*Penn\*Miner, 2003) (version 3.0.4a) and compared with the predicted model based on dietary intake (refer to section 2.4.4.2).

#### **4.3.4 Statistical analyses**

Initially, a scatter plot comparing differences in rumen pH measured by rumenocentesis versus stomach tube was computed using Statistix (Version 7, NIH Software). The relationship between rumen pH and other rumen biochemical analyses was then explored (section 4.4.1). A three stage approach was used to analyse these data. In the first stage, the relationship between the outcome variable (rumen pH) and each of the explanatory variables was assessed using linear regression. In the second stage, each of the explanatory variables that were associated with the outcome at an alpha level of less than 0.20 were included in a fixed effects linear regression model (SPSS, Version 11.0). In the third stage, the fixed-effects linear regression model was extended to include herd as a random effect (Genstat, Version v.7.1.0.198). Our rationale for including herd as a random effect term was to allow for unmeasured, herd-level factors, such as environmental conditions or genetics, that may influence rumen pH and the other factors modelled as fixed effects. The random effects model was assessed both with and without the inclusion of L-lactate. The number of rumen samples used in the analysis without inclusion of L-lactate was 686 whilst 169 samples were used in the model including L-lactate. Both models have been included because, although the inclusion of L-lactate is valuable, the greater number of samples able to be included gives the model without L-lactate more statistical power.

Statistical analysis for group allocation and categories were performed using K-means cluster analysis and linear regression (SPSS, Version 11.0). Cluster analysis was used to divide cows into a set number of groups (n=3) based on a set of predictors, in this case biochemical analyses results. Refer to section 2.4.6.1. Predictive variables used

in the cluster analysis were converted to standardised variates before clusters were assessed. The variables identified from the cluster analysis were used to describe the categories also derived from the cluster analysis. These data were tested with a logistic regression model that included or excluded the random effect of herd as a predictive variable using Bayesian modelling (Winbugs, Version 11.4) (outlined in section 2.4.6.4). There were 114 unclassified cases in the cluster analysis due to missing data, primarily rumenocentesis data, from one or more variables. These cases were categorised into one of the three categories using discriminant analysis (SPSS, Version 11.0) (outlined in section 2.4.6.2) based on the other variables measured, including stomach tube pH. Of the 114 cases to be categorised, 13 were classified in cluster 1, 39 classified in cluster 2 and 62 classified in cluster 3.

Comparisons between categories of the mean parameter values were analysed using Kruskal-Wallis One-way ANOVA and Wilcoxon rank sum test due to inequality of variances. These non-parametric tests were also used to determine significant differences among data in Table 4.8. Data differences between amounts of rumen modifiers and antibiotics fed in Table 4.7 were analysed using One-way ANOVA because assumptions associated with this test were met.

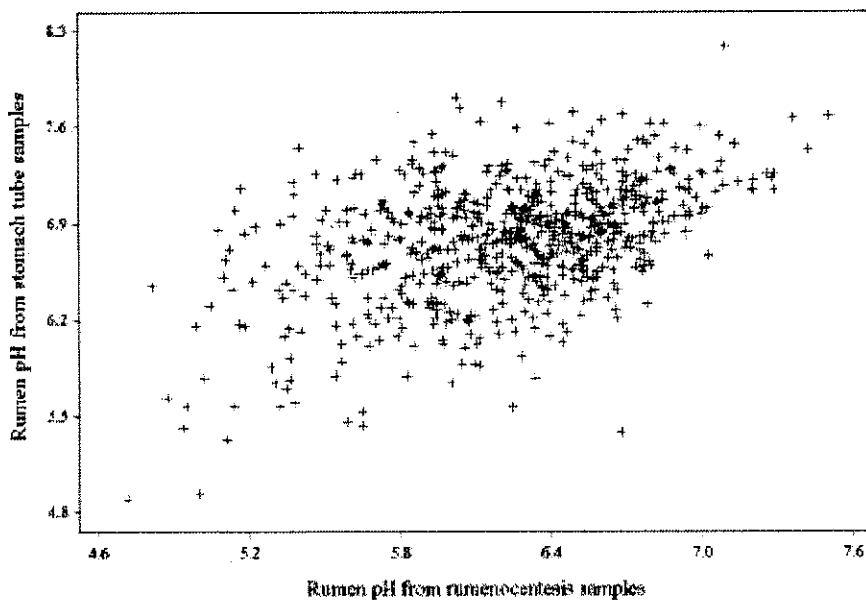
Production data summarised in Table 4.8 were collated from all animals found in categories one to three excluding animals > 100 DIM ( $n = 83$ ) and those where herd test data were not recorded. The herd test before and after the sampling date was recorded. The herd test used for the production data was the closest test recorded to the sampling date. Analyses on production data conducted used DIM, parity and number of days to herd test as covariates and controlled for the effect of herd. Although part of the selection criteria was to sample cows < 100 DIM, there were occasions where farmers accidentally held the wrong cow back after milking or made a mistake on the calving dates which was recognised after sampling was completed. Production data collected from these cows were therefore not included in analyses. Rumen biochemical data from these cows, however, were included because it was concluded that DIM would not be a confounding factor on rumen function.

## 4.4 RESULTS

### 4.4.1 Relationship between rumen pH and rumen biochemical analyses

A scatter plot is presented comparing rumen pH measured by rumenocentesis and stomach tube (Figure 4.1). Using rumenocentesis data, 16.1% of cows sampled had a rumen pH less than 5.8, whilst 31% of cows had a rumen pH less than 6.0.

**Figure 4.1.** Scatter plot comparing rumen pH measured by rumenocentesis versus stomach tube



The relationship of rumen pH (rumenocentesis) to rumen acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, caproic acid, D-lactate and ammonia was assessed by linear regression including the random effect of herd (Genstat, Version v.7.1.0.198). When individual covariates were assessed, propionic acid, isobutyric acid, butyric acid, caproic acid and D-lactate were significant ( $P < 0.05$ ) whilst acetic acid approached significance ( $P = 0.108$ ). The residual plot showed no patterns or outliers of concern and the residuals appeared to be approximately normally distributed. The estimated model was;

$$\text{Rumen pH} = 6.226 (\pm 0.0278) + 0.00378 (\pm 0.002350) \text{ acetic acid} - 0.0257 (\pm 0.00271) \text{ propionic acid} + 0.594 (\pm 0.0989) \text{ isobutyric acid} - 0.0359 (\pm 0.00823) \text{ butyric acid} + 0.153 (\pm 0.0683) \text{ caproic acid} + 0.0693 (\pm 0.02192) \text{ D-lactate}$$

Therefore, rumen pH increased with increased concentrations of acetic acid, isobutyric acid, caproic acid and D-lactate in the rumen and with decreased concentrations of propionic acid and butyric acid.

L-lactate concentration was also assessed in 183 rumen fluid samples. The concentration of D- and L-lactate in the selected rumen samples was highly correlated ( $R = 0.905$ , Pearson's correlation) and the mean concentration of L-lactate present in the samples was low ( $0.12 \pm 0.141$  mM). The relationship of rumen pH to propionic acid, butyric acid, isobutyric acid, D- and L- lactate was examined using linear regression including herd as a random effect using 169 rumen fluid samples (Genstat, Version v.7.1.0.198). Propionic acid, butyric acid, isobutyric acid and D-lactate were highly significant ( $P < 0.01$ ) whilst L-lactate was significant ( $P = 0.031$ ). The estimated model was;

$$\text{Rumen pH} = 6.144 (\pm 0.0337) - 0.03365 (\pm 0.003984) \text{ propionic acid} - 0.06082 (\pm 0.012023) \text{ butyric acid} + 1.694 (\pm 0.5494) \text{ D-lactate} - 1.422 (\pm 0.6609) \text{ L-lactate} + 1.143 (\pm 0.2013) \text{ isobutyric acid}$$

#### 4.4.2 Allocation of cows

Cows were allocated to one of three categories by cluster analysis based on results from rumen biochemical analyses. When cows were clustered into four or more categories, the category size in one or more categories became too small and category definition was poor. Three categories were identified and proposed to represent acidotic, sub-clinically acidotic and non-acidotic animals. Where one or more variables were missing, the animal remained unclassified. Cows ( $n = 682$ ) were used in the cluster category; 70 in category 1, 204 in category 2 and 409 in category 3.

##### 4.4.2.1 Significant factors for category allocation

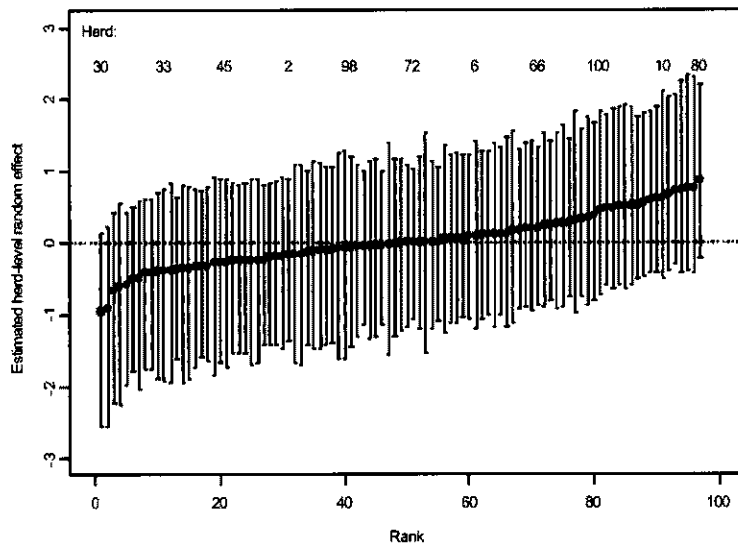
Table 4.1 shows the significant factors in determining the cluster categories. All variables were highly significant in predicting category allocation ( $P < 0.01$ ). Propionate ( $F = 332.38$ ) and valerate ( $F = 332.62$ ) were the most important factors in determining cluster categories. Also important in predicting categories were butyrate ( $F = 291.86$ ), isobutyrate ( $F = 243.50$ ), acetate ( $F = 226.17$ ) and ammonia ( $F =$

203.90). Interestingly, rumen pH and D-lactate were least important in prediction of the categories where  $F = 54.95$  and  $F = 5.02$  respectively. D-lactate was also the least significant of all the variables ( $P = 0.007$ ). The random effect of herd was not significant in the Bayesian logistic regression model (see Figure 4.2) and cluster analysis was used to provide category definition. Bayesian statistics was also used to examine the difference between the mixed model with random effect of herd included and the fixed effects model. No substantial difference was found between the two models, however, confidence intervals in the random effects model were slightly larger as expected. Some cows ( $n = 114$ ) were unclassified due to missing data, primarily missing rumenocentesis results (see Table 4.3). Data from these cattle are presented for comparative purposes (see Table 4.2).

**Table 4.1.** K-means cluster analysis ANOVA between acidotic, sub-clinically acidotic and non-acidotic animals after standardising variables to Z score ( $n = 682$ ).

	Mean Squares		D.F	F-ratio	P-Value
	Between	Within			
Acetate	131.811	0.583	2, 680	226.172	0.0001
Propionate	168.694	0.508	2, 680	332.375	0.0001
Butyrate	140.255	0.481	2, 680	291.856	0.0001
Isobutyrate	130.184	0.535	2, 680	243.502	0.0001
Isovalerate	95.694	0.733	2, 680	130.571	0.0001
Valerate	152.686	0.459	2, 680	332.620	0.0001
Caproate	70.108	0.601	2, 680	116.572	0.0001
Rumen pH	47.211	0.859	2, 680	54.950	0.0001
D-lactate	2.973	0.593	2, 680	5.017	0.007
Ammonia	120.556	0.591	2, 680	203.895	0.0001

**Figure 4.2.** Posterior means and 95% credible intervals for the herd level random effect terms from a logistic regression model of factors influencing the probability of acidosis in 100 Australian dairy herds (19/7/2001 – 21/5/2002).



#### 4.4.2.2 Cluster category one

Mean values for the three cluster categories determined using cluster analysis are given in Table 4.2. Category 1 ( $n = 70$ ) was characterised by a low mean rumen pH ( $5.74 \pm 0.47$ ), high mean total VFA concentrations ( $100.74 \pm 23.22$  mM) and low to normal mean rumen ammonia concentrations (2.46 mM). The mean propionate concentration was significantly higher in cluster 1 ( $34.19 \pm 10.17$  mM) than the other two categories ( $P = 0.001$ ) resulting in a ratio of acetate: propionate of 1.53. The mean D-lactate concentration of category 1, although significantly higher than categories 2 and 3 ( $P = 0.001$ ), was only  $0.34 \pm 0.86$  mM. Interestingly, the mean valerate concentration was significantly higher ( $P = 0.001$ ) than the other two categories, being  $2.55 \pm 1.20$  mM.

#### 4.4.2.3 Cluster category two

Cattle in cluster category 2 ( $n = 204$ ) were characterised by very different mean concentrations of almost every measure than category 1. In this cluster, the mean rumen pH ( $6.18 \pm 0.44$ ) was significantly higher than category 1 and significantly lower than category 3 ( $P = 0.001$ ). The mean total VFA concentration ( $94.79 \pm 18.13$  mM) was significantly higher than category 3 ( $P = 0.001$ ). There was no significant difference between the mean total VFA concentration in categories 1 and 2. Of the individual VFA concentrations, acetate and butyrate were significantly higher and

propionate significantly lower than category 1 ( $P = 0.001$ ). The decrease in propionate concentration in category 2 accounts for the increase in the ratio of acetate: propionate in this category (3.04). The mean D-lactate concentration was also significantly lower in category 2 ( $0.28 \pm 0.97$  mM) compared with category 1. Interestingly in this category, the mean ammonia concentration ( $7.79 \pm 3.75$  mM) was significantly ( $P = 0.001$ ) higher than in either category 1 or 3.

#### 4.4.2.4 Cluster category three

Cluster category 3 contains the largest percentage of cows in the survey (51%). This category had a significantly higher mean rumen pH ( $6.33 \pm 0.43$ ) than category 1 or 2 and lower mean total VFA and D-lactate concentration ( $P = 0.001$ ). The mean ammonia concentration for category 3 was  $3.64 \pm 2.03$  mM, significantly higher than category 1 and lower than category 2 ( $P = 0.001$ ). Mean acetate, propionate and butyrate concentrations were all significantly lower than categories 1 and 2 ( $P = 0.001$ ) ( $39.16 \pm 10.74$  mM,  $14.44 \pm 4.88$  mM and  $6.89 \pm 2.32$  mM respectively) with an acetate to propionate ratio of 2.72. Mean isobutyrate, isovalerate, valerate and caproate concentrations were also significantly lower than categories 1 and 2 ( $P = 0.001$ ).

**Table 4.2.** Means (and standard deviations) of biochemical analyses for acidotic, sub-clinically acidotic and non-acidotic cows determined using cluster analysis.

	Cluster 1 (n=70)	Cluster 2 (n=204)	Cluster 3 (n=409)	Unclassified (n=114)
pH (rumenocentesis)	5.74 <sup>a</sup> (0.47)	6.18 <sup>b</sup> (0.44)	6.33 <sup>c</sup> (0.43)	6.32 (0.5) n=24
Total VFA (mM)	100.74 <sup>a</sup> (23.22)	94.79 <sup>a</sup> (18.13)	62.81 <sup>b</sup> (15.65)	80.68 (26.78) n=110
Acetate (mM)	52.45 <sup>a</sup> (12.37)	59.15 <sup>b</sup> (11.80)	39.16 <sup>c</sup> (10.74)	48.98 (16.04) n=110
Propionate (mM)	34.19 <sup>a</sup> (10.17)	19.47 <sup>b</sup> (6.18)	14.4 <sup>c</sup> (4.88)	18.76 (8.43) n=110
Butyrate (mM)	9.13 <sup>a</sup> (3.46)	12.38 <sup>b</sup> (2.94)	6.89 <sup>c</sup> (2.32)	9.66 (4.91) n=110
Isobutyrate (mM)	0.68 <sup>a</sup> (0.22)	0.86 <sup>b</sup> (0.22)	0.52 <sup>c</sup> (0.16)	0.71 (0.31) n=110
Isovalerate (mM)	1.14 <sup>a</sup> (0.37)	1.37 <sup>b</sup> (0.49)	0.88 <sup>c</sup> (0.26)	1.08 (0.40) n=110
Valerate (mM)	2.55 <sup>a</sup> (1.20)	1.28 <sup>b</sup> (0.42)	0.82 <sup>c</sup> (0.36)	1.24 (0.98) n=110
Caproate (mM)	0.59 <sup>a</sup> (0.56)	0.27 <sup>b</sup> (0.18)	0.13 <sup>c</sup> (0.17)	0.26 (0.46) n=110
D-lactate (mM)	0.34 <sup>a</sup> (0.86)	0.28 <sup>b</sup> (0.97)	0.12 <sup>c</sup> (0.51)	0.42 (1.88) n=88
Ammonia (mM)	2.46 <sup>a</sup> (2.02)	7.79 <sup>b</sup> (3.75)	3.64 <sup>c</sup> (2.03)	4.56 (3.99) n=108

<sup>ab</sup>Column means within a row with different superscripts differ ( $P < 0.01$ ) using Wilcoxon rank sum test.

**Table 4.3.** Reasons for cows unable to be classified using cluster analysis (n=114)

<b>Reason for unclassification</b>	<b>Number of unclassified cows</b>
Missing rumen pH (rumenocentesis)	86
Missing D-lactate concentration	19
Missing ammonia concentration	2
Missing rumen pH and D-lactate concentration	3
Missing rumen VFA, D-lactate and ammonia concentration	2
All data missing	2

Table 4.4 summarises the most important variables used in the discriminant analysis to reclassify cases. Table 4.5 summarises the means and standard deviations of the variables in each category using all cases (n = 797) as determined by discriminant analysis. Only the unclassified cases were reclassified. The means reported were similar to the initial cluster analysis results.

**Table 4.4.** Stepwise statistics in discriminant analysis to classify unclassified category (variables standardised to Z score).

<b>Step</b>	<b>Variable entered</b>	<b>F-ratio</b>	<b>P value</b>
1	Valerate	332.62	0.001
2	Butyrate	297.01	0.001
3	Isobutyrate	235.02	0.001
4	Propionate	200.86	0.001
5	Ammonia	176.65	0.001
6	Rumen pH (rumenocentesis)	150.34	0.001
7	Isovalerate	131.57	0.001
8	Acetate	116.67	0.001

**Table 4.5.** Means ( $\pm$  SD) of biochemical analyses for acidotic, sub-clinically acidotic and non-acidotic animals determined by cluster analysis after reclassification of unclassified category (n = 797). Numbers in brackets indicate number of samples used.

	Cluster 1 (n=83)	Cluster 2 (n=243)	Cluster 3 (n=471)
pH (rumenocentesis)	5.73 $\pm$ 0.47 (73)	6.18 $\pm$ 0.43 (207)	6.33 $\pm$ 0.42 (427)
Total VFA (mM)	100.89 $\pm$ 23.62 (83)	95.44 $\pm$ 18.41 (243)	62.93 $\pm$ 16.03 (467)
Acetate (mM)	52.26 $\pm$ 12.52	59.54 $\pm$ 11.87	39.27 $\pm$ 11.00
Propionate (mM)	34.04 $\pm$ 9.86	19.53 $\pm$ 6.14	14.44 $\pm$ 4.95
Butyrate (mM)	9.49 $\pm$ 4.07	12.53 $\pm$ 3.21	6.88 $\pm$ 2.36
Isobutyrate (mM)	0.67 $\pm$ 0.22	0.89 $\pm$ 0.23	0.52 $\pm$ 0.16
Isovalerate (mM)	1.10 $\pm$ 0.38	1.38 $\pm$ 0.47	0.88 $\pm$ 0.26
Valerate (mM)	2.65 $\pm$ 1.28	1.29 $\pm$ 0.42	0.82 $\pm$ 0.36
Caproate (mM)	0.62 $\pm$ 0.67	0.28 $\pm$ 0.19	0.13 $\pm$ 0.17
D-lactate (mM)	0.55 $\pm$ 1.92 (81)	0.25 $\pm$ 0.90 (239)	0.14 $\pm$ 0.61 (450)
Ammonia (mM)	2.33 $\pm$ 1.92 (82)	7.79 $\pm$ 3.91 (243)	3.56 $\pm$ 2.02 (466)

#### 4.4.3 Diet summary for cluster categories

The diets of each herd containing three (37.5%) or more cows allocated to the same category were grouped. Where 3/8 cows from category 1 were found in a herd, the herd was classified as 'ACID', 3/8 cows from category 2 in a herd classified the herd as suboptimal rumen function 'SO' and 3/8 cows from category 3 in a herd classified the herd as normal function 'NA'. The number of herds used in categories ACID, SO and NA were 10, 35 and 69 herds respectively. Twenty herds met the criteria of 2 categories and were used twice in analysis; one herd was in categories 1 and 2, two herds were in categories 1 and 3 and 17 herds were in categories 2 and 3. Although there would be a variance inflation effect due to the counting of some herds

twice, this would have minimal effect on the primary herd category of interest, ACID, because the majority of the herds used twice were found in categories SO and NA. Had the herds not been included in either category, the power of the data set would have been reduced. The mean dietary parameters for each herd category are presented in Table 4.6. Significant differences were found between herd categories for ME, NDF, peNDF, NFC and starch percentage ( $P < 0.05$ ). The mean ME concentration in the diet of herd category 'ACID' was significantly greater than that of herd category 'NA' ( $P = 0.011$ ). The mean NFC percentage in the diet of 'ACID' herds was also significantly higher than either 'SO' or 'NA' herds ( $P = 0.002$  and  $P = 0.001$  respectively). Similarly, the mean starch percentage of the diet was significantly higher in 'ACID' herds compared with 'SO' or 'NA' herds ( $P = 0.004$  and  $P = 0.003$  respectively).

**Table 4.6.** Mean dietary parameters ( $\pm$  SD) of herds in each category where herds contained 37.5% or more of cows allocated to the category.

	ACID herds (n = 10)	SO herds (n = 35)	NA herds (n = 69)
DMI (kg/cow/day)	19.4 $\pm$ 3.01	18.7 $\pm$ 3.94	19.2 $\pm$ 3.74
CP%	19.9 $\pm$ 2.19	20.0 $\pm$ 2.96	19.6 $\pm$ 3.00
Undegradable protein (%CP)	35.2 $\pm$ 3.67	34.7 $\pm$ 4.71	35.0 $\pm$ 4.49
Soluble protein (%CP)	35.1 $\pm$ 2.40	34.9 $\pm$ 4.85	35.2 $\pm$ 4.21
Estimated ME (MJ/kg DM)	11.0 $\pm$ 0.37 <sup>c</sup>	10.5 $\pm$ 0.91	10.4 $\pm$ 0.79 <sup>d</sup>
NDF%	30.4 $\pm$ 4.27 <sup>ac</sup>	35.7 $\pm$ 5.98 <sup>d</sup>	36.1 $\pm$ 5.18 <sup>b</sup>
peNDF%	21.1 $\pm$ 3.12 <sup>c</sup>	24.2 $\pm$ 4.68 <sup>d</sup>	24.6 $\pm$ 4.26 <sup>d</sup>
NFC%	40.3 $\pm$ 4.44 <sup>a</sup>	33.4 $\pm$ 6.45 <sup>b</sup>	33.5 $\pm$ 5.71 <sup>b</sup>
Starch%	22.7 $\pm$ 5.37 <sup>a</sup>	16.8 $\pm$ 6.23 <sup>b</sup>	16.9 $\pm$ 5.95 <sup>b</sup>
Total Fat%	4.0 $\pm$ 0.51	4.4 $\pm$ 0.79	4.4 $\pm$ 0.81

<sup>a,b</sup> Column means within a row with different superscripts differ ( $P < 0.01$ ).

<sup>c,d</sup> Column means within a row with different superscripts differ ( $P < 0.05$ ).

The mean NDF percentage of the diet of 'ACID' herds was significantly lower to that of 'SO' or 'NA' herds ( $P = 0.015$  and  $P = 0.002$  respectively). The dietary peNDF was also significantly lower in 'ACID' herds compared with 'SO' or 'NA' herds ( $P = 0.042$  and  $P = 0.011$  respectively). All other dietary measures reported in Table 4.6 were not significant between categories.

The number of herds in each category (from Table 4.6) that fed modifiers and antibiotics were also examined. The percentage of herds feeding monensin, lasalocid, virginiamycin and tylosin and mean amounts fed from the herds feeding the product are summarised in Tables 4.7 and 4.8, respectively. Interestingly, all of the diets summarised in Table 4.6 for 'ACID' herds contained monensin at a mean rate of  $252.6 \pm 100.79$  mg. Only 65.7 and 60.9% of the diets summarised in Table 4.6 for 'SO' or 'NA' herds respectively contained monensin. The only herds feeding lasalocid and tylosin were 'NA' herds. All three herd categories included some diets containing virginiamycin with the highest percentage (50%) in 'ACID' herds. Notwithstanding this, 'ACID' herds fed virginiamycin at the lowest rate for all herd categories ( $156.8 \pm 92.13$  mg). There was no significant difference between mean amounts of monensin and virginiamycin recorded in each herd category.

**Table 4.7.** Percentage of dietary modifiers and antibiotics used in the herd categories.

% herds feeding product	'ACID' herds (n = 10)	'SO' herds (n = 35)	'NA' herds (n = 69)
Monensin	100.0	65.7	60.9
Lasalocid	0	0	7.2
Virginiamycin	50.0	28.6	24.6
Tylosin	0	0	4.3

**Table 4.8.** Mean amount ( $\pm$  SD) of modifiers and antibiotics used in the herd categories (excluding herds feeding none)

Mean amount fed	'ACID' herds	'SO' herds	'NA' herds
(mg/cow/day)			
Monensin	252.6 $\pm$ 100.79	258.5 $\pm$ 92.64	240.2 $\pm$ 71.75
Lasalocid	-	-	265.0 $\pm$ 40.07
Virginiamycin	156.8 $\pm$ 92.13	186.6 $\pm$ 46.32	195.3 $\pm$ 74.97
Tylosin	-	-	119.7 $\pm$ 39.50

#### 4.4.4 Production data summary for cluster categories

The mean number of days of the herd test to the sampling date for each of the three categories was  $11.3 \pm 8.19$  days,  $10.7 \pm 9.31$  days and  $12.0 \pm 9.66$  days for categories 1, 2 and 3 respectively. The mean parity for categories 1, 2 and 3 was  $2.08 \pm 1.53$  calvings,  $3.15 \pm 2.39$  calvings and  $3.05 \pm 2.06$  calvings respectively. Parity was significantly lower in category 1 than category 2 ( $P = 0.013$ ) or 3 ( $P = 0.005$ ). DIM was not significantly different between categories and mean values for categories 1, 2 and 3 were  $44.7 \pm 25.47$  days,  $52.0 \pm 26.68$  days and  $47.6 \pm 26.85$  days, respectively.

The mean production data for the three categories after correcting for DIM, parity and number of days from herd test to sampling date and including herd as a random effect are presented in Table 4.9. Cows were selected for inclusion into the data set if they were  $< 100$  DIM, not in category 4 (unclassified) and if the date to nearest herd test was  $< 31$  days (1 month). Milk production in category 1 was higher ( $P = 0.057$ ) than category 2 and category 2 was significantly lower than category 3 ( $P = 0.011$ ). The percentage milk fat was significantly lower in category 1 than category 2 ( $P = 0.001$ ) and 3 ( $P = 0.020$ ) but higher in category 2 than 3 ( $P = 0.022$ ). The percentage milk protein was only significantly higher in category 2 than 3 ( $P = 0.024$ ). This resulted in a significantly lower milk fat: protein in category 1 compared with category 2 ( $P = 0.001$ ) or 3 ( $P = 0.004$ ). The differences in milk volume between the categories resulted in a higher milk protein yield in category 1 compared with category 2 ( $P = 0.055$ ). There was no significant difference in somatic cell count between the 3 categories.

**Table 4.9.** Mean production data ( $\pm$  SD) for cows in categories where a herd test was recorded (corrected for DIM, parity and number of days from last herd test to sampling day, controlled for effect of herd).

Mean production data	Category 1 (n = 39)	Category 2 (n = 132)	Category 3 (n = 276)
Milk volume (L)	30.0 $\pm$ 0.97	27.9 $\pm$ 0.52 <sup>c</sup>	29.7 $\pm$ 0.36 <sup>d</sup>
% milk fat	3.32 $\pm$ 0.112 <sup>ac</sup>	3.79 $\pm$ 0.061 <sup>bc</sup>	3.61 $\pm$ 0.041 <sup>d</sup>
% milk protein	3.15 $\pm$ 0.056	3.18 $\pm$ 0.031 <sup>c</sup>	3.09 $\pm$ 0.021 <sup>d</sup>
% fat: protein	1.06 $\pm$ 0.036 <sup>a</sup>	1.20 $\pm$ 0.020 <sup>b</sup>	1.18 $\pm$ 0.010 <sup>b</sup>
Milk fat yield (kg)	1.00 $\pm$ 0.044	1.04 $\pm$ 0.024	1.05 $\pm$ 0.016
Milk protein yield (kg)	0.94 $\pm$ 0.030	0.87 $\pm$ 0.017	0.91 $\pm$ 0.011
Somatic cell count (cells/ml, log transformed)	10.9 $\pm$ 0.23	11.0 $\pm$ 0.12	11.0 $\pm$ 0.09

<sup>a,b</sup>Column means within a row with different superscripts differ ( $P < 0.01$ ).

<sup>c,d</sup>Column means within a row with different superscripts differ ( $P < 0.05$ ).

## 4.5 Discussion

### 4.5.1 Potential herd and cow selection biases

An initial estimate of 120 herds was made based on a number of anticipated risks for hypotheses of interest. However, the number of farms sampled was limited to 100 due to cost, time and labour constraints. This number represents 1.2% of Victorian and New South Wales dairy farms (Dairy Australia 2003). The 100 herds provide for determining a difference in the odds of acidosis being present for a univariate association with a statistical power of 0.6, with  $\alpha = 0.05$  for a prevalence of 30% in one group and 40% in the group of interest. Although the best outcome would have been to sample 120 herds, it was concluded that, given constraints, it was more valuable to sample more cows in each herd and less herds. Therefore, approximately 800 cows were sampled for rumen fluid and 1500 cows were assessed for body condition, locomotion and faecal score. This number of samples would not have been achieved had more herds been sampled.

It is also acknowledged that the herd sample was not completely random, particularly in the Taree and Camden regions, and it is possible that these data reflect farmers that are more sympathetic to research. The willingness to be involved could also indicate that participating farmers recognized acidosis as an important production problem. Notwithstanding this, compared to many studies that have used Dairy Herd Improvement Association (DHIA) herds only, this study offers a data set that was sampled more widely, and I consider is indicative of the dairy industry in Southern Australia. It is also the author's belief that, after sampling the herds, the majority of even the most progressive farmers did not recognize signs of acidosis in a herd and in most cases, when there was a problem, did not realize that their herd was at risk. The study was not limited to herds involved in a herd testing program. Herd testing in the Australian dairy industry involves collecting a sample of milk from a lactating cow, generally at morning milking. Samples can be collected from part or all of a lactating herd. Milk yield (L) is measured on the day, with milk components, including fat, protein and somatic cell count measured from the sample later at the laboratory. Herd testing generally occurs approximately once per month, however some dairy farmers prefer to test less often, for example, every 6 months. The two most common herd testing schemes in Australia are Dairy Express and Maffra Herd Improvement (MIA).

Eight cows were sampled from each herd for rumen fluid, blood and faeces. Garrett *et al* (1999) concluded that a minimum sample size of 12 cows per herd was appropriate. The aim of that study was to validate a field protocol for the determination of ruminal pH and develop a strategy to interpret ruminal pH data from a group of cows (Garrett *et al.* 1999). In this study, rumen pH was just one of a number of variables used to attempt to understand the syndrome of acidosis rather than evaluate an outcome, specifically low rumen pH. The sampling 8 cows in this study rather than 12 reflected cost and time restraints on each farm, given the labour available. It was also concluded that statistically, it was more valuable to sample as many herds as possible. Sixteen cows (six heifers and 10 mature cows) were initially selected where possible. There were situations where it was not possible to select 16 cows, for example, when there were only three heifers that met the selection criteria. Of the cows held after milking, the first eight eligible cows to be restrained were sampled. The sampler was blinded from knowledge of herd tests or calving dates until after sampling so as to

minimise selection biases. This blinding contributed to some cows that were more than 100 days in milk being included in sampling.

#### 4.5.2 *Grouping of herds into categories*

Data collected in the study were both cow and herd level. It was important to compare both sets of data, therefore herds were grouped into categories based on individual cow classifications determined by cluster analysis. Herds containing three (37.5%) or more cows allocated to the same category were grouped. Where 3/8 cows from category 1 were found in a herd, the herd was classified as 'ACID', 3/8 cows from category 2 in a herd classified the herd as suboptimal rumen function 'SO' and 3/8 cows from category 3 in a herd classified the herd as normal function 'NA'. The number of herds used in categories ACID, SO and NA were 10, 35 and 69 herds respectively. Twenty herds met the criteria of 2 categories and were used twice in analysis; one herd was in categories 1 and 2, two herds were in categories 1 and 3 and 17 herds were in categories 2 and 3. The classification of herds by grouping 4/8 cows allocated to the same category was explored, however this would have reduced numbers in herd groups ACID, SO and NA to 5, 21 and 53 herds respectively. Although this would have alleviated the problem of herds meeting the criteria of 2 categories, it was considered more valuable to include herds in analyses, rather than lose herds from the analysis.

#### 4.5.3 *Rumen pH prediction*

Rumen pH increased with increased concentrations of acetic acid, isobutyric acid, caproic acid and D-lactate in the rumen and with decreased concentrations of propionic acid and butyric acid. The low acetic acid and high propionic acid concentrations when rumen pH was low confirms previous *in vitro* studies showing that cattle with low acetate to propionate ratios also had low ruminal pH values, contributing to reduced methanogenesis (Lana *et al.* 1998). Rumen pH also decreased with decreasing D-lactate concentration, when including the random effect of herd. Interestingly, when L-lactate was included in the model, rumen pH decreased when L-lactate increased and D-lactate decreased. Notwithstanding this, total lactate concentration may lower rumen pH. The difference between the isomers of lactate may be explained either by possible differences in the transport system across the rumen wall or by preferences of lactate utilising bacteria for one or other of the

isomers at different rumen pH. Goad *et al* (1998) found that decreased rumen pH in hay-adapted and grain adapted steers was due primarily as result of increased VFA concentration rather than increased lactate concentration. Lactate utilising bacteria increased over time with grain challenge. When ruminal acidosis was experimentally induced in calves, D-lactate concentrations significantly and rapidly increased whereas L-lactate did not deviate from physiological concentrations (Gentile *et al.* 2004). However these studies were conducted on cattle experimentally induced with subclinical or clinical acidosis. The study described in this chapter was a field survey of cattle fed diets predominantly pasture based but adapted to concentrate, therefore rumen conditions may well be different.

#### 4.5.4 Biochemical analyses

Previous studies have described rumen acidosis using rumen pH. The cut-off pH for which sub-clinical acidosis is diagnosed is controversial with some authors suggesting 5.5 by rumenocentesis (Garrett *et al.* 1999). However *in vitro* fibre digestibility is reduced when pH drops below 6.2 (Calsamiglia *et al.* 1999; Grant 1994; Grant and Mertens 1992). In this study, 7.6% of cows had a rumenocentesis pH of less than 5.5, 16.1% of cows less than 5.8, but 43% of cows had a rumen pH of less than 6.2 (Figure 4.1). Rumen pH is known to fluctuate throughout the day depending on diet, time of feeding of concentrates and supplementation of fibre sources such as hay following milking in Australasian feeding studies. Because of the fluctuation of rumen pH during the day, diagnosis of acidosis based solely on rumen pH can be difficult. Cluster analysis was used to identify relatively homogenous groups of cases based on rumen biochemistry, including rumen pH, individual volatile fatty acid concentrations, ammonia and D-lactate concentrations in an attempt to identify a more sensitive and specific test for rumen acidosis.

The results of biochemical analyses of rumen fluid obtained were used to allocate cows to categories using cluster analysis. The mean concentrations of almost every measure differed for each of the three categories identified. Category 1 cows are consistent with a model of clinical acidosis that reflects a rapid rate of carbohydrate fermentation, producing higher volumes of milk, but poor milk fat percentage. Category 2 describes cows with a balanced dietary intake, but possibly mismatched rumen fermentation rates leading to poorer milk production. Category 3 contains cows

with similar dietary intakes to category 2, lower concentrations of VFA, and, anomalously better milk production.

#### 4.5.4.1 Cluster category one

The rumen metabolism and milk production characteristics of cows in category 1 are consistent with an acidosis model reflecting a high rate of carbohydrate fermentation, resulting in high total VFA and propionate concentrations, high to normal D-lactate concentrations and low to normal rumen ammonia concentrations. The milk production figures show slightly higher mean milk production ( $30.0 \pm 0.97$  L/cow/day) than other categories, but low milk fat percentage resulting in a significantly lower milk fat: protein percentage ( $1.06 \pm 0.036$ ).

The results in Table 4.6 confirm that herds with a proportion of cows in this cluster category ('ACID' herds) had access to highly fermentable carbohydrates (mean dietary NFC =  $40.3 \pm 4.44\%$ ), that exceed recommendations for a maximum concentration of 25 to 33% (National Research Council 2001). In this study, the most common pattern of feeding concentrates was twice daily with variable amounts and quality of pastures fed in between concentrate allocations. Rumen fluid samples were collected 2 to 4 hours following concentrate feeding where applicable, in an attempt to sample simultaneously with the maximum effect of the concentrate on rumen function (Carruthers and Neil 1997). The high NFC% in the diet combined with a mean dietary NDF percentage of  $30.4 \pm 4.27\%$ , the lowest of the three herd categories, provides conditions that favour growth of amylolytic bacteria. When the amount of concentrate or carbohydrate percentage is increased in the diet and NDF percentage decreased, the concentration of total volatile fatty acids increases (Lana *et al.* 1998; Russell 1998).

In this study, the high total VFA concentration resulted in a lower rumen pH than other categories ( $5.74 \pm 0.47$ ), a response that has been documented in previous studies (Lana *et al.* 1998; Russell 1998). Cows fed 90% concentrate had lower ruminal pH values and acetate to propionate ratios than did cows fed 100% hay diets (Lana *et al.* 1998; Russell 1998). One theory for the lower acetate to propionate ratio in concentrate fed cattle has been to ascribe this to substrate preferences of rumen bacteria. Favourable conditions for amylolytic bacteria such as *Selenomonas*

*ruminantium*, *Streptococcus bovis*, *Succinomonas amylolytica* and *Prevotella ruminicola* result in production of propionate as well as acetate. Cellulolytic bacteria in pure culture such as *Ruminococcus albus* produce large amounts of acetate. However, *Fibrobacter succinogenes* and *Ruminococcus flavefaciens*, also cellulolytic bacteria, produce mainly succinate, an intermediate in the production of propionate (Hungate 1966). More plausible, therefore is the theory proposed by Lana *et al.* (1998) suggesting that the ratio of ruminal acetate to propionate was substantially influenced by the capacity of the bacteria to produce methane *in vitro*. Lana *et al.* (1998) found that cattle with low acetate to propionate ratios also had low ruminal pH, contributing to reduced methanogenesis *in vitro*. The two most important electron sink products in the rumen are propionate and methane and there appears to be an inverse relationship between the two products (Wolin 1960).

The significantly higher valerate concentration in cluster category one compared to other categories and very substantial importance of valerate in the statistical models used to determine category differences are interesting. Valerate is normally present in very low concentrations in the rumen with the most significant producer of valerate being *Megasphaera elsdenii*, found primarily in calves and cattle receiving rations high in grain (Hungate 1966; Stewart *et al.* 1997). *Megasphaera elsdenii* utilises 60 to 95% of the lactate available in the rumen (Counotte *et al.* 1981) and valerate is a major product from this substrate (Stewart *et al.* 1997). *Megasphaera elsdenii* takes 5 to 7 days to establish rumen populations of  $5.5 \times 10^{11}$  in concentration after introduction of grain to the diet (Klieve *et al.* 2003). The significantly higher concentration of valerate in cluster category one may therefore be explained by the higher production of lactate in this category. The higher concentrations of D-lactate found in the category support this contention. The strong correlation between D and L- lactate found in a sub-sample of cows analysed indicated that inclusion of one or other lactate or total lactate was appropriate. Given the importance of D-lactate in the pathogenesis of ruminal acidosis, low concentrations of L-lactate and strong correlation with L-lactate, it was decided to use D-lactate solely for assessing status in this study, rather than use total ruminal lactate concentrations.

D-lactate concentrations in category 1 are significantly higher compared to the other categories, but are low compared to previously reported concentrations in

concentrate- induced acidosis (Crichlow and Chaplin 1985). It is, therefore, probable that these cows do not fit the traditional concentrate induced acidosis model where *Lactobacilli* are known to multiply quickly, producing large amounts of lactate (Nocek 1997). Instead, when concentrate is fed, amylolytic bacteria such as *Selenomonas ruminantium* and *Streptococcus bovis* increase in growth rate due to their substrate preference for starch and produce lactate in addition to acetate, propionate and butyrate.

Dietary CP ( $19.9 \pm 2.19\%$ ) was adequate and should not be the cause of the low to normal ammonia concentrations. The relatively low ammonia concentrations may, therefore, either reflect an increase in microbial protein synthesis and ammonia assimilation, more rapid clearance from the rumen or failure of proteolytic bacteria to deaminate available protein. Other studies have shown lower rumen ammonia concentrations associated with higher feeding rates of non-structural carbohydrate (Lana *et al.* 1998; MacGregor *et al.* 1983). Lana *et al.* (1998) found that when concentrate percentage was increased in the diet, deamination rates decreased. A decrease in rumen pH also reduced deamination *in vitro* in forage fed cattle. However, cattle fed 90% concentrate were not affected by low rumen pH, demonstrating differences in microbial populations on the two diets (Lana *et al.* 1998). The addition of monensin (350 mg/cow/day) has also been shown to decrease ruminal ammonia production by more than 30% by inhibiting amino acid-fermenting bacteria (Yang and Russell 1993). Although there was no significant difference between herd categories of the mean amount of monensin fed ( $252.6 \pm 100.79$  mg/cow/day), 100% of herds in this category were feeding monensin, possibly having some association with the lower ruminal ammonia concentration.

Virginiamycin, an antibiotic used to control acidosis in dairy herds by its activity against *Streptococcus bovis* and *Lactobacillus sp.* (Al Jassim and Rowe 1999), was fed in 50% of herds in category 1. The mean inclusion rate in the diets however, was lower ( $156.8 \pm 92.13$  mg/cow/day) than the recommended rate of 200 mg/cow/day. It is possible that without the feeding of virginiamycin, concentrations of ruminal lactate may have been much higher. However, even in the herds feeding virginiamycin, the lower than recommended rate may have reduced the effectiveness of control. Alternatively, virginiamycin may not be effective in decreasing lactate production in

this feeding system of pasture and milk bail feeding not withstanding the lower than recommended dose given.

The production summary for category 1 is presented in Table 4.9. These cows show a high rate of rumen fermentation and higher production figures ( $30.0 \pm 0.97$  L/cow/day,  $P = 0.057$ ) than other categories. The relationship between concentrate feeding and increased milk production on pasture based diets is well recognised (Wales *et al.* 2001; Walker *et al.* 2001). The significantly lower milk fat percentage after correcting for DIM, parity and days to herd test ( $3.32 \pm 0.112\%$ ) is also associated with a high level of concentrate feeding, lower dietary NDF and peNDF and lower rumen pH. This decrease was associated with a lower milk fat: protein ratio of  $1.06 \pm 0.036$  than category 2 ( $P = 0.001$ ) or 3 ( $P = 0.004$ ), supporting previous research linking rumen pH to milk fat concentration (Kolver and de Veth 2002) and dietary effective NDF to milk fat percentage (Mertens 1997). Interestingly, the mean parity of cows in category 1 was significantly lower ( $2.08 \pm 1.53$  calvings) than category 2 cows ( $3.15 \pm 2.39$  calvings,  $P = 0.013$ ) and category 3 cows ( $3.05 \pm 2.06$  calvings,  $P = 0.005$ ). This may indicate that heifers, although offered the same diet as older cows, were less likely to eat some constituents, suggesting that the type of feeding system or diet preference may be a problem. For example, if there was limited access to fibre, older cows may have had preferential intake of this (Shepherd 2003).

#### 4.5.4.2 Cluster category two

Dietary CP percentage ( $20.0 \pm 2.96\%$ ), ME concentration ( $10.5 \pm 0.91$  MJ/kg DM) and NFC% of  $33.4 \pm 6.45\%$  offered are all adequate for an early lactation dairy cow and are not significantly different to the respective values for diets offered to cows in category 3. These values coupled with a mean NDF% of the diet of  $35.7 \pm 5.98\%$  should create a stable rumen environment. Instead, the rumen analyses suggest a possible energy - protein mismatch, where ammonia is not being assimilated into microbial protein possibly because of inadequate energetic precursors being offered at the right time. The low concentration of propionate present in the rumen ( $19.47 \pm 6.18$  mM) probably resulted in lower glucose availability, consequently the lower milk production ( $27.9 \pm 0.52$  L/cow/day) in this category compared with either category 1 ( $P = 0.057$ ) or 3 ( $P = 0.011$ ).

The total VFA concentration ( $94.79 \pm 18.13$  mM) observed is adequate; previous data suggest that optimal concentrations of total VFAs are more than 95 mM (Leng and Brett 1966). However, some studies found much lower total VFA concentrations. Russell (1998) found non-lactating cows fed 10 kg DM/cow/day (NRC recommendations) of a diet of 100% hay had total VFA concentrations of 68.3 mM compared to cows fed 90% concentrate with a total VFA concentration of 83.9 mM. The total VFA concentration is less than category 1, although not significantly, with the trend possibly explained by the lower mean dietary ME concentration ( $10.5 \pm 0.91$  MJ/kg DM) and significantly lower ( $P = 0.002$ ) NFC% than 'ACID' herds.

The optimal ME concentration of the diet for an early lactation dairy cow is suggested to be 11.5 MJ/kg DM (Lean *et al.* 2003). The lower NFC% and significantly higher NDF% ( $P = 0.015$ ) and peNDF% ( $P = 0.042$ ) in the diet of 'SO' herds may also explain the low propionate concentration in the rumen and correspondingly higher acetate: propionate ratio (3.04), supporting previous studies (Russell 1998). Rumen fermentation in this category appears to have been slower with the dietary changes and would favour more cellulolytic bacteria, such as *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens*. Russell (1998) found that substrate had a much greater impact on the fermentation end products than did pH. Cows fed 100% hay diets had an acetate: propionate ratio of 4.1 compared to a ratio of 2.2 for cows fed 90% concentrate. Cattle with higher acetate: propionate ratios had higher rumen pH values which favoured methane production *in vitro* (Lana *et al.* 1998). The significantly higher acetate and butyrate concentrations ( $P = 0.001$ ) in category 2 than 1 may explain, in part, the significantly higher milk fat percentage ( $3.86 \pm 0.066\%$ ;  $P = 0.001$ ) in category 2 cows than category 1 cows. Acetate and butyrate are utilised by mammary cells for energy and the production of milk fat, particularly triglycerides (Rook 1976).

Both D-lactate and valerate concentrations in the rumen were significantly lower than category one ( $P = 0.001$ ). The lower D-lactate and higher pH indicates that either production of lactic acid is limited or transfer rates of this from the rumen are sufficiently high that accumulation is not occurring. More likely, production of lactic

acid is less because of changes in microbial population, such as a lower prevalence of *Megasphaera elsdenii*, resulting from lower dietary NFC% and higher NDF%.

Rumen pH in this category was  $6.18 \pm 0.44$ , significantly higher than category 1 but lower than category 3 ( $P = 0.001$ ). The Cornell Net Carbohydrate and Protein System model and other workers predict reductions in fibre digestion when pH falls below 6.2 (Calsamiglia *et al.* 1999; Grant 1994; Grant and Mertens 1992). One *in vitro* study found that on high quality pasture based diets, although DM digestibility was optimised at pH 6.35, a large decrease in digestibility only occurred below a ruminal pH of 5.8 (de Veth and Kolver 2001a). In this category therefore, it is unlikely that fibre digestion was compromised markedly.

Dietary crude protein percentage was not significantly different between herd categories 'ACID', 'SO' and 'NA'. All herd categories had adequate mean CP% based on National Research Council (2001) recommendations. There was also no significant difference between undegradable protein and soluble protein fractions of the CP%. Despite these similarities, ammonia concentrations were significantly higher in category 2 cows ( $7.79 \pm 3.75\text{mM}$ ) than the other two categories ( $P = 0.001$ ). Dietary protein is converted to ammonia by proteolytic bacteria. However, there may be a failure of nitrogen incorporation into microbial amino acids and hence protein. Bacteria that primarily ferment structural carbohydrates use only ammonia as a N source, whereas NSC fermenting bacteria use peptides and ammonia as an N source (Russell *et al.* 1992). Conversion of ammonia to bacterial protein requires a number of cofactors including volatile fatty acids. The total concentration of VFAs in the rumen indicates that sufficient energy is available for this process. Consequently, there appears to be a failure of nitrogen – energy matching in cows from this category and may reflect asynchronous availability of nutrients for bacterial growth. One possibility is that the diets, although adequate on a daily basis may be imbalanced in supply of fermentable carbohydrate and N supplies on an hourly basis (Shabi *et al.* 1998). Carbohydrate sources may be also being fed at different times to protein sources in the day. Feeding practices for the herds indicated that in most areas of the study, rapidly fermentable carbohydrates (wheat, triticale, barley) were fed more commonly in the bail than protein sources (lupins, canola meal and urea) and in much

higher quantities (Chapter 3). In most cases the most important protein source in the diet was pasture.

Cows were held off pasture routinely for 1 to 2 hours twice daily and up to 6 hours twice daily during milking. Studies have reported mixed effects of asynchronous diets with most studies examining effects of feeding energy and protein sources with different degradabilities. Herrera-Saldana *et al.* (1990) found that cows fed rapidly fermentable carbohydrates (barley) and slowly fermentable protein (brewers grain) or slowly fermentable carbohydrates (milo) and rapidly fermentable protein (cottonseed meal) had higher rumen ammonia concentrations and lower microbial protein yield than those fed synchronised highly fermentable diets. Results in that study were, however, not significant and numbers of cows used were low. Kolver, *et al* (1998) examined the effects of feeding pasture and concentrate either together or 4 hours apart with pasture fed first. Ruminal ammonia concentrations were significantly higher ( $P = 0.001$ ) in cows fed the asynchronous compared with the synchronous diet 3 and 5 hours after pasture was fed in the morning. Many milking parlour diets are predominantly or all cereal grain, a poor source of protein. Interestingly, the high concentration of ammonia in category 2 cows was not associated with a significantly lower milk protein percentage ( $3.18 \pm 0.031\%$ ). Mean milk protein yield was, however, lower in this category than category 1 ( $P = 0.055$ ), supporting the possibility that microbial protein production was lower in this group.

#### 4.5.4.3 Cluster category three

Some of the mean rumen measures for category 3 indicate that cows in this category may have sub-optimal rumen function. A normal rumen pH ( $6.33 \pm 0.43$ ) where fibre digestion was not compromised was coupled with low total VFA concentration ( $62.81 \pm 15.65$  mM), lower D-lactate concentration than other categories ( $0.12 \pm 0.51$  mM) and adequate rumen ammonia concentration ( $3.64 \pm 2.03$  mM). However, mean production figures for cows in this category (after correcting for DIM, parity and days to herd test) were significantly higher than category 2 ( $29.7 \pm 0.36$  L/cow/day,  $P = 0.011$ ). A significantly lower milk fat percentage ( $3.61 \pm 0.041\%$ ;  $P = 0.022$ ) than category 2 resulted in a milk fat: protein ratio of  $1.18 \pm 0.010$ , not significantly different to category 2. None of the dietary intake variables for 'NA' herds were significantly different to 'SO' herds. Notwithstanding this, there are significant

differences between many of the rumen parameters and production data. One possible explanation is the feeding regime implemented in these herds. Although the overall dietary variables are similar, these cows may have a more synchronous diet presented throughout the 24-hour period, allowing greater production. The lower total VFA concentration in this category may indicate a slower rumen fermentation relating to substrate offered compared with rumen fermentation in category 1 cows or rapid clearance from the rumen. The good production levels of this group suggest that VFA and microbial production from this group were good, but not optimal.

#### 4.6 Conclusions

These cows were subject to a wide variety of diets, feeding regimes and management strategies. These resulted in significant differences in rumen fermentation and milk production achieved. However, despite the diversity of feeding strategies employed in the study herds, the random effect of herd was not a significant factor in the bayesian model. Three very different categories of cows, therefore, were identified using cluster analysis. The first cluster category of cows, although exhibiting slightly higher milk production had some concerning trends in rumen function, possibly resulting in outcomes associated with clinical acidosis. The second cluster category of cows appear to have measures reflecting optimal rumen function, but had significantly lower milk production measures. The third category of cows, showed rumen measures associated with either a slow, but stable rumen fermentation or markedly better clearance of VFA, and also had significantly higher milk production measures than cows in category 2. This was despite dietary inputs not being significantly different for herds with a high prevalence of cows in category 2 and 3. Outcomes, as well as risk factors for these categories will be examined in future chapters. This data set is consistent with many intensive and *in vitro* studies conducted, but also presents new insights on rumen fermentation for cows fed pastures and concentrate.

#### 4.7 References

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## Chapter 5.

# ACIDOSIS AND LAMENESS IN AUSTRALIAN DAIRY HERDS

## 5.1 Summary

*Outcomes associated with acidosis were examined in a cross-sectional survey of one hundred dairy herds selected in five regions in New South Wales and Victoria. Each lactating herd was scored for body condition, rumen fill, locomotion, faecal staining and faecal consistency. Herds were classified as 'subclinically acidotic' ('ACID'), 'suboptimal' ('SO') and 'non-acidotic' ('NA') on the basis of rumen biochemical measures, when 3/8 cows examined per herd were allocated to previously defined categories.*

*Mean body condition and perineal faecal staining of herds were not associated with herd categories. The relationship between high faecal scores 4/5, indicating diarrhoea, and ACID herds was influenced by other variables, particularly pasture percentage in the diet. In a multivariate logistic regression model, with district modelled as a random effect, an increase of one percent of pasture in the diet was associated with a 5.5 percentage increase in risk of high faecal scores indicating scouring. However, a number of interactions were identified including an interaction between concentrate % and pasture % in the diet and pasture % in NA herd diets. Herds that had a greater prevalence of lame cows, with locomotion scores 3/5, had a 83% higher risk of being categorised as ACID than SO and 118% higher risk of being categorised ACID than NA.*

*This study confirmed that herd categories based on rumen function are associated with biological outcomes consistent with acidosis. While cows categorised from rumen biochemical data as acidotic had higher milk production, herds that had a higher risk of lameness also had a much higher risk of being categorised ACID than SO (83%) or NA herds (118%). Herds with a high prevalence of low rumen scores were 75% more likely to be category ACID than SO and 57% more likely to be category NA than SO when the random effect of district was evaluated in the model*

*predicting rumen fill score. The findings indicate that differences in rumen metabolism identified for herd categories ACID, SO and NA were associated with differences in disease risk and physiology. The study also identified an association between pasture feeding and higher scour scores. This study suggests that there is a challenge for farmers seeking to increase milk production of cows on pasture whilst maintaining the health of their cattle.*

## **5.2 Introduction**

While cows, and subsequently herds, can be classified as being 'acidotic' or otherwise based on rumen biochemistry, it is critical to determine whether such classifications relate to conditions that reflect previously described sequelae to acidosis. Clinical and subclinical acidosis are recognised as problems in dairy cows fed large amounts of feeds rich in rapidly fermentable carbohydrates (Crichlow and Chaplin 1985; Nocek 1997). The practice on 79% of study farms (Chapter 3), and these being representative of dairy farms in Southern Australia, of combining supply of good quality pasture, in an early vegetative state, with twice-daily concentrate feeding in the bail may increase the prevalence of these acidotic conditions. However, there is controversy about the prevalence and significance of these conditions in cattle primarily fed on pasture (de Veth and Kolver 2001; Kolver and de Veth 2002; Wales *et al.* 2001). Indicators of a possible acidotic episode in a dairy herd include scouring (Nocek 1997), laminitis causing lameness (Nocek 1997; Owens *et al.* 1998), poor rumen fill, decreased milk fat (Lean *et al.* 2000), weight loss and increased incidence of liver abscesses (Owens *et al.* 1998).

Acidosis has been implicated as a common cause of laminitis in dairy herds with lesions typical of the condition having been described by several authors (Bergsten 2000; Vermunt 1992). Laminitis can be defined as acute, sub-acute, chronic and sub-clinical depending on the severity and duration of the condition. Acute laminitis occurs after a metabolic insult causes a decreased ruminal and subsequently systemic pH, resulting in production of vasoactive substances including histamine, lactic acid, serotonin or endotoxins (Bergsten 2000; Vermunt 1992). However, ruminal pH may have returned to normal when laminitic lesions are first diagnosed as a result of the four to eight week delay between nutritional insult and the onset of laminitis.

Histamine is produced from histidine in the rumen by *Allisonella histaminiformans* and absorption of histamine into the blood is increased by low rumen pH (Russell and Garner 2003). Altered perfusion of the vasculature resulting from the action of histamine compromises the supply of nutrients to horn-producing tissues of the digit and impairs circulation in the claw tissues (Vermunt 1992).

The faeces of cows may provide indirect evidence of clinical and sub-clinical acidosis. Faecal moisture content is increased by a lactate-based osmotic overloading of the large intestine, by lactate derived from rumen metabolism (Bolton and Pass 1988). Lactate causes sequestration of large quantities of water in the rumen and can induce dehydration. Increased proportions of undigested feeds resulting from reduced microbial cellulolytic activity at a lower ruminal pH also contribute to diarrhoea (Bolton and Pass 1988).

The aetiology, effects and prevention of ruminal acidosis have been extensively researched and reviewed (Dirksen 1970; Huber 1976; Hungate *et al.* 1952; Nocek 1997; Owens *et al.* 1998; Slyter 1976; Underwood 1992). However, these studies have almost exclusively been based on individual cases of spontaneous or experimentally induced acidosis. There are no quantitative studies that have systematically examined the clinical and production effects of acidosis. This survey examines the effects of acidosis in 100 randomly selected Australian dairy herds. Herd categories in this chapter are based on K-means cluster analysis of rumen biochemical data taken from individual cows from each of the 100 herds sampled (Chapter 4). Rumen volatile fatty acids (VFAs), ammonia, D-lactate and rumen pH were significant in predicting category allocation ( $P < 0.01$ ), however, propionate ( $F = 332.38$ ) and valerate ( $F = 332.62$ ) were the most important factors in determining cluster categories. Interestingly, rumen pH and D-lactate were least important in prediction of the categories with  $F = 54.95$  and  $F = 5.02$ , respectively.

## 5.3 Materials and methods

### 5.3.1 General materials and methods

The epidemiology of acidosis was examined in one hundred Australian dairy herds. Refer to section 2.3.2 for further details on herd selection. Cows ( $n = 15$ ) from each

lactating herd (refer to section 2.3.6.2) were randomly selected and examined for body condition score, rumen fill, locomotion score and faecal score in the paddock (refer to section 2.4.1). Herds were classified as ‘subclinically acidotic’ (‘ACID’), ‘suboptimal’ (‘SO’) and ‘non-acidotic’ (‘NA’) on the basis of rumen biochemical measures (Chapter 4). Further detail on herd classification is found in section 2.4.5.1.

### 5.3.2 *Statistical analyses*

Data were initially evaluated and manipulated using an Excel spreadsheet (Microsoft Corporation) and Statistix (version 7, NIH Software). A mean BCS for each herd was calculated from the 15 cows scored from each herd. This together with the faecal staining score given to each herd was used to calculate mean scores for the herds in each category of rumen function (ACID, SO or NA), which were subsequently tested for significant differences using Kruskal-Wallis analysis of variance (KW ANOVA) (Table 5.1). Initial evaluation included examination of univariate relationships, evaluation of variables for linearity or cutpoints in responses and graphing of relationships among variables. Cutpoints were evident for some variables (faecal score, locomotion score and rumen fill score) and herds were categorised into those with a high prevalence of scouring with faecal scores 4/5, and those with a high prevalence of locomotion scores 3/5. Mean prevalences of faecal score, locomotion score and rumen fill score were compared for each category (Table 5.1). Data were further analysed using univariate LR and odds ratio (Table 5.2). Possible covariates and confounding factors for faecal score and locomotion score were examined separately using LR (Tables 5.4 and 5.6). Evidence of confounding was assessed from change in the coefficients when subsequent variables were entered. Variables with a  $P$  value  $< 0.2$  were included in LR multivariate models. Biological plausibility of variables was also considered in the inclusion of variables. For example, although footbath was highly significant ( $P = 0.001$ ) on univariate analysis, it was not included in the final model because it was regarded as being associated rather than causing a high prevalence of lameness. The unit of interest in this chapter is the prevalence of conditions within a herd.

Multivariate LR models were developed and assessed for fit using Hosmer-Lemeshow statistics. Multivariate models were weighted by number of cows in each herd classified in category 1. District was incorporated into final models as a random effect

and reported in Tables 5.3, 5.5 and 5.7 using Egret (Version 2.03.1 Cytel Statistical Software). Multivariate fixed effect models were very similar to random effects models and were therefore not reported. A random effects logistic normal model was used, based on the distribution of prevalence of rumen fill, lameness and faecal scores in herds, to develop final models.

The model for the response probability  $\delta_i$  is given by

$$\text{logit}(\delta_i) = \mathbf{x}_i \hat{\boldsymbol{\alpha}} + \phi u_i,$$

where  $\phi$  is a constant greater than zero, and  $u_i \sim \text{iid } N(0,1)$ .

The models were assessed for fit by examining graphs showing residuals for each trial in the data set.

#### 5.4 Results

Table 5.1 describes mean BCS for herd categories based on mean score of cows within those herds, mean faecal staining score and mean numbers of cows in herds with high faecal scores, locomotion scores and low rumen fill scores for herd categories.

Table 5.2 presents the risk (odds ratio) for herds in the three categories for the prevalence of cows with different scores. For example, herds with low faecal scores 1 and 2/5 were 28% more likely ( $P = 0.012$ ) to be in category ACID than herds classified SO. Herds with a high prevalence of low rumen fill scores 1 and 2/5 were 31% more likely ( $P = 0.009$ ) to be in category ACID than classified SO, whereas these herds were 26% less likely to be in category NA than SO. Significant factors (ACID and NA) from the univariate LR model used to predict prevalence of rumen fill score 1 and 2/5 (Table 5.2) were combined with district as a random effect (Table 5.3). The random effect of district was not significant in predicting the model ( $P = 0.413$ ), however on inclusion of district, herds with a high prevalence of low rumen fill scores were more 75% likely to be categorised ACID than SO ( $P = 0.001$ ) and 57% more likely to be classified NA than SO ( $P = 0.001$ ).

**Table 5.1.** Mean ( $\pm$  SD) measures of cattle performance and health associated with acidosis for the three categories.

Mean	ACID	SO	NA
Body condition score <sup>1</sup> (1 – 5)	3.0 (0.09)	3.0 (0.12)	3.0 (0.13)
Faecal staining <sup>2</sup> (1 – 5)	2.7 (1.06)	2.2 (0.99)	2.0 (0.68)
Cows with faecal scores ( $\geq 4/5$ ) <sup>3</sup>	6.5 (4.99)	7.1 (5.49)	9.1 (5.43)
Cows with locomotion scores ( $\geq 3/5$ ) <sup>4</sup>	6.7 (3.77)	4.6 (2.78)	4.3 (2.79)
Cows with rumen fill scores (1 or 2/5) <sup>4</sup>	4.9 (4.01)	3.6 (4.92)	3.0 (4.31)

<sup>1</sup> $P = 0.916$  not significant (KW ANOVA)

<sup>2</sup> $P = 0.085$  not significant (KW ANOVA)

<sup>3</sup>mean ( $\pm$  SD) number of faecal pats (out of 20) from the herds examined scoring  $\geq 4/5$ .

<sup>4</sup>mean ( $\pm$  SD) number of cows (out of 15) from the herds examined for each variable

**Table 5.2.** Univariate logistic regression models of associations among herd category (category SO = reference) and score prevalence.

Score	Category	Regression coefficient	P value	Odds Ratio	95% Confidence Interval
Faecal score (1 and 2/5)	ACID	0.248	0.012	1.28	1.06 – 1.56
	NA	-0.288	0.001	0.75	0.67 – 0.84
Faecal score ( $\geq 4/5$ )	ACID	0.057	0.498	-	-
	NA	0.484	0.001	1.62	1.49 – 1.77
Rumen fill score (1 and 2/5)	ACID	0.270	0.009	1.31	1.07 – 1.60
	NA	-0.307	0.001	0.74	0.65 – 0.83
Locomotion score ( $\geq 3/5$ )	ACID	0.539	0.001	1.71	1.42 – 2.07
	NA	-0.121	0.028	0.89	0.80 – 0.99

**Table 5.3.** Random effects model for prevalence of rumen fill scores 1 and 2/5 with district as a random effect (category SO = reference)

	Regression coefficient	<i>P</i> value	Odds Ratio	95% Confidence Interval
ACID	0.557	0.001	1.75	1.24 – 2.46
NA	0.450	0.001	1.57	1.21 – 2.03
District	-0.045	0.413	-	-

#### 5.4.1 Faecal score potential confounding and interactions

Other potentially confounding factors and interactions that reflect herd management and may influence faecal scores were examined using LR. Significant factors are summarised in Table 5.4. Factors with a *P* value < 0.2 were then examined using fixed effect multivariate LR and logistic regression with district as a random effect. The random effect of district was significant ( $P = 0.035$ ) in predicting the model. The associations for herds classified as ACID were influenced by other factors, in particular pasture % in the diet. While herds with a high prevalence of high scour scores were not univariately associated with being classified as ACID, when other variables, including the effect of district, were included in the model, this factor approached significance ( $P = 0.058$ ). On inclusion of district, other significant predictors of high faecal scores were herd category NA ( $P = 0.001$ ), pasture percentage in the diet ( $P = 0.001$ ) and drench treatment ( $P = 0.021$ ). Interestingly, when pasture % in the diet was increased in NA herds, the risk of scouring decreased ( $P = 0.004$ ). Similarly, the combination of increased concentrate percentage with increased pasture percentage in the diet also increased risk of a high prevalence of scouring ( $P = 0.001$ ).

**Table 5.4.** Univariate logistic regression models for proportion of cows with faecal scores 4/5

	Regression coefficient	P value	Odds Ratio	95% Confidence interval
Drench treatment <sup>1</sup>	0.581	0.001	1.79	1.63 – 1.96
Fluke treatment <sup>2</sup>	-0.124	0.002	0.88	0.82 – 0.95
Fluke treatment (dry off) <sup>3</sup>	-0.175	0.001	0.84	0.77 – 0.91
Fluke treatment (other times) <sup>4</sup>	0.098	0.155	1.10	0.96 – 1.26
Pasture% in diet	0.044	0.001	1.05	1.04 – 1.05
Concentrate% in diet	-0.040	0.001	0.96	0.96 – 0.96

<sup>1,2</sup>0 = no drenching, 1 = drenching when needed or at set times of the year

<sup>3</sup>0 = no fluke treatment or fluke treatment at other times, 1 = fluke treatment at dry off

<sup>4</sup>0 = no fluke treatment or fluke treatment at dry off, 1 = fluke treatment at other times

**Table 5.5.** Random effects model for prevalence of faecal scores 4/5 using district as a random effect (category SO = reference).

	Regression coefficient	P value	Odds Ratio	95% Confidence Interval
ACID	0.363	0.058	1.44	0.99 – 2.09
NA	1.305	0.001	3.69	1.97 – 6.89
Pasture % in diet	0.049	0.001	1.05	1.04 – 1.06
Concentrate % in diet	-0.015	0.069	0.99	0.97 – 1.00
Drench treatment	0.260	0.021	1.30	1.04 – 1.62
Concentrate % * pasture %	0.001	0.001	1.001	1.00 – 1.00
Pasture % * NA	-0.016	0.004	0.984	0.97 – 1.00
District	-0.056	0.035	-	-

#### 5.4.2 Locomotion score potential confounding and interactions

Possible confounding factors and interacting variables for locomotion score were examined using LR and significant factors are included in Table 5.6. Time spent in the milking yard, use of a dog on the track or in the yard and walking distance per day were all not significantly associated with an increased prevalence of high locomotion scores in univariate analyses ( $P > 0.2$ ). Factors with a  $P$  value  $< 0.2$  were used in a multivariate fixed effect logistic regression with exception of the use of a footbath but not reported because of similarities with the final random effects model reported

(Table 5.7). Significant factors from the multivariate LR fixed effects model were combined in a model, using district as a random effect (Table 5.7). District was a significant factor in the model ( $P = 0.007$ ). After inclusion of district as a random effect, the significant predictors of high lameness scores were ACID category ( $P = 0.034$ ), NA category ( $P = 0.001$ ), laneway fair ( $P = 0.001$ ) and backing gate ( $P = 0.001$ ). ACID was associated with an increased risk of high lameness scores of 83% over SO herds and 118% over NA herds. Interestingly, in NA herds, the presence of a backing gate reduced the risk of high lameness scores by 45%.

**Table 5.6.** Logistic regression model relating proportion of locomotion scores 3/5 to possible confounding factors.

	Regression coefficient	<i>P</i> value	Odds Ratio	95% Confidence Interval
Laneway good <sup>1</sup>	0.146	0.068	1.16	0.99 – 1.35
Laneway fair <sup>2</sup>	-0.366	0.001	0.69	0.63 – 0.76
Laneway poor <sup>3</sup>	0.297	0.001	1.35	1.22 – 1.48
Backing gate <sup>4</sup>	0.153	0.002	1.17	1.06 – 1.28
Footbath <sup>4</sup>	0.499	0.001	1.65	1.46 – 1.86

<sup>1</sup> 0 = other condition, 1 = top quality

<sup>2</sup> 0 = other condition 1 = laneway smooth, some stone (fair)

<sup>3</sup> 0 = other condition, 1 = laneway dangerous, sharp, rocky, muddy

<sup>4</sup> 0 = no, 1 = yes

**Table 5.7.** Random effects model for prevalence of locomotion scores 3/5 using district as a random effect (SO reference for category, laneway good quality and poor quality reference for laneway).

	Regression coefficient	<i>P</i> value	Odds Ratio	95% Confidence Interval
ACID	0.606	0.001	1.83	1.29 – 2.60
NA	-0.432	0.001	0.65	0.51 – 0.83
Laneway fair	-0.388	0.001	0.68	0.57 – 0.81
Backing gate	0.617	0.001	1.85	1.35 – 2.5
Backing gate*NA	-0.601	0.002	0.55	0.38 – 0.79
District	0.084	0.007	-	-

## 5.5 Discussion

The results in this chapter provide further insights into the herd categories (ACID, SO and NA) identified using rumen biochemical data and cluster analysis in Chapter 4. There was no significant difference in BCS between categories, which is not surprising given the single score for cows of each herd. Previous Australian studies where high rates of concentrates were fed to cattle also grazing pasture and with or without sodium bicarbonate or virginiamycin also reported no significant effects on bodyweight (Clayton *et al.* 1999; Valentine *et al.* 2000). Faecal staining on the cows did not significantly differ among categories when assessed using univariate methods, possibly because of the large number of other factors influencing faecal score (Table 5.4).

Significant differences were found in the prevalence of low rumen fill scores in herds using a univariate model (Table 5.2). Herds with a high prevalence of low rumen score were more likely to have been categorised as ACID, but less likely to have been classified as NA herds than SO herds, the reference category. Interestingly, the addition of the random effect of district and the use of the logistic normal model indicated that herds with a high prevalence of low rumen fill scores were 57% more likely to be in category NA than SO ( $P = 0.001$ ), and 75% more likely to be in ACID category than SO ( $P = 0.001$ ). The logistic normal model is weighted by prevalence of cows with a rumen category in a herd, whereas the logistic binomial fixed effect model is not, and this may also influence the difference in results between the models. Diets of ACID herds were significantly lower in NDF% than the other two categories ( $P < 0.05$ ) indicating a lower fibre intake, partially explaining the tendency towards poorer rumen fill. It is also possible that cows in these herds were more prone to rumen disruption and periodic inappetence. Although there was no significant differences between dietary NDF% of SO herds and NA herds, fibre sources offered to NA herds may have been more digestible than SO herds. This is also supported by the result that NA herds were 369% more likely to have a high prevalence of scours than SO herds ( $P = 0.001$ ).

When district was included as a random effect, it was significant in predicting prevalence of high scour scores. This may reflect the timing of sampling in different regions and quality of the pasture on offer. The prevalence of high faecal scores indicating scours was significantly higher in category NA compared with category SO both on a univariate analysis (Table 5.2) and when district was included as a random effect ( $P = 0.001$ ) (Table 5.5) where category NA was associated with an increased risk of 369%. Interestingly, when pasture % was increased in NA herds, the risk of a high prevalence of scouring decreased ( $P = 0.004$ , odds ratio = 0.984). The presence of increasing amounts of pasture in NA herds may have helped stabilise rumen function in these herds. The prevalence of high faecal scores indicating scours also tended to be higher in category ACID compared with category SO ( $P = 0.058$ ) with an associated increased risk of 44%. However, the relationship between scour scores and herds categorised as ACID was influenced by other variables, particularly proportion of pasture in the diet (Table 5.5). Increased risk of high scour scores was associated with a 5% increased risk per 1% increase in pasture in the diet. Also significant was the interaction of concentrate % and pasture % in the diet. Increased risk of high scour scores was associated with a 1% increased risk per 10% increase in the multiple of concentrate % and pasture % in the diet. This finding may highlight the importance of digesta flow rates in producing a higher scour score. Drenching with an anthelmintic was associated with increased risk of high scour scores of 30% ( $P = 0.021$ ), but it is unclear whether this is a cause or effect of scouring. A history of reasons for drenching, such as high faecal egg counts or routine practice, was not obtained.

The prevalence of high locomotion scores, indicating lame herds was examined (Tables 5.6 and 5.7). District was included in analysis reported in Table 5.7 and was significant in predicting high lameness scores ( $P = 0.007$ ). Again, this may have reflected temporal changes in feeding and climate over the sampling period for the five areas. Herds with a higher prevalence of lameness were 83% more likely to be categorised as ACID than SO ( $P = 0.001$ ) and 118% more likely to be categorised as ACID than NA ( $P = 0.001$ ) when district was included as a random effect (Table 5.7). This result supports previous research linking acidosis to laminitis and lameness (Nocek 1997) and highlights a very strong association between lameness with acidosis. Acidosis has been implicated as a common cause of laminitis in dairy herds

with lesions typical of the condition having been described by several authors (Bergsten 2000; Vermunt 1992). Histamine is produced from histidine in the rumen by *Allisonella histaminiformans* and absorption of histamine into the blood is increased by low rumen pH (Russell and Garner 2003). Altered perfusion of the vasculature resulting from the action of histamine compromises the supply of nutrients to horn-producing tissues of the digit and impairs circulation in the claw tissues (Vermunt 1992). Clover contains high concentrations of histidine compared to many other feeds. Penkov et al (2003) found that three species of *Trifolium repens* contained 4.7 to 5.1 g.kg<sup>-1</sup> DM of histidine. This can be compared with ryegrass (*Lolium* spp.) containing 3 g.kg<sup>-1</sup> DM of histidine and kikuyu (*Pennisetum clandestinum*) also containing 3 g.kg<sup>-1</sup> DM (Reeves 1997). Therefore, herds grazing pastures containing a high percentage of clover may be at higher risk of acidosis. Dietary risk factors including pasture type are examined in Chapter 6.

Interestingly, the use of a backing gate was associated with an 85% higher risk of lameness in herds (Table 5.7) supporting previous research (Chesterton 1989; Chesterton *et al.* 1989). Notwithstanding this, the presence of a backing gate in NA herds reduced the risk of high lameness scores by 45%. Because NA herds in general were at less risk of high lameness scores, cows in these herds may have had better hoof health and the presence of a backing gate may have reflected improved farm practices. If the herds had laneways in fair condition that were smooth, but had occasional stones these were at 32% less risk of lameness than herds subject to either top quality or poor quality laneways. This finding may indicate that cows are careful when walking on these laneways and have the opportunity to avoid damage to feet or be the result of chance. The use of a footbath was significantly associated with lameness (Table 5.7) but was probably an indication of the farmer's awareness of a high prevalence of lameness rather than being causal and was, therefore, not included in the final random effects model.

## 5.6 Conclusions

This is the first epidemiologic study to systematically examine the effects of differences in rumen function reflecting acidosis on disease conditions in dairy cows. This study confirmed that herd categories based on rumen function are associated with biological outcomes consistent with acidosis. While cows used to define category ACID herds had higher milk production, herds with a high prevalence of lameness had an 83% higher risk of being categorised as ACID than SO and 118% higher risk of being classified ACID than NA. Herds with a higher prevalence of low rumen fill scores were 57% more likely to be categorised NA than SO, and 75% more likely to be categorised ACID than SO ( $P = 0.001$ ). The findings indicate that the differences in rumen metabolism identified leading to the different categories were reflected in differences in disease risk and physiology. The significant association between scour scores and pasture feeding was also identified. This study confirms the challenge of increasing milk production of cows on pasture whilst maintaining health, particularly lameness, of cattle.

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## Chapter 6.

# THE EPIDEMIOLOGY OF ACIDOSIS IN DAIRY HERDS IN NEW SOUTH WALES AND VICTORIA: PREVALENCE AND RISK FACTORS

## 6.1 Summary

*Risk factors associated with acidosis in pasture-based dairy herds were examined in a cross-sectional survey of 100 randomly selected herds in five areas of NSW and Victoria. The lactating cow diet from each herd was recorded and pasture samples taken for analysis of nutrients where applicable. Diets were validated against production figures using CPM dairy. Herds were classified as 'subclinically acidotic' ('ACID'), 'suboptimal' ('SO') and 'non-acidotic' ('NA') on the basis of rumen biochemical measures, when 37.5% of 8 cows examined per herd were allocated to the previously defined categories (Chapter 4). Presence and amounts of constituents fed and total diet analysis variables were examined using multinomial logistic regression and Pearson Chi-square. Prevalence of feeding wheat and amount of wheat fed tended to be greater in herd category ACID than NA herds ( $P = 0.083$ ,  $P = 0.055$  respectively). Legume dominant pastures (clover, shaftal or lucerne) were significantly more prevalent in ACID herd diets than NA herd diets ( $P = 0.008$ ) whilst ryegrass dominant pastures were significantly less prevalent in SO herd diets than NA herd diets ( $P = 0.02$ ). Pasture texture was also scored on a 1 to 5 scale where 1=soft lush pastures with minimal effective fibre and 5=forage sorghum or maize with very high effective fibre. Prevalence of score 1 pastures was significantly greater in ACID herds than NA herds ( $P = 0.004$ ), confirming pasture type results. Importantly, NDF%, NFC% and carbohydrate B1 % (% starch + % sugar in the diet) were all important predictors of ACID herds compared with NA herds. ACID herd diets contained a significantly higher NFC% ( $P = 0.002$ ) and CHO B1% ( $P = 0.008$ ) but lower NDF% ( $P = 0.004$ ) than NA herds. However, most importantly, using multivariate modelling, category ACID was best described simply by the NDF%: NFC% ratio. There was no significant difference in NDF%, NFC% and CHO B1%.*

between SO herds and NA herds ( $P > 0.6$ ), however SO herds tended to feed more fibrous by-product ( $P = 0.159$ ) and straw ( $P = 0.144$ ) than NA herds.

## 6.2 Introduction

Concentrate induced acidosis is a well documented disease of dairy cattle that can lead to rumenitis, metabolic acidosis, lameness, hepatic abscessation, pneumonia and death (Huber 1976; Lean *et al.* 2000; Nocek 1997; Owens *et al.* 1998; Underwood 1992). Research into the disorder has almost entirely been based on experimentally inducing acidosis using the abrupt introduction of substantial concentrations of carbohydrates for short periods in individual or small numbers of animals (Crichlow and Chaplin 1985; Hungate *et al.* 1952; Keunen *et al.* 2002; Krajcarski-Hunt *et al.* 2002). Induction of acidosis has been effectively achieved by replacing predominantly forage diets high in effective fibre with diets low in effective fibre containing rapidly fermentable carbohydrate sources such as wheat (Krajcarski-Hunt *et al.* 2002). These strategies produced both clinical signs of acidosis and related ruminal and systemic biochemical disturbances (Dirksen 1970; Slyter 1976). Type of grain and amount of processing may also be risk factors for acidosis. Opatpatanakit *et al.* (1994) found that grains were fermented in the order from most rapid to least rapid of wheat > triticale > oats > barley > maize > rice, sorghum. Processing of grain also increases efficiency of starch fermentation and microbial degradation of starch in the rumen (Hale 1973; Theurer 1986). This can lead to a depression in rumen pH, for example, flatly rolled barley may decrease rumen pH more than barley rolled coarsely ( $P < 0.15$ ) (Yang *et al.* 2001a).

The Australian dairy industry is predominantly pasture based. The practice on 79% of study farms (Chapter 3), and these being representative of dairy farms in Australia, of combining supply of good quality pasture in an early vegetative state with twice-daily concentrate feeding in the bail may increase the prevalence of acidosis. Data from 23 pasture-based studies reported a mean daily ruminal pH of  $6.15 \pm 0.21$  and a range from 5.6 to 6.7 across studies (Kolver and de Veth 2002). The diets collated in that study contained at least 47% pasture and an average pasture content of 81% across all treatments. Pasture quality and quantity varies in Australia with season and area. Pasture that is lush, leafy with a high leaf to stem ratio and growing rapidly may

increase risk of acidosis (Westwood and Lean 2001). These diets appear to lack physically effective fibre, which stimulates chewing activity (Mertens 1997).

Increasing proportion of NDF in the diet and increasing length of particle size of forage increases chewing time (Beauchemin 1991). However, Maekawa *et al.* (2002) found that increased chewing time did not increase total daily saliva production, and therefore buffering capacity of the rumen. Instead, cows offered silage and concentrate separately ate more concentrate than silage (F:C = 43:57), and, consequently had a lower mean rumen pH of 5.77 compared to cows fed a TMR diet (F:C = 50:50) that had a mean rumen pH of 5.99 ( $P = 0.09$ ). These authors concluded that feeding separate ingredients compared with TMR increased the risk of acidosis, because cows selectively ate a higher proportion of concentrate. Further, Shepherd (2003) showed, using simulation modelling, that many cows within large herds will have limited access to grazed forage. This problem arises under circumstances where cows that are milked early in the milking process have access to pasture before those milked later. These cattle will consequently also have inadequate intakes of fibre even if forage sources have appropriate fibre content. Therefore, combining concentrate with lush pasture in the Australian system may be a factor increasing risk of acidosis because of selective eating and uneven access to pasture.

The addition of ionophores and antibiotics to the diet are also common practices on Australian dairy farms. In the present study, 60% of farms fed sodium monensin and 24% of farms were feeding virginiamycin (Chapter 3). Monensin has been found to reduce risk of acidosis *in vitro* (Nagaraja *et al.* 1987) and prevent clinical acidosis in cattle with an experimentally induced acidosis (Avery *et al.* 1980; Nagaraja *et al.* 1981). Rumen pH was higher in monensin treated animals (Horn *et al.* 1981; Nagaraja *et al.* 1981). Virginiamycin, an antibiotic with primarily gram positive activity is effective in reducing lactic acid production *in vitro* (Nagaraja *et al.* 1987) by removing *S. bovis* (Hedde *et al.* 1980), the organism primarily responsible for lactic acid production on starch-based diets. Al Jassim and Rowe (1999) found *S. bovis*, which produced only L-lactate was very sensitive to virginiamycin *in vitro* at concentrations of 2 µg/ml. Effects seen from the use of virginiamycin include higher rumen pH, lower *Lactobacillus* and *S. bovis* counts and lower L-lactate concentrations (Clayton *et al.* 1999; Coe *et al.* 1999), higher faecal pH (Clayton *et al.* 1999) and a

higher concentration of propionate produced as a percentage of total VFAs on a 22% concentrate diet (Hedde *et al.* 1980). *S. ruminantium*, a gram negative bacterium, also produced D- and L-lactate, and was very resistant to virginiamycin even at 8 µg/mL (Al Jassim and Rowe 1999). This suggests that on predominantly pasture diets or diets where *S. ruminantium* can be well established in the rumen, virginiamycin may not be as effective in controlling the risk of acidosis.

In the present study, dietary components were related to previously defined categories of dairy herds that reflected rumen biochemistry of cows within these herds in order to better define the risk factors for acidosis in herds.

## 6.3 Materials and Methods

### 6.3.1 General materials and methods

One hundred Australian dairy herds were selected in five areas of NSW and Victoria. Refer to section 2.3.2.1. A questionnaire was completed for each herd detailing the lactating cow diet on the sampling day (refer to sections 2.3.3 to 2.3.5). The amount of concentrate fed to cows in the milking bails was confirmed by weighing two samples. Amount of wasted feed was estimated visually from wastage on the ground on the sampling day. Some feed samples with unknown composition were analysed at Dairy One Forage Testing Laboratory. Refer to section 2.4.4.1 Where samples were not analysed, similar feed analyses were used to estimate the feed value of the sample. The composition of pellets fed was checked with the supplying feed company. Grass samples were collected from pasture of the same or similar composition to the pasture grazed by the lactating herd on the questionnaire day. Diets were validated using the ration formulation program CPM dairy (Cornell\*Penn\*Miner, 2003) (version 3.0.4a). Refer to section 2.4.4.2. Pasture texture was scored on a 1 to 5 scale as outlined in section 2.4.4.3.

### 6.3.2 Classification of herds

Herds were classified into one of three categories; 'subclinically acidotic' ('ACID'), 'suboptimal' ('SO') and 'non-acidotic' ('NA') on the basis of rumen biochemical measures. Refer to section 2.4.5.1. for further detail.

### 6.3.3 Statistical analyses

Data were examined using SPSS version 11.0 and multinomial logistic regression (Tables 1 and 3). Where  $P$ -values were not obtained using this model, Stat Xact version 4.0.1 (Cytel Software Corporation, 1999) was used to obtain  $P$ -values using likelihood ratios. Selected feeds were analysed using Pearsons Chi-square test (Table 6.2) where no herds were feeding the product in a specific category. Oats, sorghum, rice, urea, whole cotton seed, miscellaneous protein, brewers grain, cereal meal, citrus pulp, miscellaneous commodities, lasalocid, tylosin and bentonite were omitted from Table 6.3 due to lack of numbers in categories. Pasture type and texture as predictors of herd category were examined using multinomial logistic regression with pasture type treated as categorical variables and texture score treated as a continuous variable (Table 6.4). A multivariate logistic regression, weighted by number of acidotic cows in a herd, was used to examine effect of dietary variables on herd category ACID compared with categories SO and NA using the statistical package Egret (Version 2.03.1).

## 6.4 Results

Table 6.1 describes significance of presence of feeds in herds of different categories with the reference category as NA. For example, herds in category ACID were more likely to feed wheat ( $P = 0.083$ ) than herds in category NA (Table 6.1). In fact, nine of the ten ACID herds fed wheat (Figure 6.1). There was no significant difference in the presence of millrun between category ACID and NA ( $P = 0.766$ ) and category ACID and SO ( $P = 0.422$ ) (Figure 6.2, Table 6.1). All 10 herds in category ACID fed monensin (Figure 6.3); ACID herds were significantly more likely to feed monensin than NA herds ( $P = 0.002$ ) (Table 6.1). Only 50% herds in category ACID fed virginiamycin (Figure 6.4) and there was with no significant difference in use for category ACID herds compared with NA herds ( $P = 0.106$ ) (Table 6.1). Rice, whole cottonseed, cereal meal, citrus pulp, lasalocid and tylosin were not evaluated in Table 6.1 because few herds used these feeds. Feeding oats, sorghum, urea and brewers grain was evaluated using a Pearson Chi-square statistic and reported in Table 6.2. No significant associations between herd category and these feeds were detected ( $P > 0.2$ ).

**Table 6.1.** Multinomial logistic regression of presence of feeds (Y/N) compared with herd categories (reference category is NA, numbers in brackets indicate numbers in each category)

Feed Y/N	Category	Regression coefficient	P-value
Barley (29)	ACID (2)	-0.189	0.822
	SO (11)	0.418	0.367
Corn (17)	ACID (1)	-0.535	0.628
	SO (5)	-0.129	0.825
Oats (8) <sup>b</sup>	ACID (0)	-17.998	-
	SO (4)	0.740	0.317
Sorghum (19) <sup>b</sup>	ACID (0)	-19.291	-
	SO (7)	0.172	0.745
Triticale (35)	ACID (2)	-0.693	0.404
	SO (10)	-0.223	0.622
Wheat (68)	ACID (9)	1.876	0.083
	SO (19)	-0.150	0.720
Canola meal (26)	ACID (4)	0.875	0.217
	SO (7)	-0.105	0.837
Cottonseed meal (25)	ACID (1)	-1.000	0.360
	SO (8)	-0.019	0.970
Lupins (20)	ACID (1)	-0.639	0.562
	SO (7)	0.172	0.745
Urea (15) <sup>b</sup>	ACID (0)	-19.196	-
	SO (6)	0.322	0.575
Miscellaneous protein (23)	ACID (2)	-0.105	0.901
	SO (6)	-0.295	0.582
Brewers grain (16) <sup>b</sup>	ACID (0)	-19.247	-
	SO (6)	0.199	0.724
Millrun (42)	ACID (3)	-0.219	0.766
	SO (5)	0.341	0.422
Molasses (36)	ACID (3)	-0.021	0.978
	SO (12)	0.176	0.690
Oil/ fat (25)	ACID (2)	-0.189	0.822
	SO (7)	-0.189	0.711
Rice pollard (12)	ACID (2)	0.795	0.369
	SO (3)	-0.186	0.797
Miscellaneous commodities (22)	ACID (2)	0.074	0.930
	SO (7)	0.074	0.887

<b>Monensin (74)</b>	<b>ACID (10)</b>	18.724	0.002 <sup>a</sup>
	<b>SO (23)</b>	0.269	0.533
<b>Virginiamycin (32)</b>	<b>ACID (5)</b>	1.118	0.106
	<b>SO (10)</b>	0.202	0.666
<b>Sodium bicarbonate (44)</b>	<b>ACID (3)</b>	-0.466	0.525
	<b>SO (13)</b>	-0.145	0.735
<b>Limestone (77)</b>	<b>ACID (6)</b>	-0.354	0.611
	<b>SO (24)</b>	0.021	0.962
<b>Magnesium oxide (52)</b>	<b>ACID (6)</b>	0.668	0.333
	<b>SO (16)</b>	0.091	0.828
<b>Bentonite (29)</b>	<b>ACID (1)</b>	-1.301	0.231
	<b>SO (8)</b>	-0.320	0.506
<b>Silage (37)</b>	<b>ACID (3)</b>	-0.118	0.857
	<b>SO (11)</b>	0.190	0.584
<b>Hay (63)</b>	<b>ACID (8)</b>	1.357	0.101
	<b>SO (20)</b>	0.259	0.536
<b>Fibrous by-product (58)</b>	<b>ACID (3)</b>	-0.992	0.175
	<b>SO (18)</b>	-0.088	0.832
<b>Straw (12)</b>	<b>ACID (2)</b>	0.795	0.369
	<b>SO (3)</b>	-0.186	0.797

<sup>a</sup>P-value from likelihood ratio test

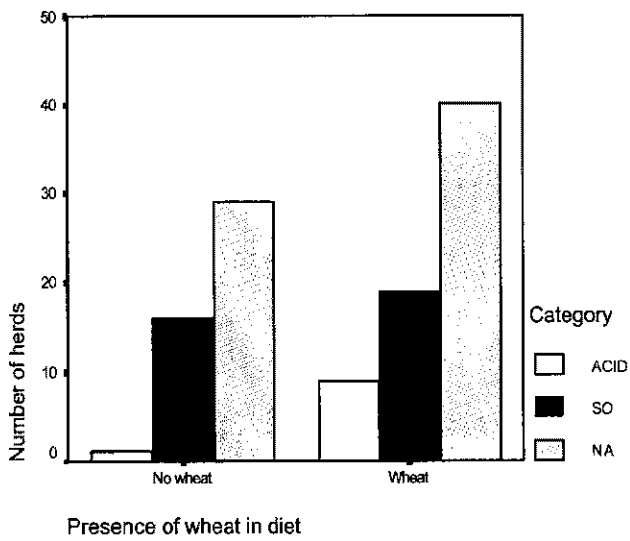
<sup>b</sup>P-value computed using chisquare statistic (Table 6.2).

**Table 6.2.** Relationship among herd categories and presence of feeds evaluated using Pearson Chisquare test.

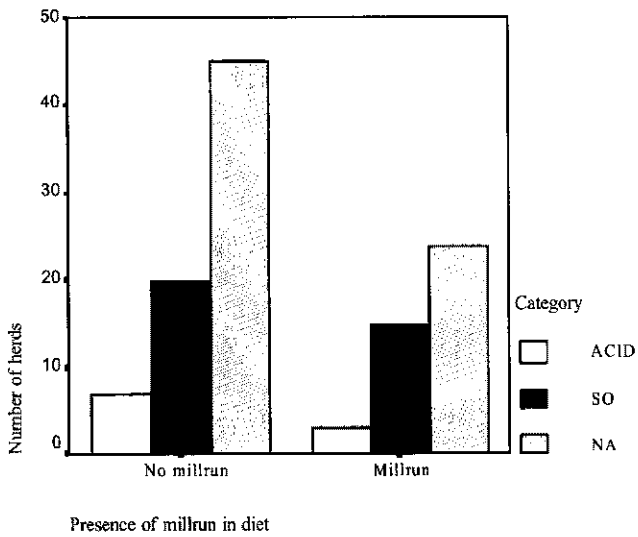
<b>Presence of feeds</b>	<b>Pearson chisquare statistic</b>	<b>P-value</b>
<b>Oats Y/N</b>	1.048	0.592
<b>Sorghum Y/N</b>	0.693	0.707
<b>Urea Y/N</b>	0.562	0.755
<b>Brewers grain Y/N</b>	0.442	0.802

<sup>a</sup>No = 0, Yes = 1

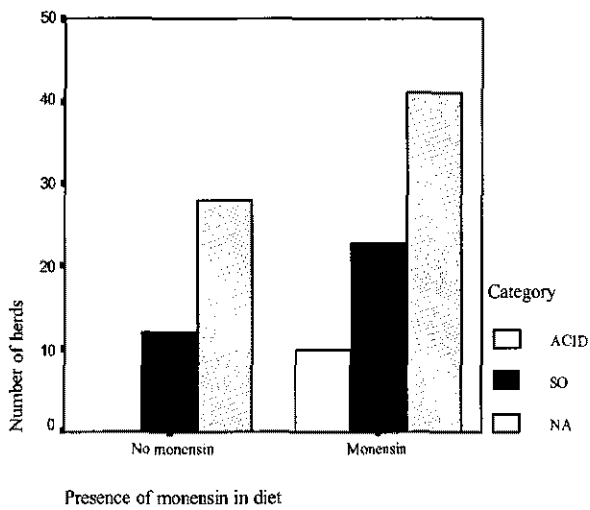
**Figure 6.1.** Number of herds in categories ACID, NA and SO feeding wheat in the diet



**Figure 6.2.** Number of herds in categories ACID, NA and SO feeding millrun in the diet



**Figure 6.3.** Number of herds in categories ACID, NA and SO feeding monensin in the diet



**Figure 6.4.** Number of herds in categories ACID, NA and SO feeding virginiamycin in the diet

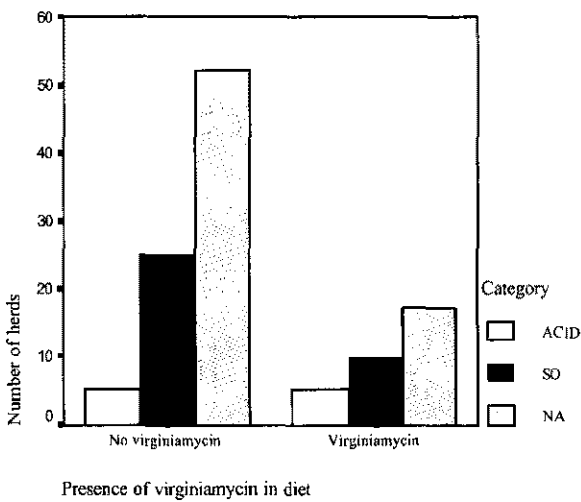


Table 6.3 describes significance of amounts of feeds and dietary variables in the diets of cows and in the study in predicting categories of herds with the reference category as NA. Amount of wheat fed was the only feed associated with category of herd; herds in category ACID fed more wheat than category NA ( $P = 0.055$ ). However, herds in category ACID were more likely to have significantly lower dietary NDF% ( $P = 0.004$ ) and significantly higher NFC% and CHO B1% than NA herds ( $P = 0.002$ ,  $0.008$  respectively).

**Table 6.3.** Multinomial logistic regression of amounts of feeds (DM) compared with herd categories (reference category is NA, numbers in brackets indicate numbers in each category)

Feed amount	Category	Regression coefficient	P-value
Barley (kg/cow/day) (29)	ACID	-0.534	0.320
	SO	-0.144	0.494
Corn (kg/cow/day) (17)	ACID	-0.029	0.974
	SO	-0.450	0.539
Triticale (kg/cow/day) (35)	ACID	0.435	0.368
	SO	0.027	0.902
Wheat (kg/cow/day) (68)	ACID	0.259	0.055
	SO	-0.017	0.822
Canola meal (kg/cow/day) (26)	ACID	0.945	0.425
	SO	-1.718	0.197
Cottonseed meal (kg/cow/day) (25)	ACID	-1.438	0.707
	SO	0.718	0.396
Lupins (kg/cow/day) (20)	ACID	-17.020	0.565
	SO	-0.481	0.542
Millrun (kg/cow/day) (42)	ACID	0.188	0.674
	SO	0.291	0.236
Molasses (kg/cow/day) (36)	ACID	19.326	0.128
	SO	1.150	0.862
Oil/ fat (kg/cow/day) (25)	ACID	0.282	0.972
	SO	1.245	0.787
Rice pollard (kg/cow/day) (12)	ACID	-0.106	0.969
	SO	-2.351	0.346
Monensin (mg/cow/day) (74)	ACID	0.002	0.660
	SO	0.003	0.390
Virginiamycin (mg/cow/day) (32)	ACID	-0.008	0.277
	SO	-0.002	0.745
Sodium bicarbonate (g/cow/day) (44)	ACID	-0.012	0.570
	SO	0.005	0.287
Limestone (g/cow/day) (77)	ACID	-0.354	0.611
	SO	0.021	0.962
Magnesium oxide (g/cow/day) (52)	ACID	0.009	0.745
	SO	0.022	0.257
Pasture (kg/cow/day) (110)	ACID	-0.084	0.292
	SO	-0.035	0.474

Silage (kg/cow/day) (37)	ACID	-0.089	0.748
	SO	-0.156	0.371
Hay (kg/cow/day) (63)	ACID	0.116	0.600
	SO	0.131	0.414
Fibrous by-product (kg/cow/day) (58)	ACID	0.079	0.768
	SO	0.177	0.159
Straw (kg/cow/day) (12)	ACID	-0.077	0.948
	SO	1.188	0.144
NDF (% of diet)	ACID	-0.200	0.004
	SO	-0.015	0.696
NFC (% of diet)	ACID	0.210	0.002
	SO	0.006	0.870
NDF%/ NFC%	ACID	-4.731	0.004
	SO	-0.091	0.883
CHO B1 (% of diet)	ACID	0.144	0.008
	SO	-0.001	0.976
% forage : % concentrate (113)	ACID (10)	-0.253	0.321
	SO (35)	-0.040	0.664

ACID category herds were more likely to be feeding legume dominant pastures (shaftel/ clover/ lucerne) than NA category herds ( $P = 0.008$ ) (Table 6.4). SO herds were at significantly lower risk than NA category herds of feeding ryegrass dominant pastures ( $P = 0.020$ ). Pasture texture was also assessed on a scale of 1 to 5 (footnotes Table 6.4) with ACID category herds less likely to be feeding pastures high in effective fibre than NA herds ( $P = 0.004$ ).

A multivariate logistic regression model was examined using univariate dietary factors with a  $P < 0.2$  and herd category ACID compared with a combination of herd categories NA and SO, weighted by number of acidotic cows in a herd. ACID herds were found to be best described by %NDF to %NFC ratio with a regression coefficient of  $-4.901$ ,  $P = 0.001$  and odds ratio of  $0.0074$  (95% CI =  $0.0004 - 0.1477$ ).

**Table 6.4.** Multinomial logistic regression of pasture type compared with herd categories (reference category is NA, numbers in brackets indicate numbers in each category)

Pasture type	Category	Regression coefficient	<i>P</i> value
Shaftal/ clover/ lucerne (10)	ACID (3)	2.411	0.008
	SO (4)	1.033	0.190
Clover: rye 50:50 (34)	ACID (3)	0.478	0.519
	SO (14)	0.669	0.118
Ryegrass (61)	ACID (4)	-0.579	0.397
	SO (13)	-0.941	0.020
Kikuyu/ paspalum (27)	ACID (0)	-20.311	-
	SO (10)	0.194	0.669
Pasture texture (131) <sup>a</sup>	ACID (10)	-1.635	0.004
	SO (40)	-0.149	0.615

<sup>a</sup> scale 1 – 5, 1=soft clover/ medic, minimal fibre, 2 = very soft ryegrass, 3 = firmer ryegrass/ lush kikuyu/ fresh oat/ fescue, 4 = mature oat/ mature fescue/ phalaris/ kikuyu, 5 = forage sorghum/ maize/ setaria

## 6.5 Discussion

Categories of herd, either ACID (acidotic), SO (sub-optimal) or NA (non-acidotic), were defined previously from individual cow data using rumen biochemical data and cluster analysis (categories 1, 2 or 3) (Chapter 4) and confirmed by outcome variables lameness, faecal score and rumen fill (Chapter 5). Mean milk production of cows in categories 1, 2 and 3 was  $33.0 \pm 0.94$  L,  $27.0 \pm 0.54$  L and  $29.1 \pm 0.37$  L, after correcting for DIM and parity, respectively. Category 1 cows had a low milk fat percentage ( $3.29 \pm 0.112\%$ , corrected for DIM and parity), resulting in a significantly lower percentage milk fat: protein in this category ( $1.05 \pm 0.036$ ) than other categories ( $P < 0.01$ ). Herds were grouped into categories ACID, SO or NA based on 3/8 cows in the herd fitting into category 1, 2 or 3. For example, if 3/8 cows in a herd were found in category 1, the herd was categorised in ACID category. Rumen metabolism of cow categories 1, 2 and 3 and milk production characteristics of herd categories ACID, SO and NA are summarised in Chapter 4.

Results in this chapter define dietary risk factors associated with these 3 herd categories. Not surprisingly, the majority of individual feeds were not significant predictors of category ACID or SO compared with NA. This may well be because of the small number of herds in total feeding any given feed, a function of practical limits of sampling size (Table 6.1) or the feed being a minor part of the total diet for many herds (Table 6.2). Many feeds were minor constituents of pellets or grain mixes. Notwithstanding this, presence of wheat approached significant association with ACID herds compared to NA herds ( $P = 0.083$ ), supporting previous studies that used wheat, a highly fermentable carbohydrate source, to induce acidosis (Krajcarski-Hunt *et al.* 2002; Lee *et al.* 1982). Importantly, of the herds that were feeding wheat, ACID herds were also fed significantly more wheat than NA herds ( $P = 0.055$ ). This result also supports *in vitro* research that found wheat produced significantly higher concentrations of total VFA than triticale, oats, barley, maize, rice and sorghum ( $P < 0.001$ ) (Opatpatanakit *et al.* 1994).

Of the dietary additives, category ACID herds were more likely to feed monensin than NA herds ( $P = 0.002$ ). However, of the herds feeding monensin, the mean amount fed per cow per day to category ACID herds was  $252.6 \pm 100.79$  mg and to NA herds was  $240.2 \pm 71.75$  mg (Chapter 4). Monensin has been shown to prevent clinical acidosis in cattle with experimentally induced acidosis (Avery *et al.* 1980; Nagaraja *et al.* 1981) and can increase rumen pH in treated animals (Horn *et al.* 1981; Nagaraja *et al.* 1981). However, Nagaraja *et al.* (1981) used dose rates of monensin of 1.3 mg/kg BW or 650 mg/cow/day for a 500 kg cow to increase rumen pH. Milk production responses indicate that the combined benefits of monensin fed at 250 to 400 mg/cow/day are reflected in increased milk production (Beckett *et al.* 1998; Ipharraguerre and Clark 2003; Phipps *et al.* 2000; Vallimont *et al.* 2001). The fact that the rate of monensin fed to ACID and NA herds was lower than that used in other studies showing positive benefits on milk production and prevention of acidosis may indicate that herds in category ACID acknowledged the risk of acidosis and were taking measures to control this, but were not effective in providing control of subclinical acidosis. Alternately, because these herds were higher producing these may be more likely to take up technologies, such as monensin that may increase milk production and reduce risk of acidosis.

ACID herds were also more likely to tend to feed virginiamycin than NA herds ( $P = 0.106$ ), possibly for similar reasons to feeding monensin. Category ACID and NA herds were feeding virginiamycin at a rate of  $156.8 \pm 92.13$  mg/cow/day and  $195.3 \pm 74.97$  mg/cow/day (Chapter 4). The recommended feeding rate for virginiamycin is 20 mg/kg of DM in complete diets (Rogers *et al.* 1995) or 0.5 mg/kg body weight/day (Thorniley *et al.* 1994), equivalent to 300 mg/cow/day when DMI is 15 kg DM/day. Virginiamycin is effective in reducing lactic acid production *in vitro* (Nagaraja *et al.* 1987) by removing *S. bovis*. The dose rate used by ACID herds was well below recommended rates and therefore may have reduced the effectiveness of control. However, on pasture-based diets, *S. ruminantium*, also a producer of D- and L-lactate and very resistant to virginiamycin (Al Jassim and Rowe 1999), can be well established in the rumen. Virginiamycin may therefore not be completely effective in decreasing lactate production in this linkage between pasture and milk bail feeding not withstanding the lower than recommended dose given.

Several studies have examined rumen measures in relationship to acidosis in cows on pasture (Kolver and de Veth 2002; Wales *et al.* 2001). Wales *et al.* (2001) found that cows offered 40 kg DM/day of perennial ryegrass (*Lolium perenne* L.)- white clover (*Trifolium repens* L.) pasture had an average daily rumen pH of 5.89. This indicates that good quality, lush pasture may depress rumen pH, predisposing cows to acidosis. In this study, neither ACID herds or SO herds were feeding significantly more pasture than NA herds ( $P = 0.292$ ,  $P = 0.474$  respectively). However, when type of pasture the herds were grazing was examined in relationship to herd categories, both legume dominant pastures and ryegrass dominant pastures were significant factors in predicting categories. ACID category herds grazed more legume dominant pastures than NA category herds ( $P = 0.008$ ) and these pastures had the lowest NDF% and highest NFC% of all pasture categories (Chapter 3). This result was confirmed on analysis of pasture texture (Table 6.4) in which ACID herds grazed pasture of a lower score on a 1 to 5 scale than pastures fed to NA herds ( $P = 0.004$ ). This particular scale of pasture texture has not been used before to assess acidosis risk associated with pasture. These results indicate that the scale may be a useful tool in assessing herd risk of acidosis.

Forage to concentrate (F:C) ratio was not a significant risk factor for herd category ACID compared with NA ( $P = 0.321$ ), confirming previous studies finding F:C per se had no effect on rumen pH (Yang *et al.* 2001b). Interestingly, previous research reported increased F:C in the diet of cows decreased milk production ( $P < 0.05$ ) (Yang *et al.* 2001b). In this study, however herds in category SO were not significantly more likely to have higher F:C than herds in category NA, despite lower milk production than other categories.

ACID herd diets had significantly lower NDF percentages ( $P = 0.004$ ) and higher NFC percentages ( $P = 0.002$ ) than NA herds. The ACID herds were also significantly more likely to contain a higher carbohydrate B1% (starch % + sugar %) of the diet than NA herds. Lana *et al.* (1998) and Russell (1998) found that when the amount of concentrate or carbohydrate percentage was increased in the diet and NDF percentage decreased, the concentration of total volatile fatty acids in the rumen increased. This supports the biochemical data relating to cows characterising category ACID, which had significantly higher total volatile fatty acid (VFA) concentrations than cows used to define category NA ( $P < 0.01$ , Chapter 4). Importantly, multivariate modelling of ACID herds compared with a combination of SO and NA herds using dietary factors with a  $P < 0.2$  indicated ACID herds were best described only by the %NDF: %NFC ratio ( $P = 0.001$ ) with an odds ratio of 0.0074. Amount of wheat, although highly significant on univariate analysis was highly confounded by this factor in the multivariate model and dropped out of the final analysis.

Interestingly, no significant differences in relationship were found between SO herd diets and NA herd diets for NFC, NDF and CHO B1% contents in the diet. Biochemical characteristics used to define cows and consequently SO and NA herds and milk production data from SO and NA herds indicate dietary differences (Chapter 4). Cows used to define NA herds (category 3 cows) had a significantly lower total VFA concentration ( $62.8 \pm 15.65$  mM) than cows characterising SO herds (category 2 cows) ( $62.8 \pm 15.65$  mM,  $P < 0.01$ ). However, mean milk production for category 3 cows ( $29.1 \pm 0.37$  L, corrected for DIM and parity) was significantly higher than category 3 cows ( $27.0 \pm 0.54$  L) ( $P = 0.002$ ). A significantly lower milk fat percentage for category 3 cows ( $3.65 \pm 0.044\%$ ;  $P = 0.007$ ) than category 2 cows resulted in a milk fat: protein ratio of  $1.18 \pm 0.013$ , not significantly different to

category 2. Examination of diets between herd categories NA and SO revealed no significant differences for CP%, ME MJ/kg DM, NFC% and NDF% content (KW-ANOVA) (Chapter 4). These results have been confirmed in this chapter which found that SO herds were not more likely to have lower NDF% or higher NFC% in the diet than NA herds. It was proposed in chapter 4 that the difference between the two categories was an energy: protein mismatch in suboptimal cows (making up 3/8 cows in SO herds). Results in this chapter suggest that the energy: protein mismatch may, in part, reflect less digestible fibre in the diet of SO cows. SO herds tended to feed greater quantities of both fibrous by-products ( $P = 0.159$ ) and straw ( $P = 0.144$ ) than NA herds. SO herds were also less likely to be grazing ryegrass dominant pastures than NA herds ( $P = 0.020$ ), but tended to graze ryegrass: clover 50:50 pastures ( $P = 0.118$ ) or legume dominant pastures ( $P = 0.190$ ) over NA herds. Both clover dominant and clover: ryegrass 50:50 pastures in this study contained lower NDF% ( $29.1 \pm 1.86\%$ ,  $35.1 \pm 5.01\%$  respectively) and higher NFC% ( $35.1 \pm 1.85\%$ ,  $26.5 \pm 4.89\%$ ) than ryegrass dominant pasture that contained  $42.1 \pm 4.27\%$  NDF and  $21.5 \pm 4.57\%$  NFC (Chapter 3). This may indicate that fibre fed in SO herds was not capable of supporting similar milk production to NA herds.

## 6.6 Conclusion

This chapter describes dietary risk factors associated with acidosis in 100 Australian dairy herds. The majority of individual feed constituents fed were not significant factors in predicting herd categories, however ACID herds fed higher amounts of wheat than NA herds ( $P = 0.055$ ). ACID herds were also more likely to feed legume dominant pasture compared to NA herds ( $P = 0.008$ ), confirming the hypothesis that feeding high quality pasture may predispose herds to acidosis. ACID herds were significantly more likely to be fed diets higher in NFC% and lower in NDF% and higher in the B1 carbohydrate fractions (% sugar + % starch in the diet) than NA herds. Furthermore, using multivariate modelling, category ACID was best described simply by the NDF%: NFC% ratio. Therefore, one of the most important risk factors for acidosis in Australian dairy herds is simply the dietary NDF% to NFC% ratio. This should become an important diagnostic tool for dairy producers, veterinarians, and support personnel in the dairy industry. There was also evidence to support observed differences in ruminal biochemistry between cows in NA and SO herds

because SO herds tended to have a higher prevalence of poor forages and fibrous by-products in the diet and less prevalence of ryegrass pastures.

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## Chapter 7.

### CLINICAL ACIDOSIS IN A GIPPSLAND DAIRY HERD

#### 7.1 Summary

*A Gippsland dairy herd was sampled as part of a wider randomised cross-sectional study that examined the prevalence, risk factors for, and effects of, acidosis on rumen function of dairy cattle. Three herds on the farm were involved in the study; the transition herd (cows 3 weeks prior to calving), the very fresh lactating herd ( $1 < \text{DIM} < 10$ , herd 1) and the fresh lactating herd ( $10 < \text{DIM} < 120$ , herd 2) including a small lame herd fed separately. The transition cows were fed 2 kg DM triticale /cow/day and hay with an estimated total dry matter intake of 4.8 kg/cow/day. The lactating cow diet included 6.75 kg DM triticale /cow/day with total concentrate fed being 8.1kg DM /cow/day in the milking parlour. Silage, lucerne cubes, hay and pasture (herd 2 only) was also fed to the lactating cows with the estimated total DMI for cows herd 1 and 2 being 13.7 kg and 20.8 kg/cow/day respectively. Three primiparous and five multiparous cows in early lactation ( $<100$  days in milk) were randomly selected from each of two lactating herds: herd 1 and 2. Rumen fluid was sampled from each cow by both rumenocentesis and stomach tube. The rumenocentesis samples were tested for pH at the time of sampling. Stomach tube samples were frozen and subsequently tested for volatile fatty acid, ammonia and D- and L- lactate concentration. In the very fresh herd, there was a high prevalence of severe lameness and scouring, coupled with a mean rumen pH 5.67, low mean total volatile fatty acid concentration 71.0 mM and high mean concentrations of L- and D- lactate, (7.71 mM) and (7.31 mM), respectively. Cows in the fresh herd had moderate signs of scouring and lameness. A lame herd comprising approximately 50 cows separated from the fresh herd was also present on the farm. The mean rumen pH of the fresh herd was 5.74 and mean rumen concentrations of volatile fatty acids, ammonia, L- and D-lactate were within ranges considered normal.*

*The transition diet failed to supply sufficient energy and protein for maintenance of cows of this live weight in late gestation. The diet fed to the very fresh herd was low in*

*effective fibre and contained an excessive content of non-structural carbohydrate in the form of processed, rapidly fermentable grain. The sudden change from the transition diet to the diet fed to the very fresh herd probably also precipitated the outbreak of acidosis. This case report provides unique detail about acidosis including information on diets and rumen parameters of an outbreak of acidosis in a pasture-fed herd.*

## **7.2 Introduction**

Rumen lactic acidosis is a clinical disorder of cattle that can result in rumenitis, metabolic acidosis, lameness, hepatic abscessation, pneumonia and death (Lean *et al.* 2000). Acidosis appears to be an increasingly common problem in dairy herds and can be under-diagnosed due to lack of awareness. Acidosis presents in both the clinical and sub-clinical form. Signs often associated with sub-clinical acidosis include: decreased milk fat content, feed conversion efficiency, feed intake and rate and extent of roughage degradation (Lean *et al.* 2000); increased laminitis causing lameness (Nocek 1997; Owens *et al.* 1998); liver abscessation (Owens *et al.* 1998); scouring (Nocek 1997), and a higher incidence of left and right displacements of the abomasum. Clinical acidosis presents over a range from mild to severe. Cattle with mild clinical acidosis may have anorexia, decreased milk production and scouring (Underwood 1992). Clinical signs of sub-acute and acute acidosis include lameness, depression, dehydration, toxemia, and 'downer cow' syndrome (Bolton and Pass 1988). Acute acidosis may result in severe metabolic acidosis, recumbency, coma and death in 8 to 10 hours (Underwood 1992). Clinical signs in the acute form develop within 8 hours and precede the onset of metabolic clinical acidosis, which peaks in 36 hours (Underwood 1992). Other sequelae to acute ruminal acidosis include hypocalcaemia resulting from calcium malabsorption, polioencephalomalacia from an induced thiamine deficiency and rumenitis leading to liver abscessation (Bolton and Pass 1988). The bacteria thought to be most commonly responsible for liver abscessation are *Fusobacterium necrophorum* and *Archaeobacterium* spp; both are able to reach the liver through the portal circulation via damaged ruminal epithelium (Bolton and Pass 1988).

Both clinical and subclinical acidosis are problems in the dairy industry and develop in cattle that have ingested large amounts of unaccustomed feeds rich in ruminally fermentable carbohydrates (Crichlow and Chaplin 1985; Nocek 1997). The common practice in the Australian dairy industry of combining supply of good quality pasture with twice-daily concentrate feeding in the bail may be increasing the potential for these conditions. Feeding pasture in an immature, early vegetative state with high concentrations of water-soluble carbohydrates and less effective fibre may increase the risk of both sub-clinical and clinical acidosis.

This case report details clinical signs, laboratory results confirming the diagnosis and indirect indicators of acidosis in a Gippsland (Victoria, Australia) dairy herd. Acidosis has been well documented in the past, particularly in experimental cases and in individual cattle (Dirksen 1970; Huber 1976; Owens *et al.* 1998; Slyter 1976), but this report presents detail on clinical acidosis in a herd, as well as detailed evaluation of the diets and feeding strategies involved and measures of rumen function in the cows affected.

### 7.3 Case report

A Gippsland dairy herd was tested, as part of a wider study on the effects of acidosis on cattle health and production, in May 2002. The wider study protocol involved careful collection of farm management and dietary information, an estimation of the prevalence of lameness and scouring in the cattle, and collection of rumen fluid from eight cows < 100 DIM. The observation of signs consistent with a sub-acute clinical acidosis in the herd prompted a more extensive sampling of rumen fluid on this farm and further investigation of the herd that is presented in this case report. The aim of collecting the additional information was to provide a case report on the presentation, clinical signs, diet and rumen function in spontaneous clinical acidosis in a pasture fed dairy herd. Treatment and prevention protocols for acidosis are outlined in this report, but were not implemented in this herd due to study restrictions.

The area of the farm was 538 ha and there was a total of 1110 Friesian cows in the herd, weighing an estimated average 550kg. The weather on the sampling day was fine although some rain had been recorded in the area in previous weeks.

Notwithstanding this, the paddocks were not waterlogged and the farm laneways were in very good condition. Most of the pastures had not been recently renovated and the predominant pasture type was Victorian perennial ryegrass (*Lolium perenne*) with approximately 188 kg DM/ha pasture available to the cattle. Parasite control included treatment of the whole herd once a year in May with moxidectin (Cydectin™, Fort Dodge Australia, Pty Ltd) and triclabendazole (Fasinex™, Novartis Animal Health Australasia, Pty Ltd).

On the day of the visit, there were 1020 lactating cows and 80 transition cows, defined as cows in the 3 weeks before calving, on the farm. The lactating cows were split by the farm manager into three herds for management purposes: 20 very fresh cows (1 < DIM < 10, herd 1), 550 fresh cows (10 < DIM < 120, herd 2) and 450 stale (> 120 DIM, herd 3) cows. Approximately 50 cows from the fresh herd were run separately due to lameness. The lactating cows, (stale and fresh cows combined) produced an average of 20.25 L/cow/day, containing 4.26% fat and 3.5% protein. The somatic cell count in milk was 195,000 cells/ml for the herd. This case report provides details of the very fresh herd (herd 1), fresh herd (herd 2) and transition herd.

### 7.3.1 Herd 1

The very fresh herd (< 10 DIM) was fed both in the milking parlour and in the sacrifice paddock following both milkings. No pasture was offered to this herd. The bail diet consisted of 6.75 kg DM crushed triticale (*Triticale hexaploide*), 0.71 kg DM faba beans (*Vicia faba*), 0.63 kg DM canola meal and 250 g of a mineral pellet that included 250 mg of sodium monensin (Rumensin™, Elanco Animal Health®) per day. The amount of concentrate fed in the bail was validated by weighing three separate allocations from the grain dispenser on the rotary milking platform. The cows were offered 9 kg DM of pasture silage per head per day and 2.4 kg DM per head per day lucerne cubes. There was considerable wastage of the silage, which was fed on the ground, and it was estimated that the cows ate 3 kg DM/cow/day of this feed. The total DM intake/cow/day was estimated at 13.7 kg. An assessment of lameness developed by (Sprecher *et al.* 1997) using a simple scoring system was used in the herd (Appendix, Table 3). With this system, a number of individual cows from the herd are scored for locomotion on a scale of 1 to 5 where 1 is clinically normal, 3 is moderately lame with the cow standing and walking with an arched back and 5 is

severely lame, showing pronounced arching of the back and refusal to bear weight on one limb. The majority of the cows (80 to 90%) in the very fresh herd were moderately to severely lame, with scores 3/5 to 4/5. Some cows presented with hot, swollen feet, were reluctant to stand, and when walking, looked as if they were 'walking on hot coals'. Severe scouring was noted in some animals. When the rumen fluid obtained by rumenocentesis and stomach tube was examined, several of the samples were white and milky in appearance, with a sweet odour.

### 7.3.2 Herd 2

The fresh herd (herd 2) was fed both in the milking parlour and in the paddock following both milkings. The estimated total DMI was 20.8 kg/cow/day. The bail diet (8.3 kg DM) consisted of the same concentrates that the very fresh herd was fed in the milking parlour. In the paddock after each milking, cows were offered on average 3 kg DM/cow/day pasture silage using a mixer wagon. The silage was visually assessed as old but not moulded, nor as having off-odours. It contained significant amounts of stalk and was long chopped. Fresh, very short chop length (< 2.5 cm) lucerne cubes were fed through the mixer wagon at an estimated 3 kg DM/cow/day. Cows were also offered, while in the paddock, 1 kg DM of old home-grown pasture hay containing a large amount of fibrous stalk. The cows were offered (to simulated grazing height) 5.5 kg DM/cow/day (188 kg DM/ha) of ryegrass pasture of low density, fed at the one to two leaf per tiller stage. The ryegrass was measured from five randomly selected 0.1 m<sup>2</sup> quadrants, excluding fertility patches in the pasture, clipped before grazing to a height estimated from the pasture height grazed on the day.

In the preceding 12 months two cases of abomasal displacement and one case of epistaxis had been diagnosed. On the sampling day, on reference scales of 1 to 5, the mean BCS of 15 cows randomly sampled was 3.15 (Edmondson *et al.* 1989) (Appendix, Table 2), rumen fill 3.33 (1=flat, 5=fully distended) and faecal staining on the cows 4, indicating a high prevalence of severe scours. Faeces in this herd were scored predominantly 3 to 4 on a 1 to 5 scale ranging from dry with excessive amounts of fibre from cows on a predominant hay diet (score 1) to scouring with no form and a large constituent of water from cows on a diet of minimal fibre (score 5). Of 15 randomly selected cows scored for locomotion from herd 2, 10 cows scored 1/5, three cows scored 2/5 and two cows scored 3/5. The prevalence of lameness in

the fresh herd is indicated in Plate 1. Cows photographed are lying or standing with heads lowered from the normal position in order to take weight off the hind limbs, or with feet placed underneath the body in an attempt to relieve pressure from all four limbs. Faecal pats were assessed in the paddock as being on average soft and less well formed, with evidence of more liquid than ideal and containing undigested grain, and were scored predominantly 3 and 4 on the five point scale.

**Plate 7.1.** Fresh herd in paddock (herd 2)



### 7.3.3 *Transition herd*

The transition herd showed no signs of scouring or lameness, however examination of the transition herd was critical to identifying the risk factors for acidosis and, in particular, examining the sequence of change in diets that could precipitate a sudden change in access of the cows to rapidly fermentable carbohydrates. Before calving the transition cow ration as fed/cow/day included 2 kg crushed triticale, 250 g of feed containing anionic salts, minimal pasture and approximately 2.4 kg poor to medium quality ryegrass hay available ad libitum. Although the hay was not dusty or mouldy, it did not look fresh and contained a significant amount of stalk. The 80 cows had been grazing a 6 ha paddock with poor quality, dirty, low density Victorian ryegrass for 4 weeks prior to sampling. Cows ate approximately 0.375 kg DM/cow/day of ryegrass and the estimated total DM intake per cow per day was 4.8 kg.

### 7.3.4 Laboratory methods and results

Samples of the pasture on offer were sent to the Dairy One Forage Laboratory for testing by near infrared reflectance spectroscopy. Refer to section 2.4.4.1. The nutrient content of the daily rations for the fresh and very fresh herds and the transition herd were calculated using CPM dairy (Cornell\*Penn\*Miner, Version 3.04a, 2003) (refer to section 2.4.4.2) and are included in Table 7.1. Target values for optimal dietary content of rations are also provided in Table 7.1.

**Table 7.1.** Analysis of fresh herd diets (per day), transition cow diet and reference values

Nutrient	Very Fresh Herd Diet	Fresh Herd Diet	Transition Diet	Recommended values for an early lactating dairy cow <sup>a, b</sup>
ME (MJ/kg DM)	11.6	10.6	10.4	11.5
NFC %	44.4	36.2	38.8	36
CP %	18.1	19.4	14.5	18
NDF %	31.6	36.2	40.6	30
peNDF %	21.1	25.5	33.0	24

<sup>a</sup> TJ Lean, personal communication.

<sup>b</sup> Lean *et al.* (2003)

Eight cows from the very fresh and fresh herd were systematically obtained from the cohort of cows that were < 100 DIM by selecting every second cow from herd 1 and every 50<sup>th</sup> cow from herd 2 and that met the standard criteria used (refer to section 2.3.7). A standard pH meter (model ECpHScan-WP3) was used to measure the pH of ruminal fluid taken by both stomach tube and rumenocentesis, in the field immediately after each sampling. The pH measurement from the rumenocentesis sample was used for all statistical analyses. For rumen fluid collection and processing technique, refer to sections 2.3.8.3 and 2.3.10.3. Rumen pH measurements and biochemical analyses are outlined in sections 2.4.2 and 2.4.3.

Laboratory analyses of rumen fluid for the two herds are displayed in Table 7.2. Herd 1 (very fresh herd) had a low mean rumen pH and sub-optimal rumen function. This was demonstrated by low total VFA concentrations and high D- and L- lactate concentrations. Ammonia concentrations in this herd were within the normal range (> 2.8 mM) (Satter and Slyter 1974). Herd 2 (fresh herd) had a low mean rumen pH, but

had adequate rumen function on laboratory results. Laboratory results from herd 1 confirmed a diagnosis of clinical acidosis in the very fresh cows.

**Table 7.2.** Rumen function parameters in herd 1 and 2 compared with normal values

	Normal	Herd 1	Herd 2
Mean pH (rumenocentesis)	>5.8 <sup>a</sup>	5.67	5.74
Mean total VFA concentration (mM)	>95 <sup>b</sup>	71.0	97.9
Mean ammonia concentration (mM)	>2.8 <sup>c</sup>	9.2	6.1
Mean L-lactate concentration (mM)	<0.15 <sup>d</sup>	7.71	0.14
Mean D-lactate concentration (mM)	<0.28 <sup>d</sup>	7.31	0.12

<sup>a</sup> de Veth and Kolver (2001)

<sup>b</sup> Leng and Brett (1966)

<sup>c</sup> Satter and Slyter (1974)

<sup>d</sup> E Bramley et al, unpublished.

## 7.4 Discussion

### 7.4.1 Differential diagnosis

Differential diagnoses for this herd based on the clinical signs observed included sub-clinical/clinical acidosis, causes of significant numbers of lameness such as infectious pododermatitis, injury to the feet and legs, and causes of a high prevalence of scouring including parasitism, salmonella, yersiniosis, winter dysentery, and pestiviral infection. Other more unusual causes of such a high prevalence of illness, including other bacterial or viral disease, were also considered.

Herd history supported a diagnosis of acidosis. There was a significant prevalence of lameness, with approximately 50 obviously lame cows, coupled in the presence of good quality tracks, concrete and movement management. There was no evidence of foot rot, but there had been recent cases of abomasal disorders and epistaxis. Clinical signs seen in both herds included lameness, scouring and loose faeces with a large liquid component. Physical qualities of the rumen fluid such as a milky colour and sweet smell from some of the samples indicated a poorly functioning rumen. Laboratory findings of low rumen pH and suboptimal total VFA concentrations coupled with very high D- and L- lactate concentrations in the very fresh cows (herd 1) confirmed the diagnosis. The lack of evidence of other signs consistent with other

differential diagnoses, the consistency of clinical signs, diet content and change, and ruminal findings, lead to a presumptive diagnosis of ruminal acidosis in this herd.

#### 7.4.2 *Ruminal acidosis*

Ruminal acidosis reflects a decrease in pH in the rumen from an inciting cause. Access to preformed acids in feeds such as silage, a failure to produce buffering with endogenously derived buffers such as salivary bicarbonate, production of large amounts of weak VFAs (acetic acid, butyric acid and propionic acid) and production of lactic acid in the rumen may all contribute to lowering ruminal pH. (Lean *et al.* 2000) Pastures low in NDF and high in non-structural carbohydrates and supplementation of pastures with silages and grain may also expose cattle that are predominantly pasture-fed to the risk of acidosis. The aetiology, effects and prevention of ruminal acidosis have been extensively researched and reviewed (Dirksen 1970; Huber 1976; Hungate *et al.* 1952; Nocek 1997; Owens *et al.* 1998; Slyter 1976; Underwood 1992). Current understanding suggests that rumen microbes multiply rapidly with the feeding of concentrates, producing large amounts of the weak VFAs. Rumen pH is lowered, promoting a favourable environment for the growth of *Streptococcus bovis* and *Lactobacillus* sp (Nocek 1997). The production of lactic acid by these bacteria further reduces rumen pH resulting in fermentation stasis, absorption of D and L- lactate and occurrence of metabolic acidosis (Nocek 1997). It is important to note that cows on good quality pasture alone may have pH < 5.8 (Wales *et al.* 2001) and the pathogenesis of acidosis of cows fed high quality pasture supplemented with concentrates requires further evaluation.

Clinical acidosis was generally considered in Australia to affect one to a few cows in a herd, in association with unusual sudden dietary changes such as access to additional concentrate. Sub-clinical acidosis often goes unrecognised and undiagnosed until significant herd involvement and obvious clinical signs are evident, by which time large financial losses and long-term health issues such as whole herd lameness problems may be inevitable. Herds with clinical cases often have significant numbers of animals with sub-clinical acidosis. The cut-off pH at which sub-clinical acidosis is diagnosed is controversial, with some authors suggesting 5.5 by rumenocentesis (Garrett *et al.* 1999). Many authors, including those of the Cornell Net Carbohydrate and Protein System model, predict that *in vitro* fibre digestibility is reduced when pH

drops below 6.2 (Calsamiglia *et al.* 1999; Grant 1994; Grant and Mertens 1992). One *in vitro* study however, found that on high quality pasture based diets, although DM digestibility was optimised at pH 6.35, a large decrease in digestibility only occurred below a ruminal pH of 5.8 (de Veth and Kolver 2001).

#### **7.4.3 Dietary factors influencing the presentation**

The presumptive diagnosis of clinical acidosis and subclinical acidosis in this case report was based on herd and diet history, clinical signs and laboratory results. Dietary factors associated with acidosis in herd 1 (very fresh herd) included a poor transition ration combined with a lactating cow ration high in non-structural carbohydrates and low in effective NDF. The poor transition diet was probably critical to causing this herd problem, providing insufficient DM, energy and protein intake for maintenance. The objectives of a good transition ration are to reduce fibre levels progressively from those fed to dry cattle, to allow introduction of concentrates used in the lactating cow diets and encouraging, by provision of similar feeds in sufficient quantity, rumen microflora to adjust to a change of diet. Instead, there was an abrupt change in diet at calving. The lactating cow diet contained very large amounts of concentrate including crushed grain and inadequate effective fibre intake as evidenced by low NDF% and peNDF% in the lactating ration. This was combined with a lack of effective rumen buffering or rumen modifiers in comparison to the amount of concentrates consumed. The use of monensin at a rate of 250 mg/cow/day did not control the problem in herd 1 (very fresh herd). The transition diet can also be used to introduce other dietary controls for ruminal acidosis, including monensin 300 to 450 mg/cow/day, virginiamycin 200 mg/cow/day and tylosin 150 mg/cow/day. Sodium bicarbonate should not be fed in the transition period before calving, because the very high sodium content can predispose cattle to hypocalcaemia, but feeding magnesium oxide as a buffer could be considered before and after calving.

#### **7.4.4 Lameness**

Significant lameness was noted in both herds 1 and 2 (Plate 7.1). The farmer had also separated the cows he recognised were lame in herd 2 to a "lame herd". Lameness is a common problem in the Australian dairy industry, with a reported incidence of 7.5% during lactation in Australia (Harris *et al.* 1988). Many environmental and nutritional factors have been implicated in the onset of foot lameness. Environmental conditions

on the property were good. The fresh herd were walking on average only 1.4 km/day with the very fresh herd in a paddock next to the dairy. Foot rot was not a common diagnosis in the herd. Clinical signs of lameness, particularly in herd 1, included swollen fetlocks in multiple feet, a finding consistent with laminitis, although individual claws were not examined. Lameness in herd 2 was more subtle with the majority of lame cows showing lameness by their stance and walk. Acidosis has been implicated as a common cause of laminitis in dairy herds. Lesions associated with laminitis include solar haemorrhage and abscessation (Bergsten 2000; Dewes 1978; Tranter and Morris 1991); bruising of the solar corium (Dewes 1978; Harris *et al.* 1988); under-run sole (Bergsten 2000; Tranter and Morris 1991); and white line lesions (Bergsten 2000; Tranter and Morris 1991). Laminitis can be defined as acute, sub-acute, chronic and sub-clinical depending on the severity and duration of the condition. Acute laminitis occurs after a metabolic insult causes a decreased ruminal and subsequently systemic pH. However, ruminal pH may have returned to normal when laminitic lesions are first diagnosed as a result of the 4 to 8 week delay between nutritional insult and the onset of laminitis. Therefore, particularly in herd 2 in this case study, cows with laminitis may have normal ruminal pH results, demonstrating the importance of taking multiple rumen pH tests.

#### 7.4.5 Scouring

Scouring was a significant problem in this herd. The faeces of cows can provide indirect evidence of clinical and sub-clinical acidosis. Faecal moisture content is increased as a result of a lactate-based osmotic overloading of the large intestine, with the lactate coming from the rumen (Bolton and Pass 1988). Lactate also causes sequestration of large quantities of water in the rumen and can induce dehydration. Increased proportions of undigested feeds resulting from reduced microbial cellulolytic activity at a lower ruminal pH also contribute to diarrhoea (Bolton and Pass 1988). Notwithstanding this, faecal pH is poorly correlated with ruminal pH due to fermentation and buffering in the hind gut (Clayton *et al.* 1999) and should be interpreted with caution. Other differential diagnoses for scouring in this herd may include a high parasite burden, Salmonella, pestivirus or other bacterial or viral disease. Faecal samples were not taken for faecal egg counts, but the whole herd had been drenched regularly with compounds effective against intestinal parasites and liver fluke, although not within the preceding 12 months.

#### 7.4.6 *Milk components*

The bulk milk fat percentage was satisfactory for a Holstein-Friesian herd (fat 4.26%, protein 3.5%). Measurements were taken from the pooled milk from all herds and it is probable that the stale herd had a significantly higher milk fat percentage than the fresh herds. Unfortunately, individual herd tests were not conducted. Ruminal pH is positively associated with milk fat concentration (Kolver and de Veth 2002), therefore repeated assessment of bulk milk vat recordings of fat percentage as an indicator of acidosis in a herd may be useful. However, the relationship between ruminal pH and milk fat concentration is not absolute, being confounded by stage of lactation, dietary fat content, and body fat mobilisation (Westwood and Lean 2001).

#### 7.4.7 *Treatment*

Treatment was not undertaken in this herd, however, we have briefly reviewed actions that would be appropriate to the case. Treatment of clinical acidosis can be difficult depending on the severity of the case. Individual cattle can be treated successfully, however if a significant proportion of the herd is involved, treatment is often logistically difficult and prioritisation of cases based on severity, labour availability and expertise and value of the cattle is critical.

Treatment of mild cases of acidosis includes reducing the amount of grain fed and feeding hay to stimulate saliva flow. Additional therapy includes oral antacids such as magnesium hydroxide or sodium bicarbonate at 1 g/kg BW and oral electrolyte solutions, preferably containing additional sodium bicarbonate to treat metabolic acidosis (Underwood 1992). Severe cases should be treated by markedly reducing the amount of grain fed, giving IV fluids (for example hypertonic saline) and access to water or balanced electrolyte solutions not containing lactic acid. Oral antacids as described above may be used, however lavaging the rumen with a wide bore stomach tube in combination with transfaunation from a healthy animal is preferable (Underwood 1992). Antibiotics including penicillins, tylosin, potentiated sulphonamides and tetracycline should be given to reduce the risk of liver abscessation. Other supportive treatments include flunixin meglumine (1 mg/kg) for endotoxaemia, antihistamines to control histamine production, and calcium/magnesium solutions either IV or SC to counteract secondary metabolic disorders

(Underwood 1992). Thiamine (10 mg/kg) every 24 to 48 hours for up to three doses may also be helpful to prevent polioencephalomalacia (Underwood 1992). Cattle that are successfully treated in the acute stage often show secondary problems such as laminitis. In this herd, most cases requiring treatment would have responded to a combination of oral antacids, treatment with flunixin meglumine and antihistamines. A reduction in the amount of grain in the lactating cow diets and an increase in total feed including grain and silage in the transition diet was indicated.

#### 7.4.8 Prevention

Control of future outbreaks of acidosis in the herd could be achieved by review and modification of the diet. Adequate amounts of effective fibre (24% peNDF of the total diet) should be offered. Lucerne cubes, although a good quality fibre source, provide less effective fibre than lucerne hay due to short particle length. Type, amount and processing of carbohydrate sources should be reviewed. Some grain types are more likely to precipitate acidotic events than others (Opatpatanakit *et al.* 1994). Factors that increase risk include rapid rates of fermentation and excessive processing. Significant differences of *in vitro* fermentation rates between grain species ( $P < 0.001$ ) have been found with wheat > triticale, oats > barley > maize > rice, sorghum (Opatpatanakit *et al.* 1994). Fermentation rates can also be different within species with wheat varieties Halberd, Hartog and Eagle having the highest *in vitro* fermentation rates (Opatpatanakit *et al.* 1994). In this case preventive measures were not recommended due to trial protocol, however an increase in effective fibre and a reduction in NFC in the very fresh herd diet, coupled with an increase in the amount fed of the total transition diet, exclusive of feed additives, would have been recommended.

#### 7.5 Conclusions

Sub-clinical and clinical acidosis is a significant problem in the Australian dairy industry. The herd described in this paper was only one of many cases identified during a survey of dairy farms in southern Australia. Currently many herds are suffering acidosis at certain times of the year, particularly in winter/ spring, with the problem undiagnosed through lack of farmer awareness or veterinary input. In this case, clinical acidosis in the lactating herds, particularly the very fresh herd, probably

resulted from the dramatic change of diet from the transition herd to the lactating cow ration. This case provided an opportunity to relate the clinical signs of acidosis to detailed measures of rumen function. It also highlights the importance of assessing diets of all cattle on the farm rather than only the animals of concern.

## 7.6 References

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## Chapter 8.

# GENERAL CONCLUSIONS

## 8.1 Introduction

Rumen lactic acidosis is a clinical disorder of cattle that can result in rumenitis, metabolic acidosis, lameness, hepatic abscessation, pneumonia and death (Lean *et al.* 2000). Clinical and subclinical acidosis are recognised as problems in dairy cows fed large amounts of feeds rich in rapidly fermentable carbohydrates, that have been well documented and reviewed in the literature in the past (Dirksen 1970; Huber 1976; Nocek 1997; Owens *et al.* 1998; Slyter 1976; Underwood 1992). Sources of carbohydrates used to experimentally induce acidosis in cattle have usually been highly fermentable grains such as wheat. It is common practice in Australian dairy herds to combine feeding of good quality pasture, in an early vegetative state, with twice-daily concentrate supplementation in the bail. Because limited research has been conducted on acidosis in pasture fed cows, there is controversy about the prevalence and significance of acidosis in the Australian dairy industry (de Veth and Kolver 2001; Kolver and de Veth 2002; Wales *et al.* 2001). Small studies ( $n < 122$ ) have found that highly digestible pasture, when fed with concentrate, increases bacterial growth rates and production of acetate, propionate and butyrate (Kolver and de Veth 2002). Rumen pH subsequently decreases and is negatively associated with total VFA concentrations ( $P < 0.001$ ;  $R^2 = 0.3$ ) (Kolver and de Veth 2002). Some intensive studies in Australia have examined the effects of feeding of starch-based concentrates to cows on pasture (Clayton *et al.* 1999; Lean *et al.* 2000; Opatpatanakit *et al.* 1994; Wales *et al.* 2001). However, no extensive surveys of the industry have been conducted.

There is an increased awareness of the problem of acidosis in the Australian dairy industry with more dairy producers and support personnel recognising the potential for financial cost and illness that are associated with the disease. This project aimed to better define acidosis under Australian grazing conditions, identify both outcomes of the disease and risk factors leading to acidosis in herds and heighten awareness of the

problem in the dairy industry. The project involved 100 dairy farms from five prominent dairy areas of NSW and Victoria, and is perhaps one of the largest field studies of dairy nutrition undertaken in Australia. A survey of feeding practices and other relevant health disorders was conducted on each farm resulting in 100 herd evaluations with full nutritional analyses, including analysis of pasture grazed by the lactating herd on the sampling day. The lactating herds on each farm were examined, resulting in approximately 1500 cow evaluations for body condition score, locomotion, rumen fill and faecal characteristics. Eight lactating cows (< 100 DIM) had samples of rumen fluid, blood and faeces taken, resulting in approximately 800 cow evaluations of rumen fluid. The thesis has been divided into five sections that examine different aspects of the survey conducted and how each relates to the problem of acidosis in the Australian dairy industry. Data were examined both on a cow and herd level.

## 8.2 Feeding practices

The feeding practices of the study herds were summarised in Chapter 3. Although alternative feeding practices such as total mixed rations are becoming more prevalent, the industry is still pasture-based in Australia, given that 79% of herds fed pasture in combination with either grain or pellets twice daily at milking. Rumen buffers, modifiers and antibiotics were commonly used in the study herds, but often at lower than recommended rates for both control of acidosis and production benefits. The addition of monensin to the diet was a particularly prevalent practice; it was fed by 60% of study farms. However, all areas except Camden and Taree were feeding monensin at a mean rate of less than 250 mg/cow/day. Milk production responses indicate that the combined benefits of monensin fed at the label dose of 200 to 450 mg/cow/day are reflected in increased milk production, suggesting that benefits for many herds included in the study may be limited by underfeeding. Virginiamycin was also used by 24% of study herds at a mean rate of less than 250 mg/cow/day in all areas. The recommended feeding rate for this product is 0.5 mg/kg BW/day, or 300 mg/cow/day for a 600 kg cow (Thorniley *et al.* 1994). Interestingly, 100% of herds categorised as ACID fed monensin and 50% of herds categorised as ACID fed virginiamycin, a higher percentage than herd categories SO or NA. This may not only indicate that ACID herds were higher producing and more rapid adopters of

technology than herds in other categories, but also recognised acidosis as a problem. Notwithstanding this, the mean rate fed for both products to ACID herds was less than recommended rate and was associated with subclinical acidosis. The problems with supplement feeding and using correct dose rates in the industry at present, are highlighted in this chapter. There is a general lack of awareness of correct dose rates, the implications of under dosing, and, indeed what is contained in grain mixes, pellets and premixes, by both dairy producers and some support personnel in the industry.

### 8.3 Rumen fluid biochemistry

Rumen fluid biochemistry of cows sampled was related to feeding practices and milk production figures in Chapter 4. Cows were categorised using K-means cluster analysis into one of three categories after ascertaining that random effect of herd was not a significant factor using a Bayesian logistic regression model. This was an important result considering the inherent differences between herds including changes in seasons and weather conditions, genetic merit and management practices. Cluster analysis was an important feature in the categorisation of the herds, allowing cows to be grouped based on similarities in a number of biochemical parameters, rather than just rumen pH. Predictive variables used in the cluster analysis were converted to standardised variates before clusters were assessed. Variables used in cluster analysis to give category definition were based on VFAs, rumen pH, D-lactate and ammonia. Previously, rumen pH has been the focus of classifying a cow as acidotic or otherwise. However, rumen pH is known to fluctuate throughout the day and may not be the most appropriate tool for diagnosis of the disease. Rumen lactate concentrations have also been shown to rise appreciably in cows experimentally induced with acidosis. All variables in this study were significant in predicting category allocation ( $P < 0.01$ ). However, propionate ( $F = 332.38$ ) and valerate ( $F = 332.62$ ) were the most important factors in determining cluster categories. Interestingly, rumen pH and D-lactate were least important in prediction of the categories where  $F = 54.95$  and  $F = 5.02$ , respectively, indicating that future research may be better focused on propionate and valerate concentrations as an indication of acidosis, particularly for pasture-based diets. These statistical findings are consistent with the known biology. However, valerate is a product of lactate metabolism in the

rumen and pH is an outcome measure where the  $H^+$  concentration is influenced by VFA, ammonia and lactic acid concentrations.

Herds were also defined in one of three categories based on rumen biochemical values of the cows found in each herd. Where  $\frac{3}{8}$  cows from category 1 were found in a herd, the herd was classified as 'ACID',  $\frac{3}{8}$  cows from category 2 in a herd classified the herd as suboptimal rumen function 'SO' and  $\frac{3}{8}$  cows from category 3 in a herd classified the herd as normal function 'NA'. The number of herds used in categories ACID, SO and NA were 10, 35 and 69 herds respectively, with 20 herds meeting the criteria of 2 categories. Defining herds into one of three categories allowed valuable comparisons between cow level data, such as rumen function and milk production figures and herd level data, such as some outcome data and dietary risk factors.

#### 8.4 Prediction of rumen pH on pasture-based diets

The relationship of rumen pH, measured on rumenocentesis samples to other variables measured was assessed by linear regression including the random effect of herd using Genstat (version v.7.1.0.198). Although previous prediction of ruminal pH from pasture-based diets has been attempted by collating results from 23 small studies, large variation between studies meant future predictions may have been unreliable (Kolver and de Veth 2002). This is the first time that rumen pH has been able to be predicted from biochemical variables using a large data set (686 cows) on pasture-based diets. When individual covariates were assessed, propionic acid, isobutyric acid, butyric acid, caproic acid and D-lactate were significant ( $P < 0.05$ ) whilst acetic acid approached significance ( $P = 0.108$ ). The residual plot showed no patterns or outliers of concern and the residuals appeared to be approximately normally distributed. The estimated model was;

**Rumen pH = 6.226 ( $\pm 0.0278$ ) + 0.00378 ( $\pm 0.002350$ ) acetic acid – 0.0257 ( $\pm 0.002709$ ) propionic acid + 0.594 ( $\pm 0.09887$ ) isobutyric acid – 0.0359 ( $\pm 0.008228$ ) butyric acid + 0.153 ( $\pm 0.0683$ ) caproic acid + 0.0693 ( $\pm 0.02192$ ) D-lactate**

Therefore, rumen pH increased with increased concentrations of acetic acid, isobutyric acid, caproic acid and D-lactate in the rumen and with decreased concentrations of propionic acid and butyric acid.

L-lactate, assessed in 169 rumen samples was also included in the model with herd included as a random effect. The concentration of D- and L-lactate in the selected rumen samples was highly correlated ( $R = 0.905$ , Pearson's correlation). Propionic acid, butyric acid, isobutyric acid and D-lactate were highly significant ( $P < 0.01$ ) in the model whilst L-lactate was significant ( $P = 0.031$ ). The estimated model was;

**Rumen pH = 6.144 ( $\pm 0.0337$ ) - 0.03365 ( $\pm 0.003984$ ) propionic acid - 0.06082 ( $\pm 0.012023$ ) butyric acid + 1.694 ( $\pm 0.5494$ ) D-lactate - 1.422 ( $\pm 0.6609$ ) L-lactate + 1.143 ( $\pm 0.2013$ ) isobutyric acid**

Although both models are very similar with the sign of each variable the same and the magnitude of the coefficient of each variable differing little, the first model has considerably greater statistical power with 686 samples included compared with 169 samples.

## 8.5 Category 1 cows/ ACID herds

Rumen metabolism and milk production characteristics of cows in category 1 were consistent with an acidosis model reflecting a high rate of carbohydrate fermentation. Biochemically, cows in this category were characterised by low rumen pH, high total VFA concentration, low acetate to propionate ratio and low to normal rumen ammonia concentration. Interestingly, valerate concentration was significantly higher in cows in this category than the other two categories. Valerate, normally present in very low concentrations in the rumen is produced by *Megasphaera elsdenii* on high grain diets, a bacterial species that utilises 60 to 95% of the lactate available in the rumen. The significantly higher concentration of valerate in cluster category 1 cows may therefore be explained by the higher production of lactate in this category. In light of these results it is, therefore, probable that these cows do not fit the traditional concentrate-induced acidosis model where *Lactobacilli* are known to multiply quickly, producing large amounts of lactate. Instead, when concentrate is fed,

amylolytic bacteria such as *Selenomonas ruminantium* and *Streptococcus bovis* increase in growth rate due to their substrate preference for starch and produce lactate in addition to acetate, propionate and butyrate, lowering rumen pH.

Milk production was slightly higher, although not significant, but milk fat percentage and milk fat to protein ratio was lower for these cows (K-W One-way-ANOVA). This was explained on examination of the diet of herds where 3/8 cows were classified in category 1. These herds were classified as 'ACID' herds. Diets fed to ACID herds were significantly higher in NFC% and CHO B1% but lower in NDF% than for the other two categories. Risk factors were examined using multinomial LR (SPSS version 11.0 for windows). When examining risk factors for this category, ACID herds tended to be more likely to feed wheat and in greater amounts than NA herds. Legume-dominant pastures (clover, shaftal or lucerne) and lush pastures with minimal effective fibre (texture score 1) were significantly more prevalent in ACID herd than NA herd diets. However, using multivariate modelling, category ACID was best described simply by the NDF%: NFC% ratio; ACID herds were much more likely to have a lower NDF% to NFC% ratio than both NA herds and SO herds.

Chapter 5 examined possible outcomes of acidosis including lameness, scouring and poor rumen fill on a herd level using KW ANOVA and LR, and Statistix (version 7, NIH Software). District was included in models as a random effect. Results from this section of the study concluded that ACID herds had an 83% higher risk of lameness than SO herds and 118% higher risk of lameness than NA herds. While the relationship between acidosis and lameness is well recognised, this is the first study to quantitatively demonstrate this association in pasture fed herds. ACID herds also had a 75% higher risk of low rumen fill scores than SO herds and 18% higher risk than NA herds using LR and district as a random effect. The relationship between scour scores and herds categorised as ACID was influenced by other variables, particularly proportion of pasture in the diet. Using LR with district as a random effect, herds with a high prevalence of high scour scores tended to be more likely to be in category ACID than SO ( $P = 0.058$ ). However, pasture % in the diet ( $P = 0.001$ ) was also important in the model, highlighting the importance of pasture feeding on scour score. The risk of an increased prevalence of high scour scores increased by 5.5% per percentage increase in pasture.

## 8.6 Category 2 cows/ SO herds

Category 2 cows were characterised biochemically by an adequate rumen pH and total VFA concentration but significantly higher rumen ammonia concentration than other categories. Propionate concentrations were also significantly lower in this category compared with category 1, resulting in a higher acetate to propionate ratio. The low propionate concentration probably resulted in lower glucose availability, and consequently lower milk production than category 1 or 3 cows. Milk fat percentage in this category was significantly higher than cows in categories 1 and 3. The high rumen ammonia concentration may indicate a lack of assimilation into microbial protein, possibly reflecting asynchronous availability of nutrients for bacterial growth and a failure of nitrogen to energy matching in the rumen. Diets of category 2 cows were adequate in CP%, ME (MJ/kg DM), NFC% and NDF% for an early lactation dairy cow, and were not significantly different to category 3 cows. Therefore, although adequate on a daily basis diets may be imbalanced in supply of fermentable carbohydrate and nitrogen supplies on an hourly basis (Shabi *et al.* 1998). On examination of dietary risk factors (Chapter 6), SO herds were less likely to be grazing ryegrass dominant pastures than NA herds, tending to graze ryegrass: clover 50:50 pastures or legume dominant pastures over NA herds. Both clover dominant and clover: ryegrass 50:50 pastures in this study contained lower NDF% and higher NFC% than ryegrass dominant pasture (Chapter 3). SO herds also tended to feed more fibrous by-product and straw than NA herds. This may indicate that fibre fed in SO herds was less digestible and not capable of supporting similar milk production to NA herds.

## 8.7 Category 3 cows/ NA herds

Some of the mean rumen measures, for example low total VFA concentrations, for category 3 indicated that cows in this category may have had sub-optimal rumen function. However, mean milk production figures for cows in this category were significantly higher than category 2, and mean milk fat: protein ratio not significantly different. Although the overall dietary variables between SO and NA herds were similar, these cows may have a more synchronous diet presented throughout the 24-hour period, allowing greater production. The lower total VFA concentration in this

category may indicate a slower rumen fermentation reflecting substrates available compared with rapid rumen fermentation in category 1 cows. It is possible that there was a more efficient production of rumen bacteria, rather than VFA for cows in this group. It appears likely, that there is even greater production potential in these cows given a change of diet.

Support for this hypothesis is provided by results discussed in Chapter 5. Herds with a high prevalence of low rumen scores were 57% more likely to be category NA than SO herds when the random effect of district was evaluated in the model predicting rumen fill score. Herds with a high prevalence of high scour scores were 369% more likely to be in category NA than SO with random effect of district in the model. This also suggests that the fibre fed to NA herds was more digestible than that fed to SO herds. SO herds tended to feed greater quantities of both fibrous by-products ( $P = 0.159$ ) and straw ( $P = 0.144$ ) than NA herds.

The rumen biochemistry and production differences between category 2 and 3 cows, although partially explained by the outcome and dietary risk factor results need further definition, particularly because 30% of cows from this survey were found in category two which appears to reflect sub-optimal rumen function.

## 8.8 Scoring systems

Several new scoring systems were used in the survey. These were rumen fill score, faecal staining score and evaluation of pasture texture. While these scoring systems need further validation, it is apparent that rumen fill score and pasture texture score can be related to physical outcomes of acidosis. Both scoring systems are based on a 1 to 5 scale and are a quick and easy assessment for both veterinarians, advisors and farmers to use in the field. Rumen fill was scored by examining each cow from directly behind and comparing the left para-lumbar fossa (rumen fill) to the right para-lumbar fossa using a continuous scale where 1 = flat and 5 was a fully distended rumen. Pasture texture was scored using freeze dried pasture samples where 1 = very soft grasses such as clovers and legumes with minimal evident fibre 2 = very soft ryegrass, 3 = firmer ryegrass/ lush kikuyu/ fresh oat/ fescue, 4 = mature oat/ mature

fescue/ phalaris/ kikuyu and those scored 5 included coarse forages such as maize and forage sorghum, high in fibre.

### **8.9 Clinical acidosis case study**

Also included in the thesis was a case study of clinical acidosis from one severely affected herd in the study (Chapter 7). This case study showed clearly the production and health related issues acidosis can cause both on a cow and herd level. It also showed that acidosis can be a significant problem in Australian dairy herds. Physical outcomes were confirmed by rumen biochemistry and related back to dietary formulation and ration delivery failures. Suggestions for treatment, control and prevention of the disease were highlighted.

### **8.10 Future directions**

Further validation of the practical scoring systems outlined in section 8.8 for rumen fill and pasture texture evaluation is warranted. Both of these scoring systems are likely to be useful tools in herd assessment for veterinarians, advisors and farmers to use in the field. Nutritional asynchrony was suggested as a possible causal factor for the rumen and production results seen in herd category SO. It would be worth using the extensive data collected to attempt to back-validate this theory. The results from the case study could also be cross validated with the results summarised in chapters 4, 5 and 6. Another very useful practical outcome that could be further investigated would be the determination of a suggested target for NDF : NFC ratio. Rumen fluid samples from selected cows in the study are being analysed using real-time PCR techniques to define rumen bacterial populations as an extension project. The extraction of bacterial DNA from rumen fluid is difficult and needs further development. Once real-time PCR has been successfully used to further define rumen bacterial populations on specific diets, further DNA studies aimed at identifying or modifying existing bacterial species to specifically mop up lactate may be useful. Controlled trials examining the effects of 100% pasture feeding using various varieties of annual ryegrass and clover may also be helpful in the selection of which varieties to grow for maximum prevention of acidosis.

### 8.11 Summary

In summary, the survey presented in this thesis provides a unique examination of acidosis in 100 Australian dairy herds and 797 dairy cows, the majority of which were fed diets predominantly pasture-based. Three distinct categories of cows were identified, with 10% showing signs of acidosis, 30% showing sub-optimal production and 60% categorised as not acidotic with better production figures. Production outcomes and health-related issues associated with acidosis were confirmed and risk factors associated with being in each of the three categories identified. New scoring systems were used, although further validation of these is warranted. Importantly, these results have given the dairy industry a better understanding of acidosis on pasture-based systems where previous research has been lacking. Both dairy support personnel, veterinarians and farmers need to begin looking at a whole herd approach to acidosis; both in identifying the problem as well as treatment, control and prevention of the disease.

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## APPENDIX

### Form 1. Questionnaire for farmers

FARM..... DISTRICT..... DATE.....

### QUESTIONNAIRE

#### *PART 1 – FARMER DEMOGRAPHICS*

1. How long have you been associated with dairying? .....years
2. How long have you been on this property? .....years
3. How old were you at your last birthday? .....
4. Where do you obtain your dairy cattle nutrition information from?  
(rank 4 most important to you in order (1-4))
  - i) journals
  - ii) newspapers
  - iii) radio/TV
  - iv) vet
  - v) nutritional advisor
  - vi) other dairy people
  - vii) other

#### *PART 2 – FARM EVALUATION*

1. What is the total size of the home farm? ..... hectares/ acres
2. What is the total size of the milking area (immediately accessible)?.....hectares/acres
3. How much of this land comes under the following categories?
  - i) improved pasture ..... hectares / acres
  - ii) irrigated pasture ..... hectares / acres
  - iii) fodder crops (for example lucerne) ..... hectares / acres
4. How many dairy cattle in total are you running in the herd?  
(include dry, lactating and transition cows).....

5. How many of the dairy herd are in each of the following categories?
- i) lactating cows .....
  - ii) dry cows .....
  - iii) transition cows.....
6. How much milk did the herd produce yesterday? .....litres/ gallons
- Does this include calf milk?                      Y/N
- If 'no', how much calf milk did the herd produce yesterday? .....litres/ gallons
7. What was the somatic cell count on the last factory test? .....
8. At the last factory test, what was the percentage fat .....%
- percentage protein.....%

*PART 3 – NUTRITION*

1. Who are responsible for making major nutritional decisions on the farm? (circle the most appropriate)
- i) Owner/ manager
  - ii) Nutritional advisor
  - iii) Herdsman
  - iv) Share-farmer
2. Do you employ a nutritionist/ nutrition company to give advice on nutritional practices on farm?                      Y/N
- If 'yes', who .....
3. Do you feed concentrate (grain and byproducts) to your animals at any stage?                      Y/N
- If 'yes', which animals do you feed concentrate to? (tick as appropriate)
- i) lactating cows .....
  - ii) dry stock .....
  - iii) springers .....
  - iv) calves .....

*PASTURES*

4. What was the percentage of farm renovated (totally worked up) this year?.....%
5. How much land was re-sown this year? ..... hectares / acres
6. Did you plant any ryegrass varieties in the last 12 months? Y/N

If 'yes', what varieties were planted?.....

Which cows grazed the new plantings of ryegrass? (tick as appropriate)

Lactating cows..... Transition cows..... Dry cows.....

7. What are the approximate areas of new plantings for each variety of ryegrass?
- variety/s.....area planted .....hectares / acres
- variety/s.....area planted .....hectares / acres
- variety/s .....area planted .....hectares / acres

8. What fertiliser was used on this new pasture in the last 4 months?
- .....

9. Is this normal practice for established pasture on the farm? Y/N

If 'no', what fertilizer, (P,N,K,S) was used on existing pasture in the last 4 months?

.....

What rates were the fertilisers applied?

fertiliser..... date applied..... amount.....area.....(ha/a)

fertiliser..... date applied..... amount.....area.....(ha/a)

fertiliser..... date applied ..... amount.....area.....(ha/a)

**Form 2. Collection on farm**

**FARM** ..... **DISTRICT**..... **DATE**.....

**WEATHER CONDITIONS**

1. Is the weather sunny..... overcast/ no rain..... raining lightly.....  
raining heavily.....
2. Maximum temperature today.....(degrees celcius)

**COWS**

1. Average condition score estimate of lactating herd.....  
transition herd .....(record on scoring sheet)
2. Breed of cows .....(estimate % herd)

**LACTATING COWS**

1. Amount fed to each cow in bail (per day)..... (kg)  
(check by weighing)
2. Total amount fed to herd outside (per day) (not including pasture)..... (kg)
3. Feeding system: bail..... outside.... mixer wagon.... other (describe).....

**CONCENTRATES**

1. Type of concentrate:
  - i) Full Pellet (tick)..... Source.....  
Amount total/day..... (kg) Amount/cow/day .....(kg)
  - ii) Grain (tick)..... Whole ..... Crushed ..... Other (inc soda).....  
Type: Amount/cow/day..... Amount total/day.....
    - a) Wheat..... Amount.....(kg)
    - b) Barley..... Amount.....(kg)
    - c) Oats..... Amount.....(kg)
    - d) Triticale..... Amount.....(kg)
    - e) Sorghum..... Amount.....(kg)
    - f) mix - Name.....  
- Amount .....(kg)  
- Percentage breakdown.....  
.....

- iii) By Products (tick as appropriate) (per cow/day) (total per day)
  - a) Brewers grain..... Amount.....(kg)
  - b) Corn gluten feed ..... Amount.....(kg)
  - c) Mill run..... Amount.....(kg)
  - d) Whole cotton seed ..... Amount.....(kg)
  - e) Other ..... Amount.....(kg)
  - f) Other..... Amount.....(kg)
  
- iv) Protein meals (tick as appropriate) (per cow/day) (total per day)
  - a) Canola..... Amount..... (kg)
  - b) Lupins..... Amount.....(kg)
  - c) Cotton seed meal..... Amount.....(kg)
  - d) Other..... Amount.....(kg)

2. Trace element inclusions

- i) Boluses ..... type .....
- ii) In Company Feed Name of product.....  
 Trace elements included and amount.....(per cow/day) (total per day)
  - Name.....Amount.....
  - Name.....Amount.....
  - Name.....Amount.....
  - Name.....Amount.....
  - Name.....Amount.....
  
- iii) Pre-mix (specify) .....  
 Trace elements included and amount.....(per cow/day) (total per day)
  - Name.....Amount.....
  - Name.....Amount.....
  - Name.....Amount.....
  - Name.....Amount.....
  - Name.....Amount.....
  
- iv) Lick blocks (specify).....

3. Buffer Inclusions (per cow/day) (total per day)

- i) MgO (causmag) ..... Amount.....
- ii) Limestone ..... Amount.....
- iii) Bentonite ..... Amount.....
- iv) Sodium bicarbonate ..... Amount.....

4. Rumen Modifier Inclusions (per cow/day) (total per day)

- i) Rumensin ..... Amount.....
- ii) Other Monensin ..... Amount.....

- |      |               |             |
|------|---------------|-------------|
| iii) | Bovatec ..... | Amount..... |
| iv)  | Eskalin ..... | Amount..... |
| v)   | Tylan .....   | Amount..... |

### OTHER FEEDS

#### 1. Silage

- |      |   |                            |
|------|---|----------------------------|
| i)   | Type: grass..... maize..... lucerne ..... | sub clover..... other..... |
| ii)  | Quality.....                              | .....                      |
| iii) | Total amount fed to cows today.....       | (kg)                       |
| iv)  | Total amount wasted by cows today.....    | (kg)                       |
| v)   | Estimated total amount eaten today .....  | (kg)                       |

#### 2. Hay

- |      |   |                     |
|------|---|---------------------|
| i)   | Type: grass..... lucerne..... sub clover..... | oat..... other..... |
| ii)  | Quality.....                                  | .....               |
| iii) | Total amount fed to cows today.....           | (kg)                |
| iv)  | Total amount wasted by cows today.....        | (kg)                |
| v)   | Estimated total amount eaten today .....      | (kg)                |

#### 3. Straw

- |      |  |       |
|------|--|-------|
| i)   | Type: oat ... other.....                 |       |
| ii)  | Quality.....                             | ..... |
| iii) | Total amount fed to cows today.....      | (kg)  |
| iv)  | Total amount wasted by cows today.....   | (kg)  |
| v)   | Estimated total amount eaten today ..... | (kg)  |

#### 4. Other feeds- .....

### PASTURE

#### 1. Type of pasture predominantly grazed .....

#### 2. Type of pasture the cows are grazing today

- |      |                  |                 |
|------|------------------|-----------------|
| i)   | Ryegrass .....   | cultivar/s..... |
| ii)  | Fescue .....     | cultivar/s..... |
| iii) | Sub-Clover ..... | cultivar/s..... |
| iv)  | Other .....      | cultivar/s..... |

#### 3. Type of pasture the cows grazed yesterday

- |      |                  |                 |
|------|------------------|-----------------|
| i)   | Ryegrass .....   | cultivar/s..... |
| ii)  | Fescue .....     | cultivar/s..... |
| iii) | sub-clover ..... | cultivar/s..... |
| iv)  | other .....      | cultivar/s..... |

4. Height pasture is grazed to today (cm) .....
5. Maturity of pasture grazed today (leaf stage) .....
6. Density of pasture (1-5 where 1= low, 5=high).....
7. Size of paddock grazed today .....(acres)
8. Pasture sample taken (tick)..... time taken .....

### **TRANSITION COWS**

1. Amount fed to each cow in bail (per day).....(kg)
2. Total amount fed to herd outside per day (not including pasture).....(kg)
3. Feeding system: bail..... outside..... mixer wagon..... other (describe).....

### **CONCENTRATES**

1. Type of concentrate:
  - i) Full Pellet (tick)..... Source .....
  - Amount total /day..... (kg) Amount/cow/day.....(kg)
  - ii) Grain (tick)..... Whole ..... Crushed .....Other (inc soda).....
  - Type: Amount/cow/day..... Amount total/day.....
  - a) Wheat..... Amount.....(kg)
  - b) Barley..... Amount.....(kg)
  - c) Oats..... Amount.....(kg)
  - d) Triticale..... Amount.....(kg)
  - e) Sorghum..... Amount.....(kg)
  - f) mix - Name.....
  - Amount .....(kg)
  - Percentage breakdown.....
  - .....
  - iii) By Products (tick as appropriate) (per cow/day) (total per day)
  - a) Brewers grain..... Amount.....(kg)
  - b) Corn gluten feed ..... Amount .....(kg)
  - c) Mill run..... Amount .....(kg)
  - d) Whole cotton seed ..... Amount .....(kg)
  - e) Other ..... Amount .....(kg)
  - f) Other..... Amount .....(kg)

- iv) Protein meals (tick as appropriate)(per cow/day) (total per day)
- |                          |                  |
|--------------------------|------------------|
| a) Canola.....           | Amount..... (kg) |
| b) Lupins.....           | Amount.....(kg)  |
| c) Cotton seed meal..... | Amount.....(kg)  |
| d) Other.....            | Amount.....(kg)  |

4. Anionic feeds/ salts etc (per cow/day) (total per day)
- |  |             |
|--|-------------|
| i) Anionic salts (type).....                         | Amount..... |
| ii) Anionic feed (biochlor, kickstart, transit)..... | Amount..... |
| iii) Rumensin.....                                   | Amount..... |
| iv) Bovatec.....                                     | Amount..... |
| v) Eskalin.....                                      | Amount..... |
| vi) Tylan.....                                       | Amount..... |

#### OTHER FEEDS

5. Silage
- |  |      |
|--|------|
| i) Type: grass..... maize..... lucerne..... sub clover..... other..... |      |
| ii) Quality.....   |      |
| iii) Total amount fed to cows today.....                               | (kg) |
| iv) Total amount wasted by cows today.....                             | (kg) |
| v) Estimated amount eaten total today.....                             | (kg) |
6. Hay
- |  |      |
|--|------|
| i) Type: grass.....lucerne.....sub clover..... oat..... other..... |      |
| ii) Quality.....   |      |
| iii) Total amount fed to cows today.....                           | (kg) |
| iv) Total amount wasted by cows today.....                         | (kg) |
| v) Estimated amount eaten total today.....                         | (kg) |
7. Straw
- |  |      |
|--|------|
| i) Type: oat ... other.....                |      |
| ii) Quality.....                           |      |
| iii) Total amount fed to cows today.....   | (kg) |
| iv) Total amount wasted by cows today..... | (kg) |
| v) Estimated amount eaten total today..... | (kg) |
8. Other feeds- .....

#### PASTURE

1. Type of pasture predominantly grazed .....

## 2. Type of pasture the cows are grazing today

- i) Ryegrass ..... cultivar/s.....
- ii) Fescue ..... cultivar/s.....
- iii) Sub-Clover ..... cultivar/s.....
- iv) Other ..... cultivar/s.....

## 4. Type of pasture the cows grazed yesterday

- i) Ryegrass ..... cultivar/s.....
- ii) Fescue ..... cultivar/s.....
- iii) Sub-clover ..... cultivar/s.....
- iv) Other ..... cultivar/s.....

5. Height pasture is grazed to today (cm) .....

6. Density of pasture (1-5) where 1=low and 5=high .....

7. Maturity of pasture grazed today (leaf stage) .....

8. Size of paddock grazed today .....

## CATTLE HEALTH

1. Is this a fluke area ? Y/N

If 'yes', treatment with flukicide Y/N  
 Type..... How often.....

2. Drenching history (parasite treatment regime)

i) Are the dry cows drenched Y/N how often.....  
 Date last dose.....type.....

ii) Are the lactating cows drenched Y/N how often.....  
 Date last dose.....type.....

3. Estimated score of faecal staining on cows in lactating herd (circle)

- 1- All cows clean around tail
- 2- Mild staining of switch/ tail 15-20%
- 3- Marked faecal staining, some explosive scours, <50% affected
- 4- High prevalence of severe scours
- 5- >80% showing evidence of scouring

- 4. Faecal score of cow dung in paddock of lactating herd and transition herd (record on sheets) ..... (tick)
- 5. Rumen score 1-5 for lactating herd and transition herd (record on sheets) .....(tick)
- 1- flat
- 5- fully distended

LAMENESS EXAMINATION

- 1. Locomotion scoring - use Zinpro scoring chart to score lactating and transition herds (record on sheets) .....
- 2. Condition score of lane-way to dairy
  - i) dangerous and sharp Y/N
  - ii) rocky/ muddy Y/N
  - iii) smooth – some stone Y/N
  - iv) top quality Y/N
- 3. Condition of holding yard at dairy
  - i) smooth concrete Y/N
  - ii) concrete with criss-cross grooves Y/N
  - iii) concrete with parallel lines Y/N
  - iv) other ..... Y/N
- 4. Maximum time held in holding yard of dairy (approximate) on day (per milking in mins) .....
- 5. Use of backing gate Y/N
 

If yes, are there use of bells .....or electric wires?.....
- 6. Is the farmer/ herd manager impatient when bringing the cows in for milking (Are the cows at the back of the herd pushed excessively up the laneways)? Y/N
- 7. Is a foot bath used ? Y/N
 

If yes, what is used in it?.....How often is it used?.....
- 8. Is a dog used on the track? Always..... Sometimes..... Never .....
- 9. Is a dog used in the shed? Always.....Sometimes..... Never.....
- 10. If the herd contains Friesians, what is the approximate percentage of Friesians with white feet?.....





## DEFINITIONS OF SCORING SYSTEMS USED

### Condition score

Use chart 'Body Condition Scoring Dairy Cattle' (Edmondson et al, 1989) and score cattle on a 1.00 – 5.00 scale

Where herd size <50, score 15 even (ID) numbered cows.

Where herd size 51-100, score 15 cows with ID numbers divisible by 3.

Where herd size >100, score 15 cows with numbers divisible by 5.

### Faecal score

Score 15 faeces from lactating cow paddock and transition cow paddock on a 1-5 scale

- 1- No formation of cowpat on ground, scouring on ground as cow walks.
- 2- Minimal formation of cowpat on ground.
- 3- Softer, less formed and evidence of more liquid.
- 4- Less formed than (5) but still holding shape, may contain whole grain.
- 5- Firm cow pat, well formed, no evidence of excessive liquid component.

### Rumen score

Score the same 15 cows that were condition scored for rumen fill on a 1-5 scale.

- 1- flat
- 5- fully distended

### Locomotion score (lactating cows only)

Score the same 15 cows that were condition scored for locomotion, based on 'Locomotion Scoring of Dairy Cattle' (adapted from Sprecher *et al* (1997)) using the 1-5 scale.



































**Table 1.** Essential and Non-essential Amino acids.

<i>Essential amino acids</i>	<i>Non-essential amino acids</i>
Histadine	Alanine
Isoleucine	Glutarate
Leucine	Aspartate
Lysine	Glutathione
Methionine	Asparagine
Phenylalanine	Serine
Threonine	Glycine
Tryptophan	Arginine
Valine	Proline
	Tyrosine
	Cysteine

**Reference**

Gilbert, HF (1992). *Basic Concepts in Biochemistry. A Student's Survival Guide.* McGraw-Hill Inc., New York.

Table 2. Body condition scoring chart (Edmondson *et al*, 1989)

	1	2	3	4	5	6	7	8
SEVERE UNDERCONDITIONING (emaciated)	<p>SCORING PROCESSES (SP) (anatomy varies)</p> <p>individual processes distinct giving a saw tooth appearance</p> 	<p>SCORING PROCESSES (SP)</p> <p>deep depression</p> 	<p>TRANSVERSE PROCESSES</p> <p>very prominent, &gt; 1/2 length visible</p> 	<p>OVERHANGING SHELL (scapula ribs)</p> <p>distinct shell, gullit, tucked</p> 	<p>TUBER COXA (hocks) &amp; TUBER SACR (pans)</p> <p>extremely sharp, no tissue cover</p> 	<p>BETWEEN THE HOOKS</p> <p>severe depression, narrow of flesh</p> 	<p>TAILHEAD TO PINS (anatomy varies)</p> <p>bones very prominent with deep V-shaped cavity formed under tail</p> 	
FRAME OBVIOUS	<p>SCORING PROCESSES (SP)</p> <p>individual processes evident</p> 	<p>SCORING PROCESSES (SP)</p> <p>obvious depression</p> 	<p>TRANSVERSE PROCESSES</p> <p>1/2 length of process visible</p> 	<p>OVERHANGING SHELL (scapula ribs)</p> <p>prominent shell</p> 	<p>TUBER COXA (hocks) &amp; TUBER SACR (pans)</p> <p>pointed</p> 	<p>BETWEEN THE HOOKS</p> <p>very shallow</p> 	<p>TAILHEAD TO PINS (anatomy varies)</p> <p>bones prominent U-shaped cavity formed under tail</p> 	
FRAME & COVERINGS WELL BALANCED	<p>SCORING PROCESSES (SP)</p> <p>sharp, prominent ridge</p> 	<p>SCORING PROCESSES (SP)</p> <p>smooth concave curve</p> 	<p>TRANSVERSE PROCESSES</p> <p>1/3-1/4 visible</p> 	<p>OVERHANGING SHELL (scapula ribs)</p> <p>moderate shell</p> 	<p>TUBER COXA (hocks) &amp; TUBER SACR (pans)</p> <p>smooth</p> 	<p>BETWEEN THE HOOKS</p> <p>shallow depression</p> 	<p>TAILHEAD TO PINS (anatomy varies)</p> <p>bones smooth, cavity under tail shallow &amp; fatty tissue filled</p> 	
FRAME NOT AS VISIBLE AS COVERINGS	<p>SCORING PROCESSES (SP)</p> <p>smooth ridge, the SP's not evident</p> 	<p>SCORING PROCESSES (SP)</p> <p>smooth slope</p> 	<p>TRANSVERSE PROCESSES</p> <p>square, smooth, IP's not discernible</p> 	<p>OVERHANGING SHELL (scapula ribs)</p> <p>slight shell</p> 	<p>TUBER COXA (hocks) &amp; TUBER SACR (pans)</p> <p>covered</p> 	<p>BETWEEN THE HOOKS</p> <p>slight depression</p> 	<p>TAILHEAD TO PINS (anatomy varies)</p> <p>bones rounded with fat and slight shallow depression under tail</p> 	
SEVERE OVERCONDITIONING	<p>SCORING PROCESSES (SP)</p> <p>blunt or fat</p> 	<p>SCORING PROCESSES (SP)</p> <p>rounded (convex)</p>	<p>TRANSVERSE PROCESSES</p> <p>slight barely discernible</p> 	<p>OVERHANGING SHELL (scapula ribs)</p> <p>none</p> 	<p>TUBER COXA (hocks) &amp; TUBER SACR (pans)</p> <p>pointed with fat</p> 	<p>BETWEEN THE HOOKS</p> <p>rounded</p> 	<p>TAILHEAD TO PINS (anatomy varies)</p> <p>bones buried in fat cavity filled with fat forming tissue folds</p> 	

This chart was developed by A. J. Edmondson, L. A. Lewis, L. D. Meeker, T. Farver, and S. Webster. It is reproduced courtesy of the Journal of Dairy Science.





Table 3. Locomotion scoring chart (adapted from Sprecher *et al*, (1997))

# Locomotion Scoring of Dairy Cattle\*


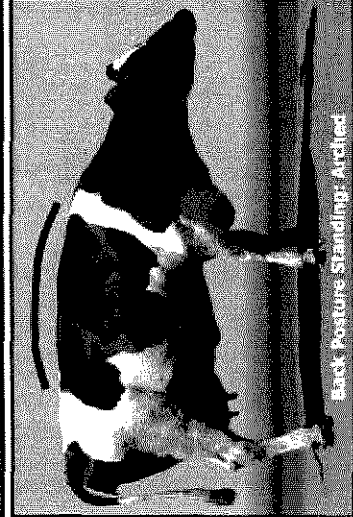


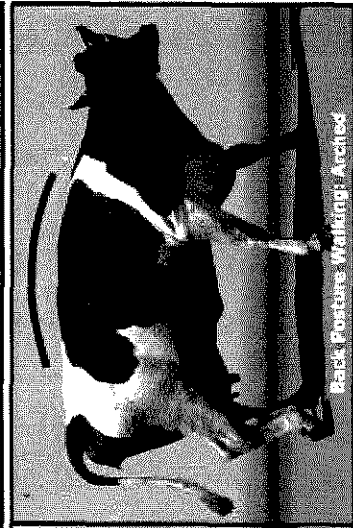

**Locomotion scoring** is based on the observation of cows standing and walking (gait), with special emphasis on their back posture. This system is intuitive and, therefore, easy to learn and implement. Use of locomotion scoring is effective for early detection of claw (hoof) disorders, monitoring prevalence of lameness (comparing the incidence and severity of lameness between herds and identifying individual cows for functional claw (hoof) trimming)



\* Adapted from Sprecher, G.J., Reisterer, D.E., Karsone, J.B. 1987. *Theriogenology* 47: 1178-1187.

<p><b>Locomotion Score 1</b></p> <p><b>Clinical Description:</b> <b>Normal</b></p> <p><b>Observation:</b> Stands and walks normally. All feet placed with purpose.</p>	 <p>Back Posture Standing: Flat</p>	 <p>Back Posture Walking: Flat</p>
<p><b>Locomotion Score 2</b></p> <p><b>Clinical Description:</b> <b>Mildly Lamé</b></p> <p><b>Description:</b> Stands with flat back, but arches when walks. Gait is slightly abnormal.</p>	 <p>Back Posture Standing: Flat</p>	 <p>Back Posture Walking: Mild</p>

Animal observations should be made on a flat surface that provides good footing for cows. Cows scoring 2 or 3 should be examined and treated to prevent more serious problems. Trimming should be done by a competent person with the goal of returning the claws to functional weight bearing and conformation.

<p><b>3</b></p> <p>Locomotion Score</p> <p>Clinical Description: <b>Moderately Lame</b></p> <p>Description: Stands and walks with an arched back. Short strides with one or more legs.</p>	 <p>Back Posture Standing: Arched</p>	
<p><b>4</b></p> <p>Locomotion Score</p> <p>Clinical Description: <b>Lame</b></p> <p>Description: Arched back standing and walking. One or more limbs favored but at least partially weight bearing.</p>	 <p>Back Posture Standing: Arched</p>	
<p><b>5</b></p> <p>Locomotion Score</p> <p>Clinical Description: <b>Severely Lame</b></p> <p>Description: Arched back, refuses to bear weight on one limb. May refuse or have great difficulty moving from lying position.</p>	 <p>Back Posture Standing: Arched</p>	
 <p>Back Posture Walking: Arched</p>	 <p>Back Posture Walking: Arched</p>	 <p>Back Posture Walking: Arched</p>

## **PAPERS AND CONFERENCE PRESENTATIONS**

### **Journal papers published**

1. Bramley E, Lean IJ, Fulkerson WJ, Costa ND. Clinical acidosis in a Gippsland dairy herd. *Australian Veterinary Journal* 83; 347 – 352.
2. Bramley E, Lean IJ, Fulkerson WJ, Costa ND. (2005). Acidosis in dairy cows. In. Combined ADSA-JAS Conference proceedings, p 433.
3. Bramley E, Lean IJ, Fulkerson WJ, Costa ND. (2005). Acidosis in pasture-fed dairy cows: Risk factors and outcomes. In. Combined ADSA-JAS Conference proceedings, p 103.

### **Journal papers submitted**

1. Bramley E, Fulkerson WJ, Lean IJ, Costa ND. Feeding practices on one hundred dairy farms in New South Wales and Victoria. *Australian Journal of Experimental Agriculture*.
2. Bramley E, Costa ND, Lean IJ, Fulkerson WJ. Acidosis and lameness in Australian dairy herds. *Australian Veterinary Journal*.

### **Conference proceedings published**

1. Bramley E, Lean IJ, Fulkerson WJ, Costa ND. (2002) The effects of acidosis on cattle health and production. *Current topics in Dairy Production*, University Printing Service, Sydney, 7; 163-164.
2. Bramley E, Lean IJ, Fulkerson WJ, Costa ND. (2003) Case study: Clinical acidosis in a Gippsland dairy herd. *Current topics in Dairy Production*, University Printing Service, Sydney, 8; 108-110.

3. Bramley E, Lean IJ, Costa ND, Fulkerson WJ. (2003) Ruminant acidosis in dairy cattle. In 'Australian and New Zealand Combined Dairy Veterinarians' Conference'. Taupo. (Ed. TJ Parkinson). Pp. 465-495. (Foundation for Continuing Education of the New Zealand Veterinary Association)
4. Bramley E, Lean IJ, Fulkerson WJ, Costa ND. (2004) Acidosis and lameness in dairy herds in New South Wales and Victoria. *Current topics in Dairy Production*, University Printing Service, Sydney, 9; 72-75.

### **Conference presentations**

1. Dairy Research Foundation Conference 2002, Camden, Australia.
2. Dairy Research Foundation Conference 2003, Camden, Australia.
3. Australian and New Zealand Combined Dairy Veterinarians' Conference 2003, Taupo, New Zealand.