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Effect of sodium bicarbonate and calcium magnesium carbonate supplementation on milk production of high producing Holstein cows

by

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Declaration

I hereby declare that this thesis, submitted for the MSc (Agric) Animal Science: Nutrition Science degree at the University of Pretoria, is my own work, and has not previously been submitted by me for a degree at any other University.

R.E. Rauch

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Summary

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Sodium discharge from dairies in California has been identified as an important contributor to soil and water pollution. The Waste Discharge Requirements General Order For Existing Milk Cow Dairies (2007) aims to minimize the amount of fixed solids, including Na, that are discharged from dairies, aiming to maximise the useable lifespan of water resources. As sodium bicarbonate (SB) contains 270 g/kg Na, SB supplementation can substantially increase Na discharge from dairies. The aim of this study was to determine the effects of SB and a potentially alternative buffer that does not contribute to Na discharge and related negative impacts on soil and water quality, (i.e., calcium magnesium carbonate (CMC)), on the performance of high producing California dairy cows. This could help establish if CMC could substitute for SB while maintaining potential benefits of SB.

It is well known that SB is a rumen buffer, but research indicates that its benefits are limited mainly to corn silage-based diets. Californian dairies use a wide range of forages, and tend not to base their diets solely or mainly on corn silage. Therefore, typical Californian lactation diets do not always conform to those reported in research publications involving SB. In addition, research parameters

such as milk yield, DM intake and dietary ADF often differ substantially between reported studies and conditions present on commercial California dairies, and results are therefore not always practically applicable. Apart from its rumen buffering capacity, SB also has an influence on dietary cation anion difference (DCAD), and can therefore elicit a response via a change in blood acid base balance of cows.

The experiment was a Latin square design with 3 treatments (i.e., control (C), SB and CMC), 3 pens of ~310 early lactation cows each, and 3 periods of 28 d. Sodium bicarbonate supplemented cows had elevated milk fat proportion, but a reduced milk yield, resulting in similar milk fat yield between SB supplemented and C cows. Based on a tendency for elevated faecal and *in vitro* rumen fluid pH, SB had a buffering effect on the gastrointestinal tract GIT, most likely in the rumen. However, it is likely that the difference in DCAD between the C and SB diets played a role in affecting milk yield and milk fat proportion, and a high intake of Na may have been the cause for a reduction in milk yield resulting in passive increase in milk fat proportion. There were no differences between C and CMC treatments, except for an elevated faecal pH of CMC cows. As CMC is not generally soluble at normal rumen pH, buffering likely occurred in the abomasum and small and large intestines. However, a lack of difference in productivity indicates that the buffering effect on the hindgut was not physiologically required.

While there were no productive benefits of SB use, it likely substantially increased Na discharge, resulting in an increase in soil and water sodicity and the associated deterioration in soil and water quality. While CMC did not improve productivity or efficiency of cows, it also did not increase Na discharge from the dairy and therefore did not contribute to soil or water sodicity. It can be concluded that SB or CMC supplementation is not advisable for diets and conditions comparable to those present in this study, i.e., high producing dairy cows fed a diet with ‘normal’ aNDF levels and relatively low proportions of corn silage and starch (334.0, 104.0 and 160.3 g/kg DM, respectively, in our study).

List of Abbreviations

aNDF	NDF assayed with heat stable amylase expressed inclusive of residual ash
ADF	acid detergent fibre
ADICP	acid detergent insoluble crude protein
ADIN	acid detergent insoluble nitrogen
BW	body weight
C	control treatment
CA	California
CCK	cholecystokinin
CLA	conjugated linoleic acid
CMC	calcium magnesium carbonate
CP	crude protein
CW	conceptus weight
DC 305	DairyComp 305
DCAD	dietary cation anion difference
DDGS	dried distillers' grains with solubles
DHIA	Dairy Herd Improvement Association
DIM	days in milk
DM	dry matter
dNDF30	digestible NDF after 30 hours in vitro incubation
EC	electrical conductivity
ESP	exchangeable sodium percentage
FCM	fat corrected milk
FPR	fat:protein ratio
GIT	gastrointestinal tract
GLM	general linear model of SAS
HPLC	high performance liquid chromatography
ME	metabolisable energy
MFD	milk fat depression
MS	maize silage based diet
MUN	milk urea nitrogen
MY	milk yield
NDF	neutral detergent fibre
NE	net energy
NIR	near infrared spectroscopy
NMS	non maize silage based diet
NPN	non protein nitrogen
NRC	National Research Council
OM	organic matter
PUFA	polyunsaturated fatty acids
SAR	sodium absorption ratio
SARA	subacute ruminal acidosis
SAS	Statistical Analysis Software
SB	sodium bicarbonate

SCC	somatic cell count
SD	standard deviation
SEM	standard error of the mean
TDS	total dissolved solids
TMR	total mixed ration
TNC	total non-structural carbohydrates
UC Davis	University of California at Davis
USA	United States of America
VFA	volatile fatty acid

List of Products

Product	Manufacturer	Description
SB	Natural Soda, 3051 West 2nd St., Rifle, CO 81650, USA	Sodium bicarbonate containing 27.4% sodium. Chemical formula: $NaHCO_3$
CMC	MIN-AD, 3131 Bell St., Suite 101, Amarillo, TX 79106, USA	Calcium magnesium carbonate (dolomitic limestone) containing 22.3% calcium and 12.0% magnesium. Chemical formula: $CaMg(CO_3)_2$

List of Programs

Program	Manufacturer	Description
DairyComp305	Valley Ag Software, Tulare, CA, USA	A program designed to manage individual and groups of animals on a dairy by processing production, health and reproductive data
SAS	SAS Institute Inc. 100 SAS Campus Drive Cary, NC 27513-2414 USA	Statistical Analysis Software used to analyse data and report means, standard errors and statistical significance of differences between treatment means

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Chapter 1: General Introduction

Addition of the buffer sodium bicarbonate (SB) to high producing dairy cow diets has become a standard procedure in many parts of the world, although locally relevant, contemporary research is often lacking. The assessed risk of subclinical acidosis and milk fat depression is a major factor in deciding for or against addition of ruminally active buffers to diets. Increases in milk fat proportion are often attributed to a rumen buffering effect (Erdman, 1988), and results from 30 studies published between 1980 and 1999, as summarised by Hu and Murphy (2005), showed that effects of SB addition to the total mixed ration (TMR) of lactating cows depends on the forage type in the diet, with beneficial effects of SB being limited to corn silage based diets. However, as forage type was confounded with dietary acid detergent fibre (ADF) levels, different responses to SB relative to the main dietary forage may be partly explained by differences in fibre content of the forages (Hu and Murphy, 2005).

Contemporary California dairies often do not conform to conditions of prior studies in which SB was found to result in increased dairy cow productivity, with important differences being forage type and inclusion rate, dry matter (DM) intake level and milk yield of cows. For example, the average milk yield and DM intake of cows in studies summarised by Hu and Murphy (2005) was 29.3 and 19.5 kg/d, compared to a range in milk yield of 32.7 to 51.3 kg/d and mean DM intake of 26.2 kg/d reported by Swanepoel et al. (2010) in a survey of 16 California dairy ‘high’ groups with a mean days in milk (DIM) of 131. In addition, mean ADF and corn silage contents of the diets were 170.6 *vs.* 214.9 and 295 *vs.* 159 g/kg DM, as reported by Hu and Murphy (2005) and Swanepoel et al. (2010), respectively.

Apart from its buffering capability, SB also affects the dietary cation anion difference (DCAD) due to the Na^+ ion. In contrast, supplementation with calcium magnesium carbonate (CMC) does not affect DCAD as Ca and Mg do not elicit an important biological effect on the balance between anions and cations. Recent research points to substantial effects of the DCAD value, defined as milliequivalents (mEq) of Na + K – Cl per unit of DM, on performance of lactating dairy cows. In a

meta-analysis of 12 studies published between 1984 and 1997, Hu and Murphy (2004) reported that milk yield and DM intake increased quadratically with DCAD, peaking at 34 and 40 mEq/100 g DM, respectively. Blood pH and HCO_3^- concentrations also increased with DCAD level, which points to an improved acid-base balance of the animals.

New environmental regulations in California limit the amount of fixed solids (FS), alternatively defined as minerals or ash content, which may be discharged from dairy farms. Sodium, as well as Ca and Mg, classify as FS; however, high levels of Na negatively affect ground and surface water for human and livestock drinking purposes and irrigation (Berg et al., 2010) while they also contribute to soil degradation which results in reduced biomass yields (Mengel and Kirkby, 2001). In contrast, Ca does not negatively affect water quality for drinking or irrigation purposes, and is applied in the form of gypsum to agricultural soils to enhance water penetration (Berg et al., 2010). Furthermore, Ca and Mg support soil flocculation (an important step in soil structure formation) and improve water and air supply to plant roots (Mengel and Kirkby, 2001). The Central Valley Regional Board adopted the General Order For Regulating Waste Discharges From Existing Milk Cow Dairies (2007) on May 3, 2007. A primary reason why the Central Valley Regional Board is addressing FS discharge in recently adopted waste discharge requirements is to identify methods to reduce the effect of specific minerals, particularly Na, on ground and surface waters so as to extend the useable lifespan of these water resources. One of the objectives is to reduce the amount of "new" FS which enter the Central Valley of California, with the objective of protecting the waters of the state from further degradation due to FS accumulation. The Order requires all dairy farms to assess their facilities' FS inputs and to identify methods to reduce the amount of FS which enters the waste stream (Berg et al., 2010). Sodium has been identified as one of the most important FS which leads to degradation of water and soil resources (Berg et al., 2010; Mengel and Kirkby, 2001).

While SB contains 270 mg/kg Na, milk and body tissue Na levels are carefully regulated so that the quantity of Na entering, and subsequently discharged from, the dairy farm increases with use of SB in dairy cow rations. Kellogg et al. (2001) reported that 0.79 of high producing dairies (n=133) surveyed in the USA use SB in their diets, which indicates that SB supplementation is commonplace in USA high producing dairy herds, although it is difficult to quantify benefits in a practical context

because of large variations in measured traits such as milk yield and milk fat proportion. Finding alternative buffers that do not contribute to Na^+ discharge, such as CMC, provided that they have similar beneficial effects on productivity as SB, may help California dairymen prevent the negative environmental impact associated with the use of SB.

The objectives of this study were to determine effects of SB and CMC on feed intake, DM intake patterns, digestibility, faecal pH, body condition score, milk yield and milk composition of high producing dairy cows, and to determine if CMC could effectively substitute for SB in the diet while maintaining the anticipated benefits of SB on productivity of the dairy cows.

Chapter 2: Literature review

2.1 Fixed solids and Californian regulations for existing milk cow dairies

2.1.1 Soil quality as affected by sodium, calcium and magnesium

Irrigation alters the water balance in soil by introducing more water and minerals; water is removed by evaporation and transpiration while minerals remain and accumulate. If the water applied is high in concentrations of Na^+ compared to Ca^{2+} and Mg^{2+} ions, and especially if the HCO_3^- ion is present, an unproductive sodic soil may result. Salt-affected soils adversely affect plants due to the high concentration of total salts (salinity) and the concentration of specific ions in the soil, especially Na^+ (sodicity). Salinity is usually measured as total dissolved solids (TDS) or electrical conductivity (EC), while sodicity is measured as the exchangeable sodium percentage (ESP) or the sodium absorption ratio (SAR) which, according to Brady and Weil (2008), is calculated as:

ESP = (exchangeable Na, $cmol_c/kg$) / (cation exchange capacity, $cmol_c/kg$) x 100

$SAR = [Na^+] / (0.5[Ca^{2+}] + 0.5[Mg^{2+}])^{0.5}$

As the ESP increases above 15%, the structure of the soil deteriorates. Sodium inhibits coagulation of soil particles, which in turn prevents flocculation and soil structure formation. Aluminium, Fe, Mg and Ca are binding materials which support flocculation and structure formation, which is of utmost importance for water storage and supply of plant roots with air and water, in which plant nutrients are dissolved. Saline soils are characterized by an excess of neutral salts such as the chloride and sulphates of Na^+, K^+, Ca^{2+} and Mg^{2+}. Soil quality is largely dependent on the proportions of these cations, and if Ca and Na proportions are relatively high and low, respectively, soil structure is acceptable. Soil sodicity hampers crop growth and results in low biomass yields (Mengel and Kirkby, 2001).

2.1.2 Waste Discharge Requirements General Order For Existing Milk Cow Dairies

The Central Valley of California is comprised of the Sacramento and San Joaquin Valleys. Land use in the Sacramento Valley is largely based on agriculture, with large areas of irrigated land and

some urban areas and dairy farms. The Sacramento and San Joaquin Valleys depend largely on ground and surface water sourced from the Sacramento Valley, This water is also an essential source for urban areas in southern California. The San Joaquin Valley is comprised primarily of agricultural land which is to a large extent irrigated, while there are large urban centers and more intensive dairy production areas compared to the Sacramento Valley. As a result of the high demands for water, the water flow into the river's outlet is reduced. Furthermore, the southernmost portion of the San Joaquin Valley consists of the Tulare Lake Basin, which lacks any natural or man-made drainage for ground and surface water. A substantial portion of the surface water is imported, mainly from the Sacramento Valley. This introduction of additional water, combined with a lack of adequate drainage, amplifies the problem of mineral concentration, as minerals are deposited with irrigation waters and cannot escape via drainage systems typical of well drained river systems (Berg et al., 2010).

A primary reason for addressing FS is to evaluate FS that are deposited and accumulate in ground and surface waters, and to identify methods which reduce the harmful impact of FS so as to extend the useable lifespan of these water resources. For example, Na and Cl are considered harmful as they reduce water quality for animal and livestock drinking purposes, as well as for irrigation. In contrast, Ca and Mg also contribute to the FS balance, but do not negatively affect water or soil quality, and are largely responsible for soil flocculation which plays a vital role in soil structure formation and the subsequent supply of water and air to plant roots (Mengel and Kirkby, 2001). For this reason, Ca is applied to agricultural soils in the form of gypsum, which improves water penetration into the soil profile (Berg et al., 2010).

The first of two main components being addressed by the Central Valley Regional Board is the introduction of "new" FS into the Central Valley from outside areas. The second relates to the accumulation of FS in localized areas which may lead to high groundwater FS concentrations and result in a situation in which this water resource becomes unusable for irrigation (Berg et al., 2010).

The Central Valley Regional Board adopted The General Order for Regulating Waste Discharges from Existing Milk Cow Dairies on May 3, 2007. This Order aims to protect the water quality of the state by minimising FS accumulation, and requires all dairies to assess their FS inputs and identify methods to reduce the amount of FS that reaches the waste stream (Berg et al., 2010). The Order

requires that "The Discharger shall submit a report which identifies sources of FS in waste generated at the dairy farm, evaluates measures that can be taken to minimise FS in the dairy waste, and certifies that they will implement the approved measures identified to minimise FS in the dairy waste" (California Regional Water Quality Control Board Central Valley Region, 2007). However, it is not possible to prevent all FS inputs on a dairy as water, chemicals, feeds, and organic bedding all contain FS, and the aim is therefore to manage FS sources to minimise accumulation of FS at the dairy (Berg et al., 2010).

2.2 Rumen buffers

2.2.1 The rumen and rumen pH

Ruminants are adapted to digesting and metabolizing forage nutrients. Feeding high grain diets results in high productivity but can lead to ruminal acidosis. Ruminal pH can drop below physiological levels (i.e., pH 5.5 to 7) when excessive amounts of rapidly fermentable (mainly non-fibre) carbohydrates are consumed by cows. The magnitude by which rumen pH declines after consuming large quantities of fermentable carbohydrates is determined by a cow's inherent capacity to buffer and absorb acids produced by rumen microorganisms (Oetzel, 2007). The first animal response to a drop in ruminal pH is to stop eating, which may be due to increased osmolality of rumen contents (Carter and Grovum, 1990) caused by the increased release of nutrients from digesta and elevated volatile fatty acids (VFA) concentrations. Inflammation of the rumen epithelium (i.e., rumenitis) may also play a role in reduced feed intake after ruminal acidosis occurs. Ruminal pH values above pH 5.5 do not appear to have an effect on appetite, but values below this strongly decrease intake (Oetzel, 2007), which may be a direct result of a low pH, although it is likely more specifically a result of the negative feedback inhibition associated with high rumen VFA and/or lactate concentrations. It has been shown that VFA infusions reduce DM intake to varying degrees, where the effect of a set amount of VFA infusion differs according to the relationship: acetate > VFA mixture > propionate > butyrate. Therefore, at the same level of energy infusion, the effect on DM intake depression increases as the molar weight of VFA infused decreases, which strongly supports

the theory that the effects of VFA infusions on DM intake depression are mediated primarily via tonicity (Faverdin et al., 1995).

Saliva is produced from several glands, being the parotid, mandibular, sublingual, labial, ventral buccal and medial buccal salivary glands. Sodium, K, bicarbonates and phosphates in saliva create its buffering ability. There is evidence that the rumen epithelium also contributes to buffering capacity by secreting bicarbonate (Van Soest, 1994). Saliva production is almost entirely dependent on chewing activity (i.e., eating and rumination), which in turn is partly dependent on the amount of structural fibre in the diet (Oetzel, 2007).

Apart from the buffering capacity of saliva, rapid absorption of VFA from the rumen contributes to the stability of rumen pH. The absorption of VFA across the rumen wall occurs passively (Bergman, 1990), and is enhanced by rumen papillae which increase the surface area available for absorption. Rumen papillae increase in length when cattle are fed diets high in rapidly fermentable carbohydrates (Dirksen et al., 1985), which presumably increases surface area and absorptive capacity, thereby preventing VFA accumulation in the rumen. If the absorptive capacity of these cells is compromised (e.g., chronic rumenitis with fibrosis), the ability to regulate rumen pH may be reduced (Krause and Oetzel, 2006). Large changes in diet composition may change mean rumen pH only slightly, but the lowest (nadir) pH values can be changed considerably. For example, Kennelly et al. (1999) reported that mean rumen pH did not differ between cows fed diets with 0.50 or 0.75 grain based concentrate, but the nadir pH was 5.9 and 5.5 for the 0.50 and 0.75 concentrate treatments, respectively. Krause and Combs (2003) found that when alfalfa silage is partially replaced by corn silage, which is higher in starch, mean rumen pH remains unaffected while nadir pH is reduced. These findings demonstrate that cattle can maintain rumen pH within physiological limits through regulation of intake, endogenous buffer secretion, microbial adaptation and absorption of VFA, except when consumption of fermentable carbohydrates results in acid production which exceeds the animal's capacity to maintain rumen pH, and therefore pH declines (Krause and Oetzel, 2006).

Rumen pH is a function of (Erdman, 1988; Raposo et al., 2006)):

- The balance between production of VFA by rumen microbes and their absorption across the rumen wall

- Water flux across the rumen epithelium
- Flow of saliva and associated buffers into the rumen
- Feed acidity
- Water outflow from the omasum into the lower gastrointestinal tract
- Feed protein content and rumen ammonia production (as ammonia can buffer VFA)

Most cellulolytic bacteria in the rumen grow best at pH values of ~6.7. Low rumen pH and excessive starch in the diet may lead to proliferation of lactate producing bacterial species (Van Soest, 1994), especially *Streptococcus bovis* (Mantovani and Russell, 2001). When rumen pH drops below 5.5, cattle develop subacute rumen acidosis (SARA). As VFA have a pKa of ~4.9, and therefore are shifting towards the undissociated (protonated) form at this pH (Oetzel, 2007; Kohn and Dunlap, 1998), this removes a free hydrogen ion and promotes uptake of VFA because only undissociated VFA can be absorbed passively through the rumen epithelium. However, gains in absorption of VFA below pH 5.5 can be negated by production of lactate. As pH declines, *Streptococcus bovis* starts fermenting glucose to lactate instead of other VFA. This is an undesirable situation because lactate has a lower pKa than VFA (i.e., 3.9 *vs.* 4.9). An additional adaptation to low rumen pH is proliferation of lactate utilising bacteria including *Megasphaera elsdenii* and *Selenomonas ruminantium*, which convert lactate to other VFA, which are subsequently protonated and absorbed. The turnover time for these organisms, however, are lower than that of *Streptococcus bovis*, and therefore this mechanism may not come into effect quickly enough to stabilize rumen pH. Additionally, periods of feed deprivation and high rumen pH may inhibit bacterial species which utilize lactate as they are poorly adapted to high rumen pH, and this increases susceptibility of lactate utilisers, and of the animal, to acidosis. Feed deprivation tends to cause cattle to over-eat once feed has been reintroduced, thereby amplifying the reduction in rumen pH (Oetzel, 2007). Low rumen pH reduces the number of species of bacteria and protozoa in the rumen, and when this occurs, the rumen microflora is less stable and has a reduced ability to maintain normal rumen pH during periods of abrupt dietary change (Garry, 1996).

2.2.2 What is a buffer?

A buffer solution is a mixture of a weak acid and its conjugate base and tends to resist changes in pH upon addition of moderate amounts of strong acid or base (Campbell and Farrell, 2006). In terms of buffer use in nutrition, it has been defined as a salt of a weak acid or hydroxide or oxide which neutralizes acids that occur in feeds or acids produced during digestion and metabolism of nutrients (Chalupa and Schneider, 1985). For a compound to be effective as a buffer under physiological conditions, it must:

- Be water soluble
- Be a weak acid, base or salt thereof
- Have a pKa value near the physiological pH of the system to be buffered (Hu and Murphy, 2005)

where pKa is defined as the pH at which an acid is half dissociated when in equilibrium (Kohn and Dunlap, 1998).

The buffering capacity of carbonates, such as SB, is based on an equilibrium between HCO_3^-, H^+, H_2CO_3, CO_2 and H_2O, as:

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 \text{ and } H_2O$$

When the concentration of H^+ increases, the equilibrium shifts towards the right (Russell and Chow, 1993), which results in a buffering effect.

Dolomitic limestone (which is composed of CMC) reacts in the following manner (Brady and Weil, 2008):

$$CaMg(CO_3)^2 + 2H_2O + 2CO_2 \rightleftharpoons Ca^{2+} + 2HCO^{3-} + Mg^{2+} + 2HCO^{3-},$$

and SB reacts according to a similar equation (Plane, 2000):

$$NaHCO_3 + H^+ \rightleftharpoons Na^+ + H_2O + CO_2$$

Where the main equilibria and pKa values are (OECD SIDS, 2002):

$$HCO_3^- \rightleftharpoons CO_3^{2-} + H^+ \qquad pKa = 10.33$$

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+ \qquad pKa = 6.33$$

While SB has a water solubility of 96 g/L at 20◦C, CMC is only sparingly soluble in water with a reported solubility of 0.32 g/L at 18◦C (OECD SIDS, 2002; European Commission – European Chemicals Bureau, 2000).

The pH of rumen fluid can be manipulated through addition of buffers or alkalizing agents. These include SB ($NaHCO_3$), sodium sesquicarbonate ($NaHCO_3 \bullet Na_2CO_3 \bullet 2H_2O$), calcium carbonate ($CaCO_3$) and magnesium oxide (MgO).

2.2.3 Acidosis, ruminal fermentation and milk fat depression

Milk fat depression (MFD) is the term used to describe abnormally low milk fat proportion (i.e., milk fat proportion below 35 g/kg) and/or yield. Milk fat contains approximately equal proportions of long and short chain fatty acids, the former being sourced directly from the blood and production of the latter occurring primarily *de novo* in the mammary gland. Milk fat depression is characterised by a reduction in both short and long chain fatty acid fractions (Van Soest, 1994), and has been linked to low rumen pH. However, there is only a weak link between this syndrome and rumen pH, and many dairy herds with very low rumen pH do not have MFD. Early studies investigating MFD (e.g., McClymont and Valence, 1962) reported a link between rumen acidosis, MFD and propionate production. Specifically, it has been shown by Bauman et al. (1971), using radioisotopes to measure VFA production in the rumen, that acetate to propionate ratios change with milk fat depressing diets due to an increase in propionate production without affecting acetate synthesis. Higher propionate levels stimulate insulin secretion, resulting in propionate's lipogenic effect on adipose tissue and competition with the mammary gland for acetate (McClymont and Valence, 1962).

However, it is now believed that MFD actually occurs when rumen acidosis inhibits bacteria that are responsible for fatty acid biohydrogenation. Therefore, more trans fatty acids are absorbed from the small intestine. The most predictable and repeatable cause of MFD is attributed to excessive intake of unsaturated fatty acids, which are not completely biohydrogenated in the rumen. Some intermediate forms of fatty acids, predominantly *trans*-10 $C_{18:1}$ and *trans*-10, *cis*-12 conjugated linoleic acid (CLA), strongly inhibit fatty acid synthesis in the mammary gland, even at very low doses of 5 g, or less, per day (Oetzel, 2007; Fuentes et al., 2009). According to Bauman and Griinari

(2003), this is caused by a reduced amount of mRNA for the enzymes critical to milk fat synthesis. During a dual-flow continuous culture fermentation experiment (Fuentes et al., 2009), the effect of pH (6.4 *vs.* 5.6) and concentrate level (forage to concentrate ratio 0.7:0.3 *vs.* 0.3:0.7) of the diet on biohydrogenation was investigated. Digestion of organic matter (OM) and fibre, total VFA and branched VFA concentrations, acetate proportion, and acetate to propionate ratio were lower, and propionate and valerate proportions were higher, for pH 5.6 compared to pH 6.4. A reduction in digestion and shift in VFA profile was likely due to inhibitory effects of low rumen pH on activity of fibrolytic bacteria. Ammonia N concentration and flow and CP degradation were lower at pH 5.6, possibly due to reduced access of bacteria and enzymes to protein within the undigested fibre fraction. There was a tendency for a lower bacterial N flow at pH 5.6 compared to pH 6.4, which was attributed to a reduction in OM digestion. Flow of non-ammonia and dietary N were higher at pH 5.6 than at pH 6.4. It was concluded that, while the rapidly fermentable carbohydrate level of the diet increases the proportion of *trans*-10,*cis*-12 CLA in the effluent, it is pH which is the main factor affecting biohydrogenation and results in accumulation of *trans*-10 $C_{18:1}$ and *trans*-10,*cis*-12 CLA fatty acids, which are related to MFD *in vivo*.

Accordingly, the National Research Council (NRC; 2001) suggested that milk fat proportion and yield are related to ingredient selection and diet formulation, with dietary polyunsaturated fatty acid (PUFA) and fibre levels being the most important. In a study examining effects of dietary fibre level as well as type and level of fat supplementation, it was shown that substantial MFD occurred only when low fibre diets where fed in combination with unsaturated fatty acid supplementation (Griinari et al., 1998). High fibre diets (i.e., forage to concentrate ratio of 0.5:0.5) supplemented with saturated or unsaturated fatty acids resulted in milk fat proportion and yield of 35.8 g/kg and 1.05 kg/d compared to 33.6 g/kg and 1.06 kg/d, respectively. However, when a low forage diet (i.e., forage to concentrate ratio of 0.2:0.8) was supplemented with saturated or unsaturated fatty acids, milk fat proportion and yield was 33.3 g/kg and 0.87 kg/d compared to 24.9 g/kg and 0.68 kg/d, respectively (Griinari et al., 1998). Therefore, dietary formulations which limit the amount of dietary PUFA, and have enough fibre to maintain adequate rumen pH, should result in ‘normal’ milk fat production (NRC, 2001).

Several measures can be used as indicators of potential SARA. Two of these are milk urea N (MUN) and milk fat to protein ratio (FPR). Normal MUN values are 3 to 5 mmol/L, while levels below 3 mmol/L may be indicative of SARA. Low MUN levels can occur when high dietary energy compared to protein levels result in increased proportional microbial ammonia use and reduced rumen ammonia levels, leading to reduced hepatic urea formation and lower MUN levels. Normal FPR is reported at 1.0 to 1.5, and the ratio decreases to below 1 during SARA (Enemark, 2009), while Hagert (1991) and Dirksen (1994) proposed the use of FPR specifically as an indicator of the dietary energy balance of the cow and suggested that a FPR of less than 1.4 was indicative of optimal, positive energy balance, while a FPR of over 2.1 occurred in a large proportion of energy deficient cows during peak lactation (Pehrson, 1996).

2.2.4 Effects of dietary supplementation of sodium bicarbonate, limestone or dolomitic limestone to the diets of lactating dairy cows

Initially, buffers were tested on milk fat depressing diets which contained 50 to 150 g/kg forage on a DM basis. Results indicated that buffers helped prevent MFD and therefore buffers have been widely recommended for diets when MFD was judged likely to occur. Careful interpretation of results is required when considering experiments involving buffer addition to the diet as buffer source, animal factors, diet sources and the interactions between them can lead to variable animal responses (Hu and Murphy, 2005).

In an attempt to compare the effects of SB with a potential alternative buffer, limestone, Teh et al. (1985; 1987) fed Holstein steers a diet supplemented with 8.0 g/kg SB or 9.6 g/kg limestone, which resulted in decreased plasma glucose without affecting plasma Ca and Mg concentrations. Rumen, duodenal and faecal pH values did not differ between control and SB or limestone addition, but rumen ammonia concentrations were lower for limestone addition compared to control and SB treatments (Teh et al., 1987). Water intake was lower for the limestone compared to SB and control treatments, but SB did not differ from control (Teh et al., 1987) while, according to Rogers et al. (1979), feeding SB to steers at a rate of 0.28 kg/d resulted in a 30% increase in water intake and liquid flow from the rumen. Estimated rumen volume, digesta disappearance rate, mean retention time, estimated liquid

volume and rumen liquid disappearance rate did not differ between SB, limestone and control treatments (Rogers et al., 1979). Rumen dilution and gastric secretions increased with SB addition (Teh et al., 1987). Studies with other species have found that Ca, K and Mg all can increase gastric secretion (Douglas, 1976; Rasmussen and Goodman, 1977; Flemstrom and Garner, 1980), which may lead to changes in levels of hormones such as gastrin, glucagon and, possibly, calcitonin (Teh et al., 1987). Dockray et al. (2001) found that Ca is a luminal stimulus for gastrin secretion, and intravenous infusion of Ca increased fluid, bicarbonate and protein secretion from the pancreas (Noel et al., 1981), while intraduodenal infusion of 25 mM Mg increased trypsin and bilirubin outputs (Malagelada et al, 1974). A decrease in pH of digesta stimulates release of cholecystokinin (CCK; Barbezat and Grossman, 1971) and therefore increases pancreatic secretion and buffering of the lower gastrointestinal tract (GIT), which may in turn lead to an increase in digestibility (Teh et al., 1987). Cholecystokinin is involved in control of satiety, gallbladder contraction, secretion of gastrin, pepsinogen, and leptin, pancreatic exocrine secretions, gastric emptying and gut motility via CCK_1 receptors (Silvente-Poirot et al., 1993; Bado et al., 1998; Wank, 1998). The family of CCK-gastrin peptides also mediates other effects via CCK_2 receptors, including gastrin-stimulated gastric acid secretion, changes in renal K and Na absorption, and glucagon secretion in the stomach, pancreas and kidney, respectively (Von Schrenck et al., 2000; Dockray et al., 2001; Lindström et al., 2001).

According to Rogers et al. (1982), SB increases rumen fluid dilution rate due to increased water intake, but limestone does not. Increased fluid dilution rate can result in increased microbial protein synthesis (Stouthamer and Bettenhaussen, 1973; Issacson, et al., 1975; Chalupa, 1979) and an elevated flow of amino acids (Harrison et al., 1975; Hemsley, 1975) and α linked glucose polymers to the duodenum (Kellaway et al., 1976). Such an increase should stimulate gastric acid secretion from the abomasum and decrease the pH of the digesta to 2.5 to 3.0. The amount of hydrochloric acid secreted by the abomasum is related to the volume and buffering capacity of the digesta arriving from the rumen. While amino acids and VFA have buffering abilities, they may also be specific stimulators of abomasal gastric secretion (Ash, 1961; Konturek et al., 1976). An increase in gastric acid secretion leads to increased pancreatic acid secretion and may improve digestion in the small intestine (Teh et al., 1987).

Rogers et al. (1982) found that cows fed a high starch diet (518 g/kg DM) supplemented with 24 g/kg DM limestone had a DM intake 2.6 kg lower than control cows, while milk production was similar at 29.5 and 29.2 kg/d for control and limestone treatments, respectively. This increased efficiency of feed utilization for the cows in the limestone treatment group (i.e., 1.56 *vs.* 1.36 kg milk/kg DM, respectively), although daily milk crude protein (CP) output tended to be lower (i.e., 998 *vs.* 1060 g/d, respectively). Sodium bicarbonate and NaCl, fed at 20 g/kg DM, did not alter efficiency of milk production, but did appear to increase milk fat proportion and yield, being especially noteworthy for SB (32.6 *vs.* 24.0 g/kg and 957 *vs.* 713 g/d for SB and control diets, respectively). Limestone did not have any effect on proportion or output of milk fat, which is consistent with studies of Emery et al. (1964) and Esdale and Satter (1972). Rogers et al. (1982) reported an increase in water consumption with 20 g/kg SB or 20 g/kg NaCl supplementation, but water consumption was lower with limestone addition to the diet compared to control. Dietary SB and NaCl addition both resulted in an increased rumen fluid dilution rate, but rumen fluid osmolalities did not differ from control, while rumen pH was elevated for the SB treatment only. An increase in fluid dilution rate may increase starch outflow from the rumen, which may lead to a reduction in the extent of fermentation (Rogers et al., 1982). According to Davis (1979), milk fat proportion declines when the ratio of acetate:propionate declines below 2.2 or when relative propionate proportion increases above 250 g/kg of total VFA, while Latham et al. (1974) have shown that cows which have a higher production of butyrate during the change to a high concentrate diet maintain a higher milk fat proportion. Rogers (1982) reported increased DM and OM digestion with SB and limestone addition, while limestone also increased starch digestion, but decreased digestion of CP. Sodium bicarbonate increased both starch and ADF digestibility compared to the control diet. If a substantial proportion of dietary starch reaches the caecum and large intestine, and is fermented there, this will result in increased microbial N production which would be voided in faeces (Rogers et al., 1982) as no digestion of microbial protein, and only limited amino acid absorption, occurs from these sites (Elliot and Little, 1977). Limestone may increase the pH of digesta in the caecum and colon which leads to increased microbial activity, enhanced starch degradation and increased microbial N excreted in faeces (Rogers et al., 1982). Elevated pH may also enhance pancreatic α-amylase activity in the small intestine, which has a

pH optimum of 6.9 (Long, 1961). Limestone may form insoluble Ca salts with long chain fatty acids (Roberts and McKirdy, 1964) and therefore reduce digestibility of fats in the diet. Limestone supplementation resulted in an increase in faecal pH (8.21 *vs.* 5.67) and decrease in faecal starch concentration (89.0 *vs.* 221 g/kg DM) compared to the control diet (Rogers et al, 1982), which supports a general hypothesis that starch concentration and faecal pH are negatively correlated.

While data relating to feedlot steers has limited application to dairy cows due to large differences in physiology and type of diet, Crawford et al. (2008) examined effects of dolomitic limestone (i.e., CMC) supplementation on feedlot steer performance and found that CMC supplementation did not affect average daily gain, DM intake, gain to feed ratio, total water intake or rumen pH of steers.

A review of 41 experiments comparing dietary neutralizing agents for lactating dairy cows conducted by Staples and Lough (1989) found that when corn silage was the main forage for early lactation cows, milk yield and milk fat proportion increased by 0.8 kg/d and 1.2 g/kg with SB supplementation, respectively. Cows in mid lactation produced 0.9 kg/d more milk and had 3.0 g/kg higher milk fat proportion compared to control cows, respectively. When hay (mainly alfalfa) was the main dietary forage, SB supplementation resulted in variable responses and, when high quality alfalfa hay was the only dietary forage in a study with early lactation cows, supplementation with 10 g/kg DM SB and 2.5 g/kg MgO did not result in an improvement in animal performance (Eickelberger et al., 1985). When the main source of fibre was cottonseed hulls, there was no change in 40 g/kg fat corrected milk (FCM) with addition of SB compared to the unsupplemented diet. Staples and Lough (1989) summarised effects of SB supplementation on body weight (BW) changes and found that, in early lactation, cows consuming an unsupplemented diet experienced a relative benefit due to a reduced loss of BW (-0.16 *vs.* -1.03 kg/cow/wk for unsupplemented and SB diets, respectively). However, in midlactation cows the effect was reversed, and cows receiving the SB supplemented diet increased their BW by 2.53 kg/cow/wk compared to 0.76 kg/cow/wk for cows consuming the unsupplemented diet. The ability of SB supplemented cows to produce more milk, and/or have a higher milk fat proportion, during early lactation as well as to have elevated BW gain during midlactation compared to unsupplemented cows, may be due to an increased DM intake for SB supplemented cows (Staples and Lough 1989).

In the review conducted by Staples and Lough (1989), an increase in rumen pH was reported for SB supplemented cows compared to the unsupplemented diets (6.04 *vs.* 5.91, respectively), and 9 of 12 studies had an increased ADF digestion with SB supplementation (482 and 426 g/kg average digestibility, respectively), although statistical significance occurred in only 4 of the experiments. However, 6 of 12 studies reported an increase in milk fat proportion of at least 10 g/kg with SB supplementation above the unsupplemented diet, and there was a negative relationship between milk fat proportion and molar proportion of rumen propionate. There also seemed to be a negative relationship between milk fat proportion and ruminal propionate proportion, even below 250 mg propionate/g total VFA, when there was a change in milk fat proportion. Effects of SB were attributed to an increase in rumen pH, increased ADF digestibility and a shift in the rumen acetate to propionate ratio. Propionate levels were believed to be a better indicator of milk fat proportion compared to acetate due to propionate's glucogenic effect on adipose tissue, which is in competition with the mammary gland for lipogenic substrates such as acetate and dietary long chain fatty acids (Palmquist, 1976). By shifting VFA production away from propionate, there may be more substrate available for milk fat synthesis in the mammary gland (Staples and Lough, 1989).

In a more recent study, the effects of SB supplementation were evaluated in a meta-analysis using data from 27 studies. Sodium bicarbonate was found to increase milk fat proportion by 2.7 g/kg and fat yield by 105 g/d in cows fed a diet with maize silage as the main or only forage in the diet (MS), but no response in milk production, milk fat proportion and yield, or milk protein proportion or yield occurred in cows fed a non maize silage based diet (NMS). This is consistent with the review of Staples and Lough (1989) which concluded that SB supplementation was only beneficial in corn silage based diets. Hu and Murphy (2005) also reported that cows fed the MS supplemented with SB consumed 1.24 kg more DM than cows fed the MS without SB addition. Rumen pH was 0.13 units higher, propionate concentration decreased and acetate:propionate ratio was higher in cows fed MS supplemented with SB compared to cows fed unsupplemented MS diets (Fig. 2.1, 2.2 and 2.3). The relationship between dietary ADF and milk fat proportion indicates a similar link (Fig 2.4) compared to increasing acetate:propionate ratios (Hu and Murphy, 2005).

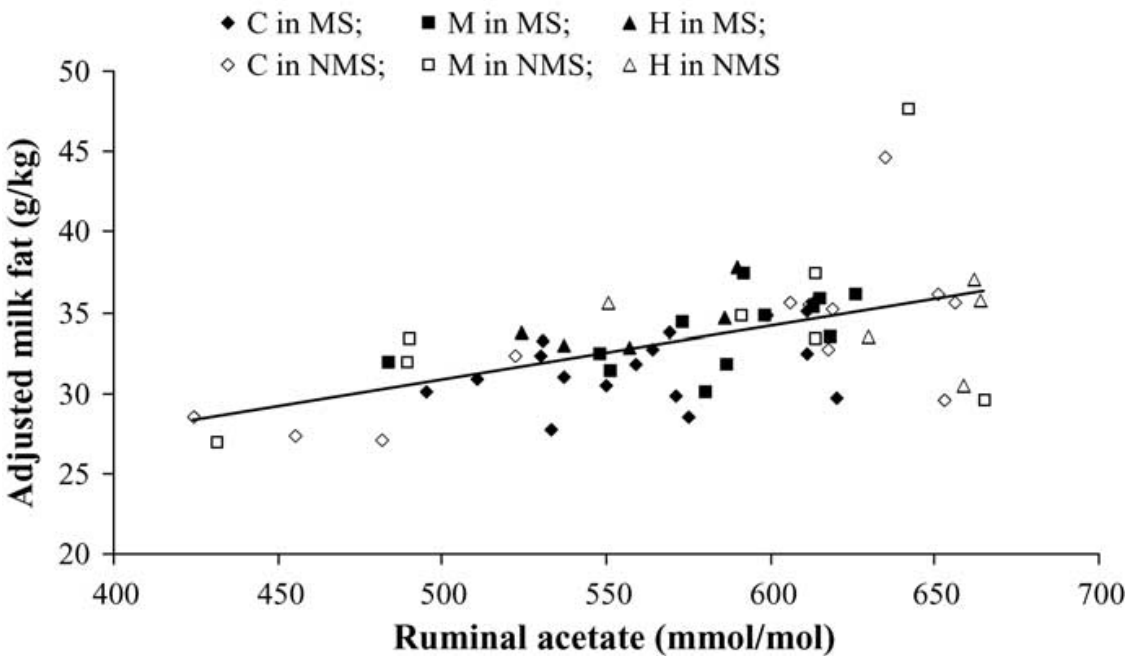

Fig. 2.1. Relationship between ruminal acetate and milk fat (adjusted for random effect of study) for all studies. The adjusted milk fat (g/kg) = 14.0730 + 0.0334×acetate (mmol/mol); among studies; $P < 0.03$; $R^2 = 0.28$; $n = 59$. C, 0.0 g/kg addition of $NaHCO_3$; M, 7.0–10.0 g/kg addition of $NaHCO_3$; H, 10.5–15.0 g/kg addition of $NaHCO_3$; MS, maize silage; NMS, forage other than MS (Adapted from Hu and Murphy, 2005).

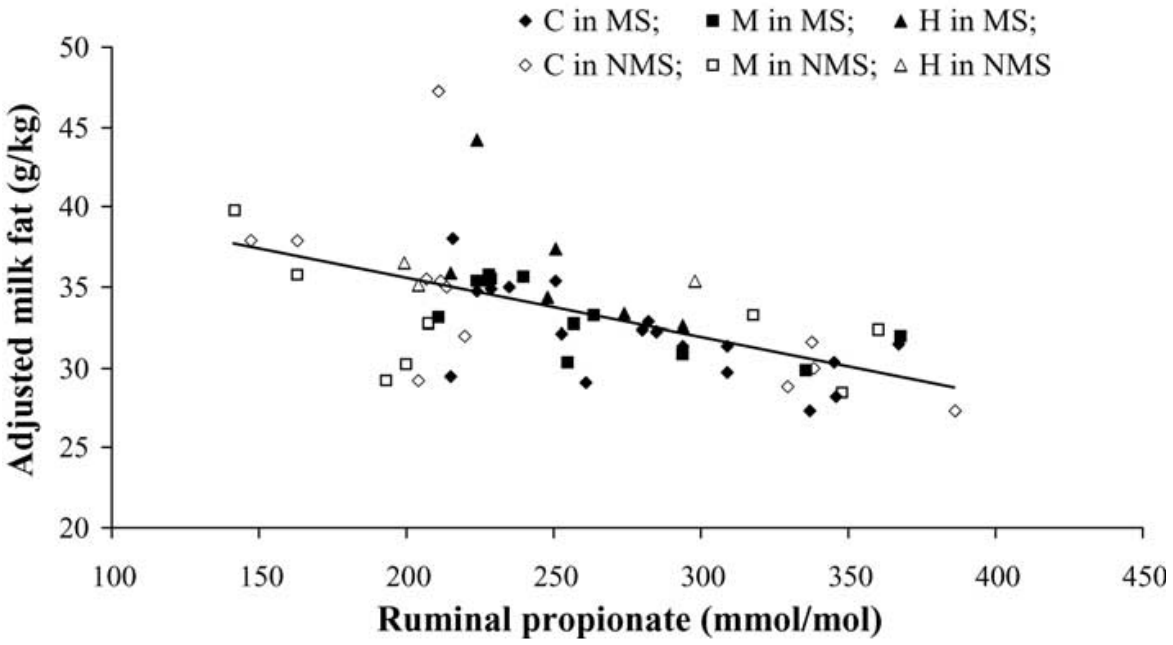

Fig. 2.2. Relationship between ruminal propionate and milk fat (adjusted for random effect of study) for all studies. The adjusted milk fat (g/kg) = 42.8600−0.0366×propionate (mmol/mol); among studies; $P < 0.01$; $R^2 = 0.34$; $n = 59$. C, 0.0 g/kg addition of $NaHCO_3$; M, 7.0–10.0 g/kg addition of $NaHCO_3$; H, 10.5–15.0 g/kg addition of $NaHCO_3$; MS, maize silage; NMS, forage other than MS (Adapted from Hu and Murphy, 2005).

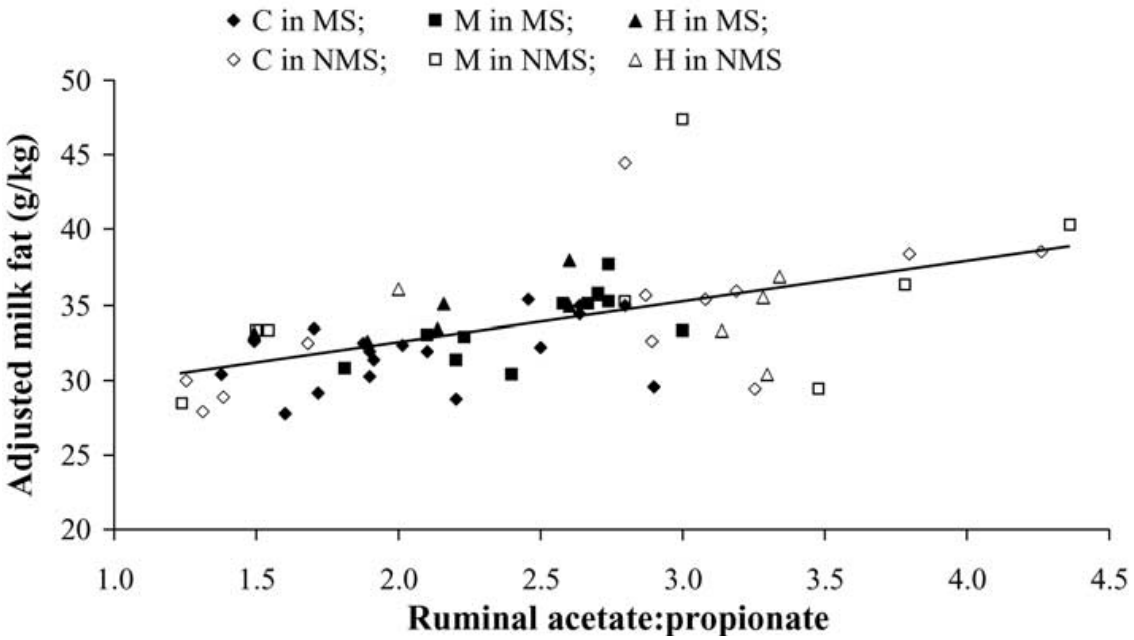

Fig. 2.3. Relationship between ruminal acetate:propionate (A:P) and milk fat (adjusted for random effect of study) for all studies. The adjusted milk fat (g/kg) = 27.0520 + 2.6930×(A:P); among studies; $P < 0.02$; $R^2 = 0.30$; $n = 61$. C, 0.0 g/kg addition of $NaHCO_3$; M, 7.0–10.0 g/kg addition of $NaHCO_3$; H, 10.5–15.0 g/kg addition of $NaHCO_3$; MS, maize silage; NMS, forage other than MS (Adapted from Hu and Murphy, 2005).

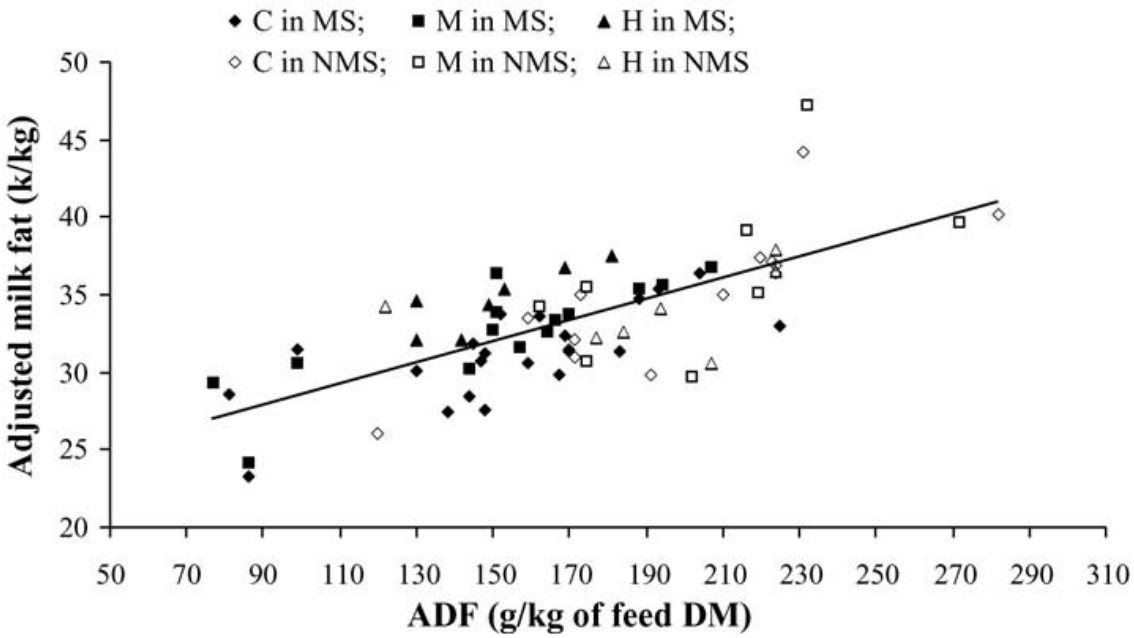

Fig. 2.4. Relationship between dietary acid detergent fibre (ADF) and milk fat (adjusted for random effect of study) for all studies. The adjusted milk fat (g/kg) = 21.5890 + 0.0687×ADF (g/kg of DM); among studies; $P < 0.01$; $R^2 = 0.52$; $n = 71$. C, 0.0 g/kg addition of $NaHCO_3$; M, 7.0–10.0 g/kg addition of $NaHCO_3$; H, 10.5–15.0 g/kg addition of $NaHCO_3$; MS, maize silage; NMS, forage other than MS (Adapted from Hu and Murphy, 2005).

While considerable research exists to the effects of limestone on dairy cow productivity, research testing the effects of dolomitic limestone (i.e., CMC) on animal productivity has been limited to feedlot nutrition (Crawford et al., 2008). This lack of research supported the use of CMC, rather than limestone, as a treatment in this study.

While SB is fed primarily to regulate rumen pH, this response is not detected in all studies, and responses in productivity may therefore be mediated via other physiological mechanisms. As Na in SB alters the dietary cation anion difference (DCAD), which has been found to affect productivity in lactating dairy cows (e.g., Hu and Murphy, 2004), DCAD may play an important role in mediating the effects of SB on productivity, and is therefore discussed in the following section.

2.3 The dietary cation anion difference (DCAD) theory

2.3.1 What is the dietary cation anion difference (DCAD)?

Leach (1979) and Mongin (1980) reviewed the non-ruminant literature and found that mineral interrelationships had important effects on the acid base status of the animal, theorizing that input and output of acid had to be balanced for an animal to maintain its blood acid-base status. Net acid intake was found to be related to differences between cations and anions in the diet, with the monovalent ions Na, K and Cl having the largest effects. It was suggested that the sum of Na plus K minus Cl (in mEq/100 g diet DM) could best predict net acid intake (Mongin, 1980). Tucker et al. (1988) named this the dietary cation anion balance, while West et al. (1991) referred to it as dietary electrolyte balance, but the term “cation anion difference” was first used by Sanchez and Beede (1991) to represent the mathematical calculation.

2.3.2 Effects of different DCAD levels on metabolic parameters and performance of lactating dairy cows

The first to examine the concept of optimal DCAD on maximizing intake and milk production of lactating dairy cows was Tucker et al. (1988), using DCAD values of -10 to +20 mEq/100 g feed DM. The diet with a DCAD of +20 mEq/100 g increased DM intake and milk yield by 11 and 9%, respectively, compared to the diet formulated for a DCAD of -10 mEq/100 g. However, as lactation

diets generally are formulated to have DCAD values above +20 mEq/100 g, it became evident that further research was required using DCAD values above +20 mEq/100 g. West et al. (1991) evaluated diets with +2.5, +15, +27.5 and +40 mEq/100 g. Milk yield and blood bicarbonate concentrations increased up to 27.5 mEq/100 g, indicating that there was an effect on blood acid base status, likely via a respiratory compensatory effect, but there was no further increase when a diet with +40 mEq/100 g was fed. West et al. (1992) then examined diets with DCAD values of +10, +21.7, +33.4 and +45.1 mEq/100 g on 16 cows during hot weather. Dry matter intake increased linearly with increasing DCADlevels irrespective if the source was Na or K. Milk yield and 35 g/kg FCM remained unaffected by DCAD or source of cations, but milk fat concentration was higher in diets supplemented with Na *vs.* K (39.2 *vs.* 36.2 g/kg). Blood pH increased linearly while blood bicarbonate increased curvilinearly, but acid base status was not affected by cation source (West et al. 1992).

Sanchez et al. (1994a) conducted an experiment with different combinations of Na, K and Cl which resulted in DCAD levels ranging from 0 to 50 mEq/100 g using the formula (Na + K – Cl – S). The basal diet was 545 g/kg concentrate, 55 g/kg cottonseed hulls and 400 g/kg corn silage (DM). The DM intake and milk yield peaked at DCAD levels of 17 to 38 and 25 to 40 mEq/100g, respectively. Blood bicarbonate increased curvilinearly and peaked at 38 mEq/100g DM.

When using the formula (Na + K – Cl) in a meta-analysis of 12 studies by Hu and Murphy (2005), milk yield increased quadratically with DCAD to maximise at 34 mEq/100 g of feed DM (Fig. 2.5) while 40 g/kg FCM also increased quadratically with DCAD, peaking at 49 mEq/100 g DM (Fig. 2.6). Milk fat proportion was not affected by DCAD but milk fat yield increased quadratically with DCAD up to a maximum at 55 mEq/100 g DM. It was hypothesized that no effect of DCAD on milk fat proportion was detected in the meta-analysis because 0.9 of the milk fat proportion treatment means were above 33 g/kg (i.e., MFD did not occur), and the diets were moderate to high in forage content. Additionally, the dilution effect of increased milk yield on milk fat proportion may partly explain why there was a lack of treatment effect of DCAD of milk fat proportion (Hu and Murphy, 2004).

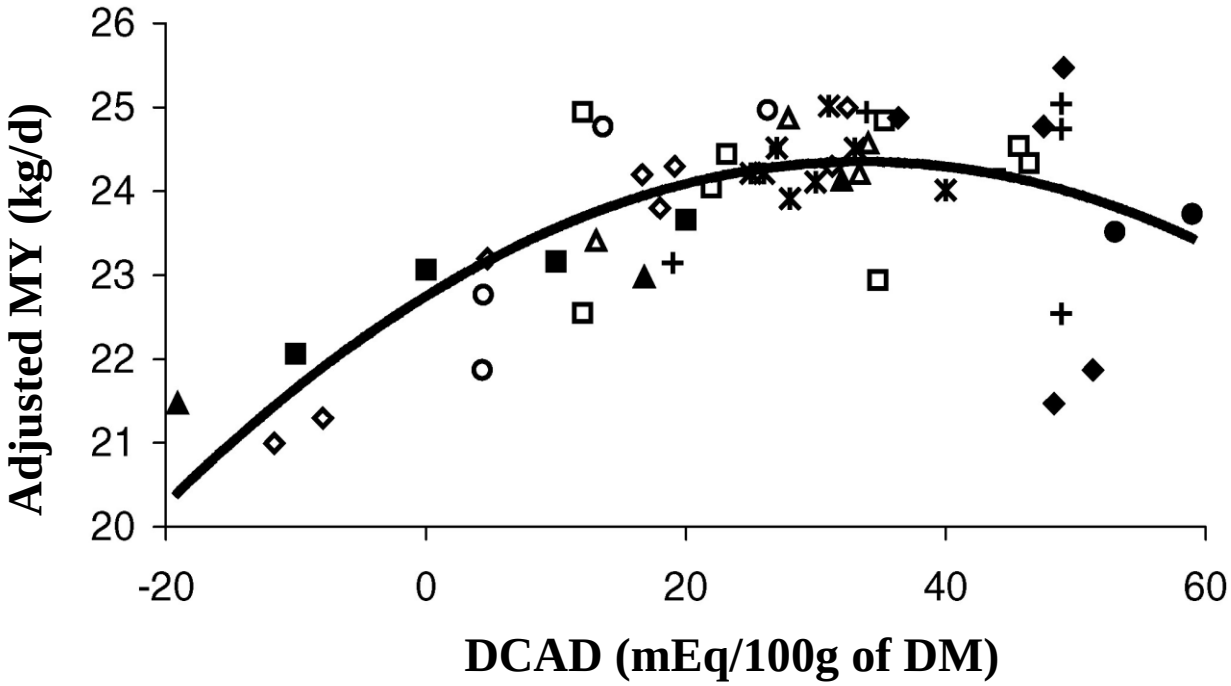

Figure 2.5: Relationship between dietary cation-anion difference (DCAD) and milk yield (MY) (adjusted for random effect of study) for studies reported in a meta-analysis conducted by Hu and Murphy (2004). The adjusted MY (kg/d) = −0.00142 × DCAD^2 (mEq/100 g of DM) + 0.0955 × DCAD (mEq/100 g of DM) + 22.75; across studies, quadratic P = 0.002; R^2 = 0.49; n = 54. Legend: Delaquis and Block (1995c) (●), Delaquis and Block (1995b) (◆), Escobosa et al. (1984) (▲), McKinnon et al. (1990) (Δ), Sanchez et al. 1997) (*), Tucker et al. (1988) (▪), Tucker et al. (1991) (+), Tucker et al. (1994) (−), Waterman et al. (1991) (O), West et al. (1991) (◊), and West et al. (1992) (□) (Adapted from Hu and Murphy, 2004).

The DM intake was maximised at 40 mEq/100 g of feed DM (Fig. 2.7). Lower DM intake with low or negative DCAD values can be attributed to the palatability (i.e., smell, taste or texture) of the anionic salt source or because of metabolic acidosis as a consequence of increased dietary anion intake. Blood pH and HCO_3^- concentration also followed a quadratic relationship with DCAD, peaking at 35 and 47 mEq/100g DM, respectively. Dietary cation anion difference did not affect milk protein proportion, but milk protein yield increased quadratically with DCAD, reaching maximum output at 40 meq/100 g DM. The effects of DCAD on milk protein and fat yields were mediated primarily through elevated milk yield. It seems clear that effects of increased DCAD with dietary buffer addition, such as SB, may play a role in preventing MFD via acid-base regulation (Hu and Murphy, 2004).

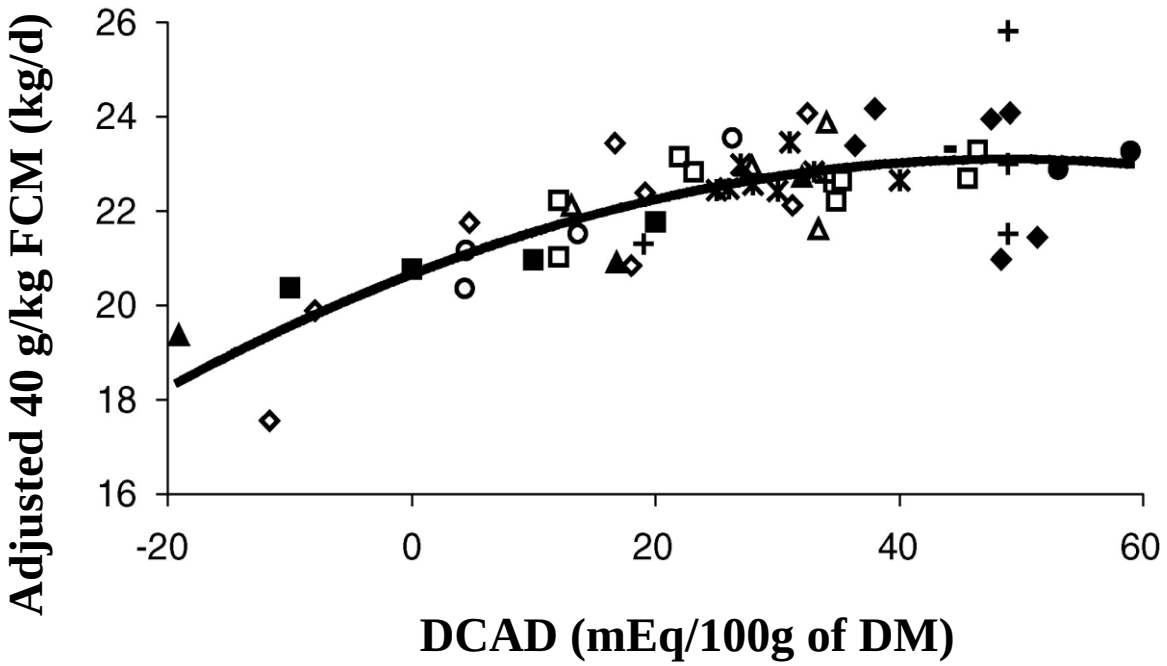

Figure 2.6: Relationship between dietary cation-anion difference (DCAD) and 40 g/kg FCM (adjusted for random effect of study) for studies reported in a meta-analysis conducted by Hu and Murphy (2004). The adjusted 40 g/kg FCM (kg/d) = −0.00102 × DCAD^2 (mEq/100 g of DM) + 0.0998 × DCAD (mEq/100 g of DM) + 20.66; across studies, quadratic P = 0.029; R^2 = 0.59; n = 54. Legend: Delaquis and Block (1995c) (●), Delaquis and Block (1995b) (◆), Escobosa et al. (1984) (▲), McKinnon et al. (1990) (Δ), Sanchez et al. (1997) (*), Tucker et al. (1988) (▪), Tucker et al. (1991) (+), Tucker et al. (1994) (−), Waterman et al. (1991) (O), West et al. (1991) (◊), and West et al. (1992) (□) (Adapted from Hu and Murphy, 2004).

The NRC (2001) recommendations for Na, K and Cl for lactating cows are 1.9, 10.2 and 2.5 g/kg of DM, respectively. This results in a DCAD of 27 mEq/100 g DM, which is lower than that found to maximise milk yield, 40 g/kg FCM and DM intake (Hu and Murphy, 2004). Therefore, current recommendations may not maximise dairy cow performance (Hu and Murphy, 2004). Block and Sanchez (2000) suggested that DCAD was a more important predictor of dietary influence on systemic acid-base status than dietary concentrations of Na, K and Cl *per se*, and so DCAD should be used as a tool in diet formulation of lactating dairy cows (Hu and Murphy, 2004). During a general linear model analysis of 10 studies, Sanchez and Beede (1996) determined that the optimum DCAD for lactating dairy cows ranged from 25 to 50 mEq/100 g DM, with milk yield, FCM and DM intake peaking at a 38 mEq/100 g DM.

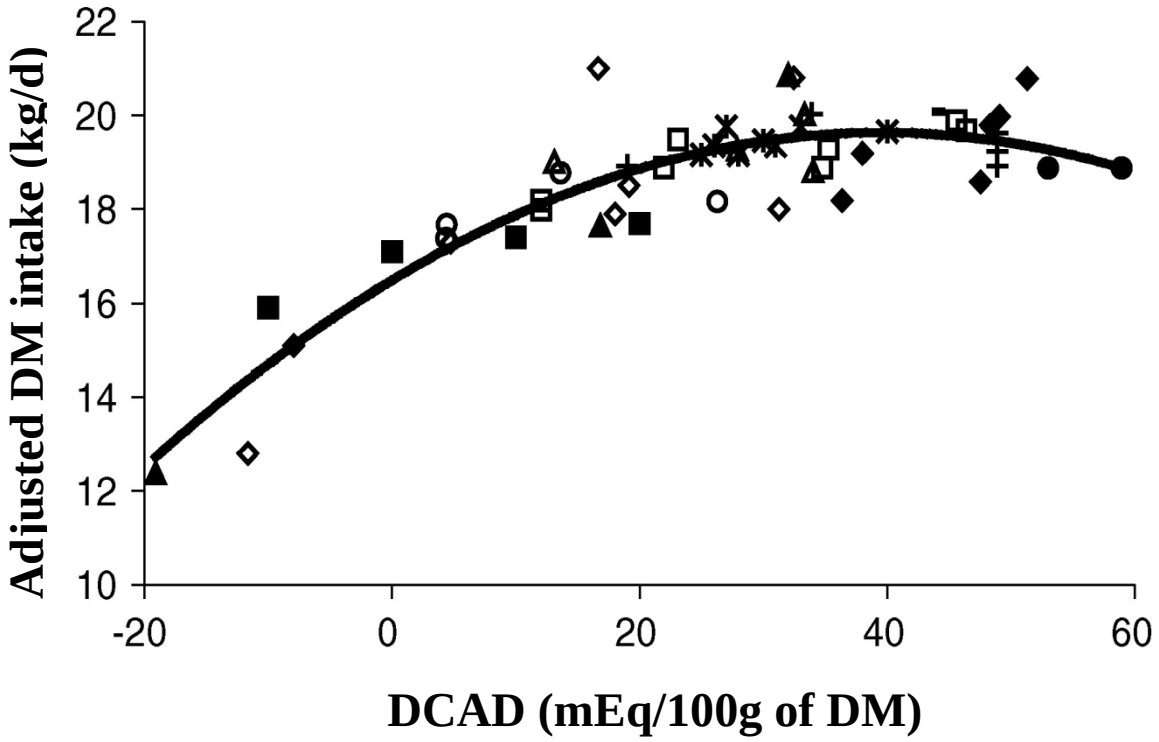

Figure 2.7: Relationship between dietary cation-anion difference (DCAD) and DM intake (adjusted for random effect of study) for studies reported in a meta-analysis conducted by Hu and Murphy (2004). The adjusted DM intake (kg/d) = −0.00201 × DCAD^2 (mEq/100 g of DM) + 0.1590 × DCAD (mEq/100 g of DM) + 16.49; across studies, quadratic $P < 0.001$; $R^2 = 0.80$; $n = 54$. Legend: Delaquis and Block (1995c) (●), Delaquis and Block (1995b) (◆), Escobosa et al. (1984) (▲), McKinnon et al. (1990) (Δ), Sanchez et al. (1997) (*), Tucker et al. (1988) (▪), Tucker et al. (1991) (+), Tucker et al. (1994) (−), Waterman et al. (1991) (O), West et al. (1991) (◊), and West et al. (1992) (□) (Adapted from Hu and Murphy, 2004).

More recently, Hu et al. (2007b) reported a linear increase in DM intake, 40 g/kg FCM and proportions and yield of milk fat and protein of early lactation (44 DIM) cows with DCAD levels of -3, 22 and 47 mEq (Na + K – Cl – S)/100 g DM. Plasma branched chain amino acid levels, and the ratio of essential to nonessential amino acids, with a higher DCAD indicated that rumen N metabolism was probably affected with increased microbial protein flow to the small intestine. In a similar experiment, Hu et al (2007a) examined effects of DCAD on performance and acid base status of cows directly after calving. However, in this study DCAD levels of 22 or 51 mEq (Na + K – Cl – S)/100 g DM resulted in similar DM intake (18.2 *vs.* 18.3 kg/d), milk yield (33.5 *vs.* 33.3 kg/d), milk composition and fat yield (1.29 *vs.* 1.33 kg/d), although net acid excretion in the urine differed suggesting it to be a much more sensitive indicator of acid load than blood acid base parameters in

postpartum cows. Consistent with Hu et al. (2007b), an elevated plasma branched chain amino acid and ratio of essential to total amino acids in cows fed the higher DCAD diet was reported, and indicated that rumen N metabolism was affected, likely resulting in increased microbial protein flow to the small intestine. It was hypothesised that a lack of response in productivity parameters to DCAD was because cows immediately after calving respond more variably to dietary changes, which makes treatment differences difficult to detect. However, considering the meta analysis by Hu and Murphy (2004) in which it was found that DM intake, milk yield and 40 g/kg FCM were maximised at 40, 34 and 49 mEq (Na + K – Cl)/100g DM, respectively, we hypothesize that the ideal DCAD to maximise these responses in the study by Hu et al. (2007b) may have been between the two DCAD treatments, which may explain the lack in response. In contrast, Roche et al. (2005) reported a positive linear response in milk fat proportion (39.6 *vs.* 41.7 g/kg) and yield (1.0 *vs.* 1.1 kg/d) in early lactation (48 DIM) pasture fed cows with DCAD treatments ranging from 23 to 88 mEq (Na + K – Cl – S)/100 g DM, respectively. This may indicate that DCAD requirements of pasture and TMR fed cows may differ (Roche et al., 2005), possibly due to differences in physical activity and/or mineral intake via increased soil consumption, and that lactation diets or supplements should be formulated for DCAD levels accordingly.

It is evident from foregoing sections that apart from its potential rumen buffering effect, SB also increases DCAD due to the presence of the Na^+ ion and may therefore elicit at least part of its physiological response via a change in acid base balance of the dairy cow. In contrast, CMC does not affect blood buffering as there is no effect on DCAD. As the dietary content of the minerals Na as well as Ca and Mg are altered when SB and CMC are supplemented, respectively, it is of interest to have a basic understanding of dairy cow requirements and physiological roles of these minerals, which will thus be discussed in the following section.

2.4 Effects of varying dietary levels of sodium, calcium and magnesium on lactating dairy cows

2.4.1 Sodium

2.4.1.1 Overview

Cattle are very efficient at Na absorption from the large and lower small intestine, and at Na resorption via the kidney. Only very small quantities of Na are stored in a form that is readily available for metabolism. Feeding Na in excess of requirements results in increased excretion, but if dietary concentrations of other macromineral electrolyte elements such as Cl are fed above requirements, additional dietary Na can improve animal performance (NRC, 2001).

2.4.1.2 Physiological roles

Sodium is the main extracellular cation (Aitken, 1976), and 0.3 to 0.5 of total body Na is found in the crystalline structure of bone and is non-exchangeable. The exchangeable fraction is responsible for regulation of extracellular fluid volume and blood acid-base equilibrium. Nerve and heart function are dependent on the appropriate balance of Na and K, and Na plays an important role in Na-K adenosine triphosphate enzyme (Na-K ATPase) responsible for creating electrical gradients for transport of glucose, amino acids and phosphate into cells and hydrogen, Ca, bicarbonate, K and Cl ions out of cells (Lechene, 1988). Sodium is also an important component of salts in saliva used for buffering acid produced during ruminal fermentation (Blair-West et al., 1970). Sodium concentrations in blood plasma are 150 meq/L, and concentrations in milk vary from 25 – 30 meq/L and are increased during periods of mastitis, but are not appreciably affected by dietary Na levels (Kemp, 1964; Schellner et al., 1971).

2.4.1.3 Sodium utilization and homeostasis

Dietary Na is assumed to be nearly completely available, and absorption occurs throughout the digestive tract. Active absorption occurs in the reticulorumen, omasum abomasum, and duodenum, and passive absorption also occurs through the intestinal walls (Renkema et al., 1962). As a result,

little Na is excreted in faeces, especially in animals that experience a dietary Na deficiency. Blood and tissue concentrations of Na are maintained by the kidneys via reabsorption and excretion, and there is a close relationship between excretion of Na and that of K and Cl. As Na is the principal effector of ion excretion, changes in renal reabsorption are main determinants of Na excretion. Endocrine control via tissue receptors and the rennin-angiotensin system, aldosterone, and atrial natriuretic factor monitor and control Na concentrations in different tissues which, in turn, control fluid volume, blood pressure, K concentrations and renal processing of other ions. Kidneys are very efficient in Na reabsorption, and secretion of Na in saliva is decreased and replaced by similar amounts of K, when dietary Na is inadequate (Van Leeuwen, 1970; Morris and Gartner, 1971).

2.4.1.4. Sodium requirements and efficiency of absorption

Common dairy feedstuffs do not contain enough Na to meet the metabolic requirements of lactating dairy cows and, therefore, supplementary sources are normally included in the diet. Apparent absorption ranges from 770 to 950 g/kg (Kemp, 1964), with NaCl being widely used as it is almost totally available. Efficiency of absorption of Na from other sources, such as SB, is also considered to be very high (NRC, 2001). Sodium requirements for lactating dairy cows producing 45 kg/d milk is 2.2 g/kg feed DM (NRC, 2001).

2.4.1.5 Responses of lactating dairy cows to varying dietary Na concentrations

Empirical data from 15 studies during cool or warm seasons indicates that DM intake and milk yield increased with dietary Na concentrations above those needed to meet requirements (Sanchez et al., 1994b, c). Dry matter intake and milk yield were curvilinear over a range of 1.1 to 12.0 g/kg, with a maximum at 7.0 to 8.0 g/kg Na on a DM basis. Maximum DM intake and milk yield at Na concentrations above those required was likely due to higher concentrations of other macro minerals, such as K, Cl, Ca and P in the diets. The interactions of Na with Cl, Na with K, and Na with P, on DM intake shows that responses to Na differed over a range of dietary levels of Cl, K and P (NRC, 2001). When lactating dairy cows were supplied drinking water with 0 or 2500 mg/L NaCl, milk yield

declined by 1.9 kg/d, and water consumption increased, but there were no effects on DM intake, digestibility or milk or blood macro mineral concentrations (Jaster et al., 1978).

Erdman et al. (1980b) examined effects of varying dietary Na concentrations (i.e., 3.1 *vs.* 5.2 g/kg DM) at varying dietary K levels (i.e., 4.2 *vs.* 8.2 g/kg DM) on midlactation dairy cows, and showed a slight decrease in milk yield and milk fat proportion, which resulted in a reduction (1.7 kg/d) in average 35 g/kg FCM of the two dietary K levels when increasing dietary Na from 3.1 to 5.2 g/kg DM. This indicates that it is important to consider not only the potential buffering effects of SB, but also the effects of increased dietary Na content when SB is supplemented, as Na over-supplementation relative to requirements can result in reduced productivity. It is also important to consider dietary K content when formulating for Na content due to the interaction between these minerals.

2.4.2 Calcium

2.4.2.1 Functions

Extracellular Ca is involved in skeletal formation, nerve impulse transmission, muscle contraction and blood clotting, and is a component of milk. Intracellular Ca is required for activity of many enzymes and acts as a secondary messenger between the exterior and interior of the cell (NRC, 2001).

When loss of Ca from extracellular fluid is more than entry, hypocalcemia may result, leading to a loss of muscle and nerve function and subsequent recumbency of the animal and the clinical condition referred to as ‘milk fever’. When vitamin D intoxification occurs, extracellular Ca levels increase, resulting in hypercalcaemia and associated soft tissue deposition of Ca (NRC, 2001).

2.4.2.2 Calcium homeostasis

Calcium is depleted from the extracellular fluid during bone formation and secretion of digestive fluids, sweat and urine, but the largest loss occurs during milk synthesis. Three main mechanisms are responsible for replacement of depleted Ca or reducing Ca losses:

- Dietary absorption
- Resorption from bone

- Resorption in the kidney during urine formation, reducing urinary Ca loss.

When plasma Ca concentration is reduced, the parathyroid gland secretes parathyroid hormone which increases intestinal absorption and bone and kidney resorption of Ca in an attempt to elevate plasma Ca levels (NRC, 2001). Passive absorption of Ca can occur in any part of the digestive tract, provided that ionized Ca in the digestive fluids at the mucosal interface exceeds 6 mM (Bronner, 1987). These concentrations can be achieved with oral Ca drenches for prevention of hypocalcaemia (Goff and Horst, 1993). However, for typical dairy cattle diets, these concentrations are not easily reached in digesta fluids, and therefore active transport of Ca appears to be the main mechanism of Ca absorption in mature ruminants. This process is controlled by 1,25-dihydroxyvitamin D, a hormone derivative of vitamin D. Extracellular Ca levels are therefore regulated to ensure a constant concentration (DeLuca, 1979; Bronner, 1987; Wasserman, 1981).

2.4.2.3 Requirement for absorbed Ca

Maintenance Ca requirements for lactating animals is 0.031 g/kg BW (Martz et al., 1990). Requirements for lactation varies with the amount of protein in the milk as a large proportion of milk Ca is associated with the casein fraction of milk protein (Lucey and Horne, 2009), As a result, Ca requirements differ according to the animal breed. For Holstein cows, the requirement for absorbed Ca per kg milk produced is 1.22 g (NRC, 2001).

2.4.2.4 Calcium absorption coefficient

The amount of Ca that needs to be fed to meet animal requirements depends on the Ca bioavailability in feedstuffs and supplementary inorganic sources. The amount of Ca absorbed is generally equal to requirements, as long as the diet contains enough Ca. Subsequently, the proportion of dietary Ca which is absorbed decreases as dietary Ca increases above requirements.

The NRC (2001) has estimated the efficiency of absorption of Ca at 300 and 600 g/kg for forage and non-forage feedstuffs, respectively. Calcium in mineral supplements generally has a higher availability than that in forages and common feedstuffs (Hansard et al., 1957). Theoretically, solubility of Ca in the mineral source is the limiting factor for Ca absorption. For example, according

to NRC (2001), the absorption coefficient of Ca in dolomitic limestone (i.e., CMC), a commonly supplemented Ca source in dairy diets, is 0.6.

2.4.2.5 Effects of physiological state

The amount of available Ca absorbed from the GIT depends on the physiological state of the animal. Most dairy cows are in negative Ca balance during early lactation (Ellenberger et al., 1931; Ender et al., 1971; Ramberg, 1974). As feed and Ca intake increase, most cows go into positive Ca balance at about 6 to 8 wk of lactation (Ellenberg et al., 1931; Hibbs and Conrad, 1983). Regression analysis of data from a study conducted by Ward et al. (1972) predicted that cows require 5 g Ca/kg milk produced in early lactation to avoid negative Ca status. However, there was no evidence to suggest that a negative Ca balance during early lactation was detrimental to the cow, as long as plasma Ca levels remained normal (i.e., enough Ca is released during bone resorption to ensure normal extracellular Ca concentration). The Ca:P ratio has little effect on absorption of Ca and P, as long as the ratio is within the range of 7:1 to 1:1 (Agricultural Research council, 1980; Miller, 1983).

2.4.2.6 Calcium excess and deficiency

In older dairy cows, Ca deficiency results in Ca withdrawal from bone to regulate Ca levels in the extracellular fluid. This can result in osteoporosis and osteomalacia, which may lead to bone fractures. Milk Ca concentration is not altered, even during a severe dietary deficiency of Ca (Becker et al., 1933).

Excess dietary Ca is not generally known to cause toxicity, but dietary concentrations of Ca higher than 10 g/kg have been associated with reduced animal performance and DM intake (Miller, 1983). Excessive levels of Ca may interfere with trace mineral absorption, especially Zn. However, feeding Ca above animal requirements may improve performance, especially when the diet is based on corn silage because Ca is a strong cation, and dietary supplementation of Ca carbonate above levels required to meet Ca needs may provide a rumen alkalizing effect and lead to improved animal performance (NRC, 2001).

2.4.3 Magnesium

2.4.3.1 Overview

Magnesium is a major intracellular cation which acts as a cofactor in enzyme reactions in all major metabolic pathways. Extracellular Mg is essential to normal nerve and muscle function, as well as bone mineral formation. Magnesium homeostasis is nearly totally dependent on absorption of adequate dietary Mg as bone Mg resorption only occurs in response to Ca homeostasis (NRC, 2001).

2.4.3.2 Absorption

In young calves, the small intestine is the main site of Mg absorption but, as the rumen and reticulum develop, they become the main, and possibly only, site of Mg absorption (Pfeffer et al., 1970; Martens and Rayssiguier, 1980; Martens and Gabel, 1986). Furthermore, Mg is secreted into the small intestine of adult ruminants, representing a loss of Mg from this part of the GIT. (Greene et al., 1983). Magnesium absorption from the rumen is dependent on rumen fluid Mg concentrations, which are important for passive and active transport, as well as integrity of Mg transport mechanisms. Magnesium transport requires a Na-linked active transport system (Martens and Gabel, 1986), which is vital if the dietary concentration of Mg is low. Animal Mg requirements are based on the assumption that the rumen wall is intact and functioning, but this is not always the case. The following sections describe when interference with Mg transport can be expected.

2.4.3.2.1 Factors affecting soluble concentration of Mg in rumen fluid

- Dietary Mg content
- Rumen fluid pH and Mg solubility. Magnesium solubility declines substantially when rumen pH rises above 6.5. Grazing ruminants tend to have a higher rumen pH due to high K levels in pasture and stimulation of secretion of saliva associated with grazing. Heavily fertilized pastures are often high in nonprotein N (NPN), which may result in the ability of bacteria to incorporate NPN into protein to be exceeded. As a result, ammonia accumulates in the rumen and may slightly elevate rumen pH. When highly fermentable carbohydrate rations are fed,

rumen pH often falls below 6.5 and Mg solubility is adequate. This may explain why feeds high in fermentable carbohydrates generally have higher Mg bioavailability (Miller et al., 1972).

- Forage can contain 100 to 200 mmol/kg of unsaturated palmitic, linoleic and linolenic acids that may form insoluble Mg salts. Some plants also contain trans-aconitic acid or citric acid. Tricarballylate, a metabolite of trans aconitic acid, can form a complex with Mg which resists ruminal degradation (Schwartz et al., 1988).

2.4.3.2.2 Factors affecting Mg transport across the rumen epithelium

- Dietary Na:K ratio. Pasture and forages are generally low in Na and therefore supplementation of the diet with Na can increase transport of Mg across the rumen epithelium when the dietary Na level is low relative to animal requirements. However, if Na levels are too high, the benefit may be negated due to increased urinary Mg excretion. High dietary levels of K can reduce absorption of Mg. The negative effects of high levels of dietary K cannot be overcome with addition of supplementary Na to the diet (Martens et al., 1988). Similarly, an increase in dietary Mg does not overcome negative effects of high K levels on the Na-linked active transport of Mg. However, an increase in dietary Mg levels may result in adequate absorption of Mg by passive uptake (Leonard-Marek and Martens, 1996; Ram et al., 1998).
- Pasture quality. Lush, high moisture pastures generally increase the rate of passage of digesta from the rumen, and this may prevent Mg concentrations from reaching high enough levels in the ruminal fluid to saturate transport sites of Mg (Martens, 1983).
- Ingestion of high amounts of Al. Aluminum in the blood can reduce parathyroid hormone secretion and result in depressed plasma Mg concentration. However, this is unlikely to occur as Al is poorly absorbed from the diet.
- Energy availability. Research has found an increase in utilization of orally administered Mg in dairy cows when combined with oral glucose, which may indicate that Mg absorption was

increased due to increased energy available for its active transport or due to a lower rumen pH caused by rapid fermentation of glucose and subsequently increased Mg solubility, or increased ammonia incorporation and subsequent reduction in the inhibitory effects of ammonia on Mg transport (Mayland, 1988; NRC, 2001).

2.4.3.2.3 Magnesium requirements of ruminants

A review published in the Agricultural Research Council (1980) concluded that the average coefficient of absorption for Mg from various feedstuffs was 294 (+/- 135) g/kg Mg. Because of the risks associated with an overestimation in efficiency of absorption of Mg, and in an attempt to provide a safety margin, the NRC (2001) assigned a coefficient of absorption of 160 g/kg, the same value adopted earlier by the Agricultural Research Council (1980).

Ammerman et al. (1972) found that Mg in magnesium carbonate has a biological availability of 437 g/kg in sheep. According to the NRC (2001), Mg in dolomitic limestone (i.e., calcium magnesium carbonate, CMC) should be considered to be unavailable. However, Rahnema and Fontenot (1983) found that Mg bioavailability from CMC in sheep was 134 g/kg Mg, which is very similar to the value of 143 g/kg DM reported by Gerken and Fontenot (1967) in steers. The coefficient of absorption for Mg from inorganic sources set by the NRC (2001) was 500 g/kg Mg. Some of the variation in estimates in Mg efficiency of absorption from forages and inorganic sources is due to the diet in the experiment. Diets high in K, N and moisture reduce efficiency of absorption of Mg, and it is recommended to reduce the assumed efficiency of absorption of Mg in diet formulation for diets that are high in K (NRC, 2001). Although a maximum tolerable level of 4.0 g Mg/kg feed DM was suggested in NRC publications prior to NRC (2001), cattle can excrete a large amount of Mg in urine and therefore Mg toxicity is not generally a problem. Potential negative effects of diets high in Mg are generally limited to a reduced DM intake and/or osmotic diarrhea (NRC, 2001).

In summary, although SB is generally supplemented to dairy cow diets to help regulate rumen pH, SB also serves as a dietary Na source. Calcium magnesium carbonate, on the other hand, is frequently added as a Ca source to dairy cow diets (NRC, 2001), although it may also be potentially beneficial as a buffer in the rumen and/or other parts of the GIT.

Objectives

The objectives of this study were to determine effects of SB and CMC supplementation on DM intake, DM intake patterns, digestibility, faecal pH, body condition score, as well as milk production and composition of high producing dairy cows, and to determine if CMC could substitute for SB in the diet while maintaining potential positive effects of SB supplementation.

Chapter 3: Materials and Methods

3.1 Dairy, animals and management

The study was conducted on Cloverdale South dairy near Hanford in the Central Valley of California (USA), which milks ~4900 Holstein cows. Three 'high' group (i.e., cows not yet confirmed in calf) free-stall pens of about 310 multiparity cows each, with an average of 63 +/- 39.3 DIM at the start of the study, were selected. Thus, a total of about 930 cows were part of the study at the onset. Cows entered the pens by random assignment from the fresh pen at ~15 DIM, where they remained until they were eligible to be moved to a 'mid' group pen based primarily on confirmation of pregnancy by palpation, as well as DIM. Cows were moved in and out of the treatment pens once a week and this averaged about 0.05 of all cows/wk. Cows which required medical attention were sent to a 'hospital' pen for treatment, after which some returned to the treatment pens. However, only cows which remained in their originally assigned pens from the start to the end of the study were considered for statistical analysis of animal response parameters. All cows were milked three times daily in a double 35 herringbone parlour.

The feedbunk and adjacent areas were fully shaded and had bunk-line misters. Each pen had 300 headgates and rubber mats on walkways, and at the feed aprons, to minimise and prevent hoof injury and associated problems. Stalls were bedded with dry composted manure which was restored weekly. Fresh feed was offered twice daily, at 06:30 and 11:30 am, and refusals from the previous day were mechanically removed and weighed just before the first feeding. During the first feeding, the headgates were put into lock position for ~50 min so that dairy personnel could perform daily routines including pregnancy diagnosis, artificial insemination and general animal inspection.

3.2 Experimental design

The experiment was a 3 x 3 Latin square design with 3 treatments and 3 pens in 3 periods of 28 d each. The three treatments were control (C), sodium bicarbonate (SB) and calcium magnesium carbonate (CMC), where the diets were identical in formulation among treatments except for addition of 8 g/kg DM buffer to the respective treatment diets. No inert material was added to the C diet to

allow for the effect of dilution of other feed ingredients with SB and CMC supplementation, as the supplements comprised a very small portion of the diet. Pens 2, 3 and 4 of the dairy were used in the study.

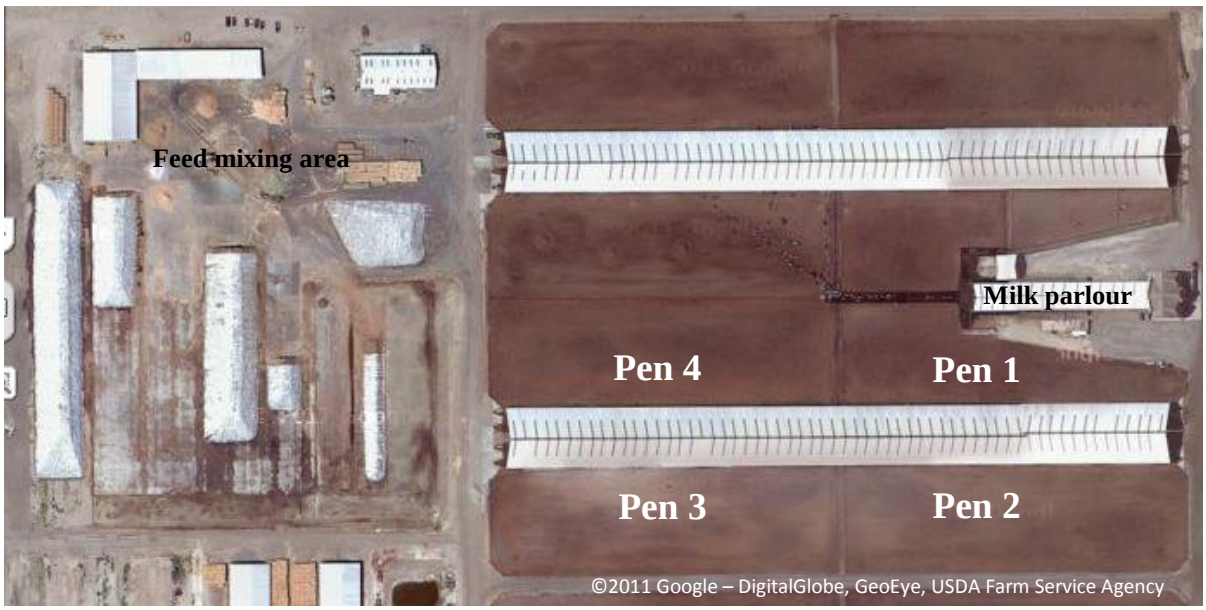

Figure 3.1: Aerial view of the design of Cloverdale South dairy near Hanford, CA, USA.

3.3 Sample and data collection

Feeds and TMR

Feed sampling was completed twice during the last week of each experimental period (i.e., days 21 and 27). Individual dietary ingredients were sampled using a plastic gloved hand, retrieving 10 to 15 random samples from each pile and emptying contents into a ziplock bag premarked with date, site and sample identification using a permanent marker. A 3.7 L ziplock bag was used for wet or bulky feeds such as silages and citrus pulp. Less bulky ingredients such as cottonseed were sampled with 1 L ziplock bags, while fine feeds such as canola meal or distillers' dried grains with solubles (DDGS) were sampled using 0.5 L ziplock bags. All hays were sampled with a manual 'golf club' style hay probe (Seifert Analytical, Lodi, CA, USA), taking 10 to 15 samples from different bales and combining the contents into premarked 0.5 L ziplock bags. All feed ingredients were frozen immediately at -20◦C.

Premix loads were mixed daily and created by combining almond hulls, canola meal, wheat straw, mineral premix, DDGS, pima cottonseed (cracked), tallow and molasses into a single mix. This premix was later added as an ingredient during final total mixed ration (TMR) mixing. Premix loads were recorded 3 to 6 times during the final week of each period by observing an electronic scale remotely connected to the vertical mixer (Model 1200 T Supreme Feed Processor, Duport TMR Equipment Co., Inc., Visalia, CA, USA). Individual dietary ingredient weights (on a wet basis) were calculated as the difference of the scale reading before and after each ingredient's addition to the mixer.

The TMR loads were mixed in vertical mixers and ingredient weights recorded as described for premixes. Sodium bicarbonate and CMC were added to the TMR after approximately two thirds of the ingredients were added to the mixer (i.e., after corn grain addition).The TMR loads were recorded an average of 4 times/pen during the final week of each experimental period, as described for premix loads, and TMR were sampled twice during the last week of each period (i.e., on days 21 and 27). The TMR sampling was completed according to guidelines of Robinson and Meyer (2010). The TMR load was sampled by following the feed truck to the pen and collecting 10 individual hand samples from evenly spaced locations using a plastic gloved hand, retrieving the TMR sample from the middle of the pile. Each sample was placed into a plastic bucket, without squeezing or shaking the sample during retrieval, to create a composite TMR sample. The TMR sample was then mixed on a cleaned flat surface using a plasterer's spatula, repeatedly turning the TMR inward from the bottom to the top. Any ingredients larger than ~2 cm in length, such as carrot tubers or citrus pulp, were cut up into smaller particles and returned to the TMR sample. The mixing continued until the sample acquired a homogenous appearance. After mixing, the sample was divided into four equal quarters, and two opposite quarters were used to make up the final TMR sample of approximately ~2.5 kg which was placed into the ziplock bag. Any excess air was squeezed out and the sample was sealed and frozen at -20◦C.

Dry matter intake

The weight of TMR offered and refused for each pen was recorded on a daily basis, and refusals where subtracted from feed offered to calculate TMR intake/pen/d. For DM intake calculations, only

the final 7 d of each period were used in order to allow the full effect of each treatment to become evident after the initial 3 wk adaptation. The TMR intake/d was divided by the average number of cows in the pen during the last 7 d of the period, and multiplied by the average DM of the corresponding TMR samples to calculate DM intake/cow/d.

Dry matter intake pattern determination

The DM intake patterns were determined by weighing the TMR at 3 pre-selected sections of each pen's feedbunk at three times during the morning, i.e., between the first and second feeding (Fig 3.2). The sections were marked by placing duct tape on the poles that designated the centre position of the respective sections. The sections were selected based upon the criteria of having one section to represent a subset of each third of the bunk, as different parts of the feedbunk may represent different cows in terms of eating behaviour and return time from the milking parlour. As there were a total of 30 sections/feedbunk/pen (i.e., 31 evenly spaced poles), the sections used for feed weighing were sections 8, 16 and 24 from the walkway (Fig. 3.2). In total, this represented 10 % of the total length of each pen's feedbunk. Shovels were used to manually collect the TMR, which was placed into large plastic bins on a portable scale. Once all the TMR from a section was collected, the weight was recorded and the TMR replaced to its original position, spreading it evenly. Each section was weighed directly after TMR was dropped from the feed truck while cows were still at milking, which was the weight recorded for time=0 (T0). The midpoint of the time when the first and last cow arrived from milking (~40 min) was taken to represent T0. The same sections were then weighed again at 110 (T110) and 240 (T240) min after T0. Thus, T0 to T110 corresponded to ~07:00 to 08:50 h, while T110 to T240 represented ~08:50 to 11:00 h.

T110 was selected because this represented the approximate time that cows were released from lock-up based on preliminary observations. To calculate TMR intake on a cow basis, the number of cows in each section were recorded, with a maximum of 10 cows/section (i.e., a capacity of 10 headlocks). Five minutes after the last cow arrived from milking, any open headlocks in the preselected sections were manually locked to prevent cows moving into the sections. T240 was selected as this was the time just before the second feeding occurred. As the TMR intake (i.e., removal of TMR) recorded during the time period between T110 and T240 represented any number of

cows from a theoretical 0 to 10 cows/section (as the headlocks were open), a correction factor was used, which was the ratio of the number of cows in the pen to the number of available headlocks in the pen. Intake/cow in each section was divided by this correction factor to make it fully representative of the number of cows in the pen at the time (i.e., the more cows that were present in the pen, the lower was the DM intake/cow/feeding space).

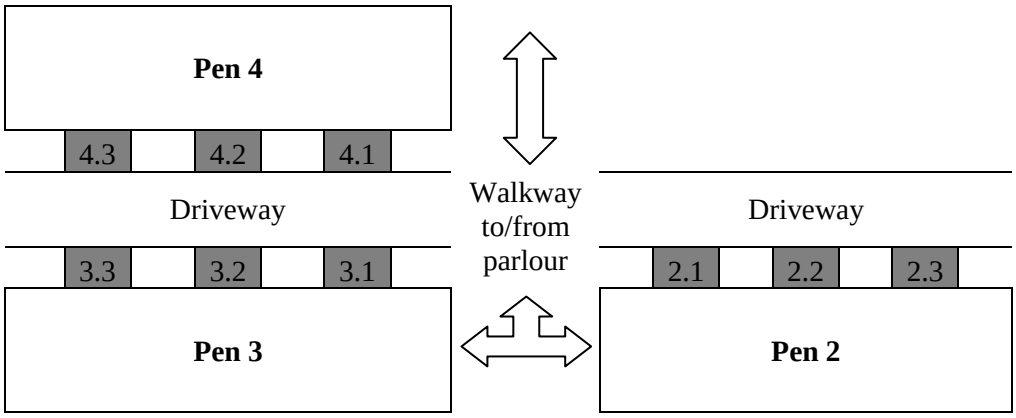

Figure 3.2: Design of pens 2, 3 and 4 of Cloverdale South Dairy indicating highlighted sections of the feedbunk used in determining DM intake patterns of cows (not fully to scale).

Milk

Dairy Herd Improvement Association (DHIA) in Hanford (CA, USA) collected milk samples at the end of each experimental period (i.e., day 28), using WB Auto Samplers (Tru-Test Incorporated, Mineral Wells, TX, USA) to create composite milk samples and record total milk yield. Milk samples from a subsample of cows were collected and preserved with Bronolab-W II and frozen for analysis of concentrations of Na, Ca and Mg. This group of cows was a subsample of the cows in the body condition score (BCS) group, and was created by sorting all the BCS cows according to DIM and selecting a representative sample to make up a total list of 12 cows.

Body condition scoring

Body condition scores (BCS) were determined using guidelines of Edmonson et al. (1989) using 8 anatomical locations of the cow and assigning an overall value between 1.00 and 5.00, with intermediate values of 0.25. The trained scorer conducted the scoring at the beginning of the experiment and at the end of each period. Cows to be body condition scored were selected from the

original cows in the pens at the start of the experiment by selecting the cows with early DIM and listing them in order of DIM. To create a similar average and spread of DIM among cows within pens, a spread of cows between 20 and 48 DIM at the start of the study were selected from each pen. Only the cows which were in their respective pens on each of 30 DairyComp 305 (a program that records and processes production, health and reproductive data) herd backups of cow locations taken during the 12 wk study were used, which resulted in a total of 112 cows which were scored at the start of the experiment and at the end of each of 3 periods. Difference in BCS for each period within cow was calculated by subtracting the initial value from the final value, and adjusting it to 30 d (i.e., units/28 x 30).

Faeces

Faecal samples were collected from a total of 49 cows in each of 3 periods for faecal pH and digestibility calculations. This group was a subsample of the cows used for body condition scoring and was the group of cows from which a smaller subsample was selected for milk Na, Ca and Mg analysis.

In vitro gas production and $dNDF_{30}$ determination

The *in vitro* gas procedure used was that of Blümmel and Ørskov (1993) with calibrated 100 ml piston pipettes of 31 mm internal diameter (Model Fortuna, *Häberle* Labortechnik, Lonsee-Ettlenschieß, Germany). Each sample was dried at 55^{o}C for 48 h, after which it was allowed to air equilibrate for 24 h. Analytical DM was determined from gravimetric loss of weight by heating to 105^{o}C for 3 h (NFTA, 2001), and final DM was calculated by multiplying initial air DM and analyitical DM. The sample was ground with a Wiley mill to pass a 1 mm sieve and 200 mg was incubated in 30 ml of buffered rumen liquor placed in a water bath at 39^{o}C, The rumen fluid was collected from a dry cow fed an all hay diet, and filtered through 3 layers of cheesecloth. Gas recordings were made at 0, 1, 2, 4, 6, 8, 10, 12, 14 and 16 h, after which readings were collected every 8 h up to 48 h. The 4 h readings are reported as these are indicative of the rapidly fermentable fraction of the ration (Groot et al., 1996), while the 24 h gas production is indicative of the relative digestible energy value of the diet (Menke and Steingass, 1988), and 48 h cumulative gas production was used as an indication of the diets' practical extent of *in vitro* digestibility (Robinson et al., 2004). Samples

were lightly swirled after every gas reading to ensure consistent mixing of the sample and the rumen liquor. All 18 TMR samples were duplicated to create 36 total samples. Digestible neutral detergent fibre (NDF) concentration at 30 h *in vitro* incubation ($dNDF_{30}$) was determined using the method of Goering and Van Soest (1970), and samples were removed at 30 h and assayed for aNDF (i.e., NDF assayed with heat stable amylase and expressed inclusive of residual ash). Digestibility was determined by the difference between times 0h and 30h, divided by the concentration at time 0h, and reported as g/kg of total aNDF.

In vitro pH buffering capacity of SB and CMC was determined using the same method as described to prepare piston pipettes with rumen liquor, in duplicate runs. Sodium bicarbonate or CMC was placed in the piston pipettes before collecting rumen fluid and 50 ml of rumen fluid was aliquoted into each piston pipette (as described above), and pH was recorded every 10 min until 60 min, mixing lightly between measurements. Three levels of SB and CMC were used (i.e., 0, 1, 2 mg/ml), with 2 mg/ml approximating our *in vivo* CMC and SB supplementation levels, based on estimated rumen size and daily CMC or SB intake.

3.4 Sample preparation and assays

Feed

The air DM content of wet feed and TMR samples were determined by gravimetric loss of free water by heating to 55^{o}C for 48 h, after which they were allowed to air equilibrate for 24 h. Analytical DM was determined from gravimetric loss of weight by heating to 105^{o}C for 3 h (NFTA, 2001), and final DM of wet feed and TMR samples were determined by multiplying initial air DM with analytical DM. A total of 6 samples per individual feed ingredient, and 6 TMR samples per treatment, were used for laboratory analyses.

Total glucose concentrations were determined by enzymatic hydrolysis of samples at 55^{o}C with amyloglucosidase for 12 h, after which it was analysed by high performance liquid chromatography (HPLC) with mass selective detection using a Phenomenex Luna NH2 (250 mm x 4.6 mm) HPLC column at a flow rate of 2.75 ml/min acetonitrile:water (78:22). As starch represents approximately

0.9 of total glucose polymers, starch content was determined by subtracting free glucose from total glucose and multiplying the value by 0.9. Glucose, fructose and sucrose where quantitatively determined by extracting samples with hot deionised water and subsequent analysis using HPLC with mass selective detection as described for total glucose, starch and total nonstructural carbohydrates (TNC) analysis (Johansen et al., 1996). Ash analysis was based on gravimetric loss by heating the sample to 550°C for at least 3 h for combustion of OM (Method 942.05; AOAC, 2005b). The aNDF analysis used sodium sulfite to remove nitrogenous matter, and a heat stable α amylase to digest residual starch which can interfere with filtration, as well as to inactivate potentially contaminating enzymes (Method 2002-4; AOAC, 2006a). Hot acid detergent solution was used to dissolve cell solubles, hemicellulose and soluble minerals to leave a residue of cellulose, lignin, heat damaged protein and some of the cell wall proteins and minerals, which is the ADF. Lignin(sa; i.e., lignin assayed with sulphuric acid) was determined gravimetrically after extracting the acid detergent (AD) residue with 720 g/kg H_2SO_4 and ashing the resulting sample. The ash value was subtracted from the ADF to give the ADFom value (Method 973.18, AOAC, 1997). Total N and AD insoluble N (ADIN) were determined by a Leco method using an induction furnace at 900°C to ignite samples, and analyzing the resulting combustion gases by thermal conductivity (Method 990.03; AOAC, 2005a). The Ranadall modification of the standard Soxhlet extraction method was used for crude fat analysis, including fats, oils, pigments and other fat soluble substances. The process involved submerging the sample into boiling ethyl ether and then lowering the solvent below the sample for a continuous flow of condensed solvent. The resulting solvent was evaporated, condensed and dried for gravimetric determination of the crude fat residue (Method 2003.05; AOAC, 2006b).

Total Se was determined by oxidation of Se-containing compounds using nitric, perchloric and sulfuric acids and reduction of selenate to selenite, and subsequent gas analysis using a Vapour Generation Inductively-Coupled Plasma Emission Spectrometer (VG-ICP; Tracy and Moeller, 1990.). Most of the minerals assayed (i.e., P, K, S, B, Ca, Mg, Zn, Mn, Fe, Cu, Mo, Na) used a nitric acid digestion method involving nitric acid/hydrogen peroxide microwave acid digestion and determination by inductively coupled plasma atomic emission spectrometry (ICP-AES; Meyer and

Keliher, 1992). Chloride analysis used water extraction and analysis by ion chromatography with conductivity detection (Jones, 2001).

Faeces

Faecal samples from each cow were measured for pH immediately after collection using an ISFET miniLab IQ128 pH-meter (Hach Company, Loveland, CO, USA). After mixing each faecal sample using a new nonsterile wooden chopstick for 5 s clockwise and anticlockwise, respectively, equivalent volumes of double deionised water and faecal material (30 ml of each) were placed into a clean container and mixed for 5 s clockwise and anticlockwise, respectively, using a new nonsterile wooden chop stick for each sample. The pH meter was recalibrated after each treatment group (pen) was sampled (~20 faecal samples) to prevent pH meter drift. After each sample was measured, the pH meter was cleaned 3 times using double deionised water and a toothbrush. A separate toothbrush was used for each treatment group (pen). The method used was similar to that described by Russell et al. (1980) and Bach et al. (2005b). Samples were immediately frozen at -20◦C and later oven dried at 55°C for 48 h, turning and breaking each sample into 4 quarters at 24 h, after which they were ground to pass a #4 Wiley Mill with a 1 mm screen. Composite samples of treatment within period were created by pooling individual cow samples by weight. Chemical analyses included the same assays as for the TMR samples in order to calculate digestibility.

Milk

Fat, true protein and lactose concentrations as well as somatic cell count (SCC) were determined using infrared (IR) spectroscopy at the Dairy Herd Improvement Association (DHIA) laboratory in Hanford, CA, USA. Milk Ca, Mg and Na analysis was done using the nitric acid digestion method involving nitric acid/hydrogen peroxide microwave acid digestion and determination by inductively coupled plasma atomic emission spectrometry (ICP-AES; Meyer and Keliher, 1992).

3.5 Calculations

The TMR intake for each pen in each period was multiplied by the average DM of the two corresponding TMR samples to give the final DM intake value for the pen, which was divided by the

average number of cows in the pen for the final week of the period to yield DM intake/cow/d. The DM intake pattern was calculated as:

(07:00 to 08:50 am):

DM intake/cow/h = [(T110 mass – T0 mass) / cows in headlock / (110*60)] * (TMR DM content (g/kg) / 1000), and

(08:50 to 11:00 am):

TMR intake/cow/h = [(T240 mass – T110 mass) / 10) / (130*60) / cf] * (TMR DM content (g/kg) / 1000)

where: cf = correction factor which is equal to (number of cows in pen / number of available headlocks)

DCAD was calculated in two ways as:

A. DCAD (mEq/kg) = ((g/kg Na/0.0023) + (g/kg K/0.00391)) – ((g/kg Cl/0.0035) + ((g/kg S/0.00321)(x2)) [Jackson et al., 2001]
B. DCAD (mEq/kg) = ((g/kg Na/0.0023) + (g/kg K/0.00391)) – (g/kg Cl/0.0035) [Hu and Murphy, 2004]

Production, body condition score and energetics were calculated as:

Milk energy (MJ/kg) = (((41.63 x g/kg fat) + (24.13 x g/kg true protein/0.934) + (21.6 x g/kg lactose) – 11.72) x 4.185) x 2.2046 [Tyrrell and Reid, 1965]

where: 0.934 is the conversion factor from true to crude protein, 4.185 converts Mcal to MJ and 2.2046 converts Mcal/lb to Mcal/kg.

Milk energy output (MJ/d) = milk energy (MJ/kg) x milk yield (kg/d)

The NE due to the loss or gain of each unit of BCS was 1448.01 MJ for cows at BCS 2 and weighing 650 kg (NRC, 2001).

NE for maintenance (NEm) = $((BW\text{-}CW)^{0.75}$ x 0.08) + NEmact [NRC, 2001]

= 43.5718 MJ/d

where: NEmact = variable to calculate NE for activity requirements

= ((distance/1000 x trips) x (0.00045 x BW)) + (0.0012 x BW)

Distance = distance from pen to milking parlour (0.5 km)

Trips = number of trips to and from the parlour/d (3)

BW = BW assumed to be 650 kg

CW = conceptus weight assumed to be 0

Digestibility:

Digestibility = 1000 - (1000 x (($g/kg\ lignin(sa)_{TMR}$ x 0.95 / $g/kg\ lignin(sa)_{Faeces}$) x ($g/kg\ nutrient_{Faeces}$ / $g/kg\ nutrient_{TMR}$)))

Assuming that lignin(sa) in the TMR is 950 g/kg indigestible and will be in faeces (Stensig and Robinson, 1997).

In vitro:

Gas production (ml/g OM) = ((gas production/h since last recording) – (*blank piston* gas production/h since last recording)) / (TMR analytical DM, g/kg) / (TMR OM, g/kg)

ME (MJ/kg DM) = 1.25 + (0.0292 × 24 h gas (ml)) + (0.0246 × g/kg fat) + (0.0143 × (g/kg CP – g/kg ADICP)) [Robinson et al., 2004]

3.6 Statistical analysis

For milk and faecal pH analyses, only those cows were used which were in their originally assigned pens for each of 30 consecutive DairyComp 305 (Valley Ag Software, Tulare, CA, USA) herd data back ups during the 12 wk study. Any cow which left the pen for any period of time was removed. All milk data was based on one morning milking per period, on the last day of each period. As cows were milked 3 times a day, milk yield was multiplied by 3 to give total daily milk yield. Data wereanalysed using the MIXED Model approach of the Statistical Analysis Software (SAS, 2000) using cow within pen as the random effect and period, pen, treatment and cow as class variables.

For assessment of DM intake, ingredient chemical analysis, TMR nutrient profile (including DCAD), TMR ingredient composition and digestibility, the General Linear Model (GLM) of SAS was used, with period, pen and treatment as class variables.

The *in vitro* fitted extent of fermentation (B) and rate of gas production (k) were determined using the nonlinear regression (nlin) procedure of the Gauss-newton model of SAS, by TMR. The model equation used was:

gas = b*(1-*e*(-k*h)).

In vitro gas production used the GLM model of SAS for the 4, 24 and 48 h values. Period, pen and treatment were used as class variables. Analysis of *in vitro* pH determination used the MIXED Model of SAS with run, material, level, tube and time as class variables and run as random effect.

Significance of differences between treatments was determined by using the PDIFF function in SAS, with $0.05 < P < 0.10$ accepted as a tendency and $P < 0.05$ as a significant difference.

The total number of cows used for statistical analysis was 430 for milk production data, 112 for BCS, 49 for faecal pH and digestibility calculations, and 12 for milk mineral composition data. As described, only those cows that remained in their respective pens for the entire 12 wk study were used for statistical analysis.

Chapter 4: Results

4.1 Ration evaluation and *in vitro* fermentation

The SB supplement contained 274.0 g Na/kg DM, with minor quantities of Ca and Mg, while CMC contained 222.7 and 120.0 g/kg DM Ca and Mg, respectively, with minor quantities of Na (Table 4.1).

Table 4.1
Dry matter and selected mineral composition (g/kg DM) of sodium bicarbonate (SB) and calcium magnesium carbonate (CMC) supplemented to the experimental diets

	SB	CMC
Dry matter (g/kg; 105^{o}C)	725	1000
Na	274.0	0.4
SD[a]	0.18	0.02
Ca	8.0	222.7
SD[a]	0.07	4.10
Mg	4.0	120.0
SD[a]	0.42	3.18

[a] Standard deviation

The nutrient profile of the feeds is presented in Tables 4.2 and 4.3, while the ingredient composition and nutrient profile of the experimental diets is reported in Tables 4.4 and 4.5, respectively.

The TMR of the treatments were very similar in nutrient composition (Table 4.5), with the exception of Na, DCAD, Ca and Mg, all due to supplementation with SB and CMC, respectively.

During *in vitro* incubation, the CMC diet had a tendency (P=0.05) to a higher gas production compared to C diet at 4 h), while there was a significantly higher gas production for CMC (P=0.01) and SB (P=0.02) supplemented diets compared to the C diet at 24 h. At 48 h, the SB supplemented diet had a tendency (P=0.06) to a higher gas production compared to the C diet. There were no differences in $dNDF_{30}$, or predicted ME and NE among diets, but there was a trend (P=0.08) for higher *in vitro* pH for the SB *vs.* C diet (Table 4.6).

Table 4.2
Average and standard deviation (SD) of the chemical analysis (g/kg dry matter) of the forage ingredients used in the experimental diets[a].

	Wheat silage	Corn silage	Alfalfa fresh chop	Alfalfa hay (HQ)[b]	Alfalfa hay (LQ)[c]	Wheat straw	Carrot tubers	Citrus pulp
Dry matter	363.2	327.2	260.8	901.8	913.3	925.7	95.7	132.9
SD	25.0	23.6	43.1	6.2	2.4	8.7	5.7	19.2
Crude protein	86.5	76.9	199.8	207.5	185.8	51.6	80.6	87.3
SD	21.9	3.9	28.2	26.2	12.4	16.1	4.4	14.9
aNDFom[d]	480.8	459.8	335.8	335.5	390.9	691.9	186.0	220.1
SD	25.9	13.2	35.3	11.1	12.0	25.4	9.9	41.4
aNDF[e]	521.8	475.5	408.5	356.2	403.5	731.0	221.0	227.4
SD	22.0	13.1	51.6	18.8	11.7	26.9	1.4	45.4
Starch	118.7	236.0	11.0	25.7	21.3	12.6	34.5	10.0
SD	75.9	11.2	5.1	4.5	2.5	5.4	4.9	5.0
Ash	111.6	71.7	182.6	116.4	111.0	131.1	117.4	50.4
SD	9.8	4.7	50.5	16.3	6.3	12.5	5.1	12.3

[a] Average for a total of six samples, two samples collected during the last week of each of three periods.
[b] High quality alfalfa hay as classified by the dairy.
[c] Low quality alfalfa hay as classified by the dairy.
[d] Neutral detergent fiber assayed with heat stable amylase expressed exclusive of residual ash.
[e] Neutral detergent fiber assayed with heat stable amylase and expressed inclusive of residual ash.

Table 4.3
Average and standard deviation (SD) of the chemical analysis (g/kg dry matter) of the concentrate ingredients used in the experimental diets[a].

	DDGS[b]	Canola pellets	Corn grain[c]	Pima cotton seed	Almond hulls	Cotton seed meal	Corn gluten pellets
Dry matter	912.7	902.7	868.2	922.5	960.8	891.3	913.0
SD	7.7	9.3	6.1	4.8	3.5	6.7	12.9
Crude protein	275.7	398.8	74.6	215.0	52.6	404.2	209.5
SD	3.3	7.9	2.0	8.2	6.7	17.7	21.0
aNDFom[d]	284.8	285.5	84.1	389.3	279.8	376.0	372.0
SD	8.4	125.5	4.8	13.8	17.2	29.1	8.6
aNDF[e]	291.7	369.3	85.6	411.3	290.0	394.8	390.9
SD	8.9	219.8	4.9	14.3	17.4	31.4	11.0
Starch	53.7	34.5	742.1	6.0	18.2	<5	136.1
SD	3.6	3.4	22.8	0.8	7.4	-	8.8
Ash	50.5	76.8	12.5	49.3	73.0	72.5	62.3
SD	0.8	2.3	1.0	2.8	7.9	1.4	7.9

[a] Average for a total of six samples, two samples collected during the last week of each of three periods.
[b] Dried distillers grains with solubles (corn).
[c] Steam-flaked.
[d] Neutral detergent fiber assayed with heat stable amylase expressed exclusive of residual ash.
[e] Neutral detergent fiber assayed with heat stable amylase and expressed inclusive of residual ash.

Table 4.4

Mean ingredient composition (g/kg DM) of the TMR fed to high producing dairy cows in the control (C), sodium bicarbonate (SB) and calcium magnesium carbonate (CMC) treatments[a]

	Treatment			
	C	SB	CMC	SEM[g]
Wheat silage (whole crop)	165.1	163.6	163.3	0.50
Corn grain (steam flaked)	130	131	134	1.0
Almond hulls[b]	109.0	108.6	108.1	0.10
Corn silage (whole crop)	104.9	104.1	103.0	0.17
DDGS[bc]	94.57	94.29	93.83	0.072
Canola pellets (solvent)[b]	61.21	61.09	60.76	0.027
Pima cottonseed (cracked)[b]	58.22	58.06	57.77	0.047
Alfalfa hay (HQ)[d]	57.5	57.5	58.2	0.22
Alfalfa hay (LQ)[e]	34.5	33.0	32.9	0.17
Corn gluten pellets	33.68	33.52	33.38	0.075
Alfalfa fresh chop (whole crop)	29.1	27.7	28.1	0.32
Cottonseed meal (solvent)	28.86	28.71	28.59	0.066
Whey (liquid)	23.1	24.2	24.4	0.39
Citrus pulp (orange and lemon)	19	16	16	1.1
Mineral premix[bf]	14.70	14.68	14.60	0.029
Molasses (liquid)[b]	14.38	14.34	14.27	0.011
Wheat straw[b]	12.75	12.71	12.65	0.016
Sodium bicarbonate (SB)	0.0	7.9	0.0	0.17
Calcium magnesium carbonate (CMC)	0.0	0.0	7.6	0.30
Carrots (pulp/whole tubers)	5.65	5.58	5.66	0.031
Tallow[b]	3.66	3.65	3.64	0.0044

[a] Based on two TMR samples collected per period per diet (i.e., 6 samples per diet).

[b] Ingredients used to create the premix

[c] Dried distillers grains with solubles (corn).

[d] High quality alfalfa hay as classified by the dairy.

[e] Low quality alfalfa hay as classified by the dairy.

[f] Premix (998.2 g/kg DM) contained (as guaranteed by the supplier) 244.9 g/kg Ca, 44.6 g/kg Mg, 7.2 g/kg P, 2.0 g/kg K, 123.1 g/kg Cl, 79.6 g/kg Na, 2.8 g/kg S, 59.63 mg/kg Co, 828.22 mg/kg Cu, 59.63mg/kg I, 1192.62 mg/kg Mn, 15.24 mg/kg Se, 3511.63 mg/kg Zn, 331.20 KIU/kg Vit A, 99.36 KIU/kg Vit D, 1.10 KIU/kg Vit E on a DM basis (Nutrius LLC, Kingsburg, CA, USA).

[g] Standard error of the mean

Table 4.5
Nutrient profile of the TMR fed to high producing dairy cows in control (C), sodium bicarbonate (SB) and calcium magnesium carbonate (CMC) treatments[a]

	Treatment			
	C	SB	CMC	SEM[i]
g/kg DM				
Dry matter	513	521	524	4.2
Crude protein	156.2	158.7	158.5	0.96
ADICP[b]	67.7	67.3	65.7	0.75
aNDF[c]	339	331	332	0.8
aNDFom[d]	324	316	316	1.1
ADF[e]	223	222	220	2.4
Lignin(sa)[f]	47	49	48	0.62
Crude fat	54.3	54.4	54.5	0.69
Starch	157	158	166	3.5
Free sugars	44	38	38	1.1
Ash	90.6	93.8	97.5	0.59
Ca	7.49[j]	7.48[j]	9.30[k]	0.079
Mg	3.18[j]	3.18[j]	4.13[k]	0.046
K	17.9	17.6	17.9	0.12
P	4.73	4.69	4.78	0.022
S	2.88	2.90	2.88	0.017
Na	3.0[j]	5.0[k]	3.1[j]	0.050
Cl	7.6	7.5	7.4	0.14
DCAD[g]	195[j]	276[k]	202[j]	5.4
DCAD[h]	375[j]	456[k]	381[j]	4.3
mg/kg DM				
Zn	76.4	78.1	77.7	0.88
Mn	41.7	43.3	43.1	0.43
Cu	17.2	17.8	17.8	0.26
Mo	1.253	1.233	1.245	0.0098
Se	0.361	0.381	0.373	0.0056

[a] Based on two TMR samples collected per period per diet (*i.e.*, 6 samples/diet).
[b] Acid detergent insoluble crude protein expressed as g/kg CP.
[c] Neutral detergent fibre assayed with heat stable amylase expressed inclusive of residual ash.
[d] aNDF expressed exclusive of residual ash.
[e] Acid detergent fibre expressed inclusive of residual ash.
[f] Lignin assayed with sulphuric acid.
[g] Dietary cation anion difference calculated as: milliequivalents/kg of (Na + K) – (Cl + S).
[h] Dietary cation anion difference calculated as: milliequivalents/kg of (Na + K) – Cl.
[i] Standard error of the mean
[j, k] Treatment means with different superscripts differ significantly ($P<0.05$).

4.2 Dry matter intake

There were no differences in intake of DM and most chemical components among treatments. However, cows in the SB treatment had higher intakes for Na compared to the other two treatments (P<0.01), while Ca and Mg intakes were higher in the CMC treatment group (P<0.01; Table 4.7). These differences reflect the higher Na and Ca/Mg levels in the SB and CMC diets, respectively. Cows consumed more DM (P<0.01) during the early morning (i.e., 07:00 to 08:50) compared to the late morning (i.e., 08:50 to 11:00; Fig. 4.1). However, there was no significant time*treatment interaction.

Table 4.6
In vitro gas production, digestible NDF after 30 h *in vitro* incubation ($dNDF_{30}$), predicted ME of the diet and pH of rumen liquor for control (C), sodium bicarbonate (SB) and calcium magnesium carbonate (CMC) supplemented diets.

	Treatment				*P*		
	C	SB	CMC	SEM[f]	C *vs.* SB	C *vs.* CMC	SB *vs.* CMC
Gas production (ml/g OM)							
4 h	73	75	78	2.4	0.44	0.05	0.23
24 h	212	225	228	5.5	0.02	0.01	0.65
48 h	246	267	251	7.2	0.06	0.63	0.14
Fermentation kinetics							
B[a]	244	268	250	7.4	0.04	0.62	0.11
k[b]	0.092	0.086	0.094	0.0027	0.12	0.57	0.04
$dNDF_{30}$ (g/kg aNDF)	499	496	493	3.9	0.64	0.28	0.53
ME[c], 1xM	12.56	12.56	12.48	0.05	0.99	0.26	0.25
3xM	11.56	11.56	11.48	0.05	0.99	0.26	0.25
NE[d]	7.17	7.17	7.12	0.03	0.99	0.26	0.25
In vitro pH[e]							
Level 1	6.44	6.60	6.47	0.023	0.11	0.46	- [g]
Level 2	6.44	6.66	6.46	0.023	0.08	0.56	- [g]

[a] Fitted extent of fermentation (ml/g OM).
[b] Rate of gas production (/h).
[c] UC Davis approach to estimate ME (MJ/kg DM) of a feed (Robinson et al., 2004). 1xM, ME requirements for maintenance; 3xM, ME requirements for lactation at 3 times maintenance energy.
[d] NE of the diets using the formula: ME x 0.62 (McDonald et al., 2002).
[e] Combined analysis of 6 pH values recorded every 10 min from 10 to 60 min after rumen liquor addition to empty piston pipettes (C), or with addition of 1 (level 1) or 2 (level 2) mg SB or CMC/ml rumen liquor.
[f] Standard error of the mean.
[g] Statistical analysis limited to C *vs.* SB and C *vs.* CMC comparisons due to experimental design.

Table 4.7
Dry matter intake and intakes of some chemical components of the TMR of high producing dairy cows in the control (C), sodium bicarbonate (SB) and calcium magnesium carbonate (CMC) treatments.

	Treatment				*P*		
	C	SB	CMC	SEM[b]	C *vs.* SB	C *vs.* CMC	SB *vs.* CMC
kg/day							
Dry matter	28.2	28.5	28.6	0.27	0.64	0.56	0.89
aNDFom[a]	9.15	9.00	9.04	0.093	0.52	0.62	0.86
Crude protein	4.41	4.52	4.53	0.026	0.16	0.14	0.83
Starch	4.4	4.5	4.7	0.12	0.82	0.36	0.46
Ash	2.56	2.68	2.79	0.016	0.06	0.02	0.07
g/day							
Ca	211.6	214.3	266.4	1.91	0.55	<0.01	<0.01
Mg	90.0	90.8	118.2	1.08	0.72	<0.01	<0.01
Na	84.2	143.5	88.2	2.71	<0.01	0.53	<0.01

[a] Neutral detergent fibre assayed with heat stable amylase expressed exclusive of residual ash.
[b] Standard error of the mean.

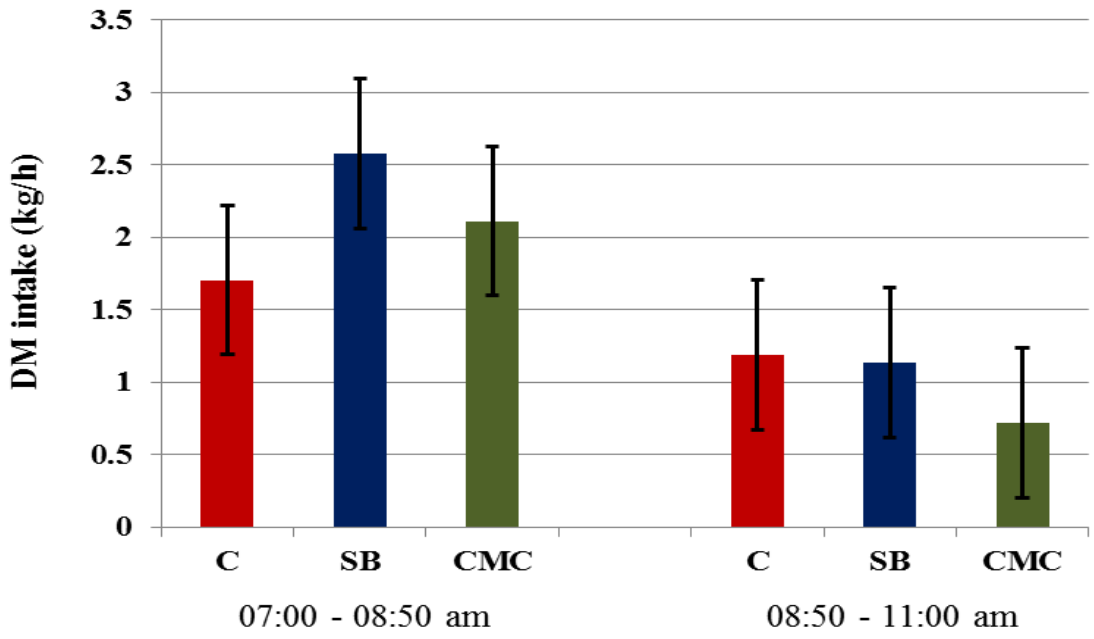

Fig 4.1
DM intake patterns of high producing dairy cows between 07:00 and 08:50 and 08:50 and 11:00 am in the control (C), sodium bicarbonate (SB) and calcium magnesium carbonate (CMC) treatments. C *vs.* SB: *P*=0.16; C *vs.* CMC: *P*=0.91; SB *vs.* CMC: *P*=0.15; time: *P*<0.0001; time*treatment: *P*=0.17; SEM=0.516.

4.3. Digestibility and faecal pH

The DM digestibility of the SB diet tended to be lower (*P*=0.05) than that of the C diet. The CP and fat digestibility was higher (*P*=0.04) for the CMC compared to the SB supplemented diet (Table 4.8). The CMC supplemented cows had higher (*P*=0.03) Na digestibilities than C cows, and SB cows tended (*P*=0.06) to have a higher Na digestibility than C cows. There was a tendency for a higher K digestibility for CMC compared to the SB (*P*=0.07) and C (*P*=0.06) groups.

Table 4.8
Faecal pH and total tract digestibility (g/kg) of the TMR fed to high producing dairy cows in control (C), sodium bicarbonate (SB) and calcium magnesium carbonate (CMC) treatments

	Treatment				*P*		
	C	SB	CMC	SEM[f]	C *vs.* SB	C *vs.* CMC	SB *vs.* CMC
Digestibility[a]							
Dry matter	656	637	649	2.2	0.05	0.27	0.12
ADF[b]	360	327	345	13.7	0.35	0.63	0.58
aNDF[c]	393	358	381	9.1	0.19	0.57	0.34
aNDFom[d]	417	382	408	11.0	0.25	0.73	0.35
CP	609	594	621	2.7	0.12	0.16	0.04
Starch	995	1000	978	11.0	0.85	0.51	0.42
Fat	842	831	860	3.2	0.22	0.10	0.04
Ash	440	439	426	11.0	0.97	0.59	0.61
Ca	332	286	300	25.1	0.45	0.59	0.80
Mg	157	111	170	27.4	0.49	0.84	0.39
Na	719	765	787	5.6	0.06	0.03	0.19
K	816	821	849	4.1	0.63	0.06	0.07
S	632	611	633	7.1	0.28	0.94	0.26
P	384	342	373	15.0	0.30	0.75	0.42
Zn	217	188	215	21.4	0.56	0.96	0.59
Cu	139	106	143	31.4	0.65	0.96	0.62
Mo	83	25	77	10.7	0.11	0.83	0.13
Se	475	489	496	15.0	0.69	0.56	0.84
Cl	751	726	728	4.5	0.10	0.12	0.84
Mn	81	60	95	28.7	0.76	0.82	0.60
Faecal pH[e]	6.60	6.65	6.76	0.027	0.09	<0.0001	<0.01

[a] Based on two TMR samples collected per treatment per period (18 samples total) and composite faecal samples pooled by pen and period.
[b] Acid detergent fibre.
[c] Neutral detergent fibre assayed with heat stable amylase expressed inclusive of residual ash.
[d] aNDF expressed exclusive of residual ash.
[e] Faecal samples collected during the last day of each period, only using cows that remained in originally assigned pens throughout the experiment (*n* = 49 cows).
[f] Standard error of the mean

Faecal pH had a tendency to be lower in cows that consumed the C diet compared to those consuming the SB diet (P=0.09), while the faecal pH of CMC cows was higher than both the SB (P<0.01) and C (P<0.0001) treatments (Table 4.8).

4.4 Milk yield, milk composition and performance characteristics

Milk and milk lactose yields (Table 4.9) were lower for SB supplemented compared to C cows (P<0.01 and P=0.02, respectively). There was a tendency (P=0.05) for SB supplemented cows to yield less milk true protein. Milk fat proportion was elevated in SB *vs.* C (P<0.01) and SB *vs.* CMC (P=0.01) supplemented cows, which corresponds to a similar trend in the milk energy concentration where SB supplemented cows produced milk with a higher (P<0.01) energy density compared to C and CMC supplemented cows. Milk protein content was elevated (P=0.04) in the SB compared to CMC supplemented cows, and showed a tendency (P=0.07) to increase in SB supplemented *vs.* C cows. There was a higher (P<0.01) fat to protein ration (FPR) in SB supplemented *vs.* C cows, and a trend (P=0.07) to a higher FPR in the SB compared to CMC supplemented cows. Cows in the SB treatment group produced milk with a higher lactose proportion compared to the CMC treatment group (P=0.0098), with no difference between SB and C treatment groups. Somatic cell count (SCC) level in milk was not affected by treatment.

Milk Ca and Na concentrations were similar among treatments, but there was a tendency (P=0.05) for a higher milk Mg concentration in C *vs.* SB supplemented cows (Table 4.10).

Table 4.9
Production performance and energy output of high producing dairy cows (*n* = 430 and 112 for milk and BCS data, respectively) in the control (C), sodium bicarbonate (SB) and calcium magnesium carbonate (CMC) treatments.

	Treatment				*P*		
	C	SB	CMC	SEM[a]	C *vs.* SB	C *vs.* CMC	SB *vs.* CMC
Yield (kg/d)							
Milk	46.2	45.2	45.7	0.36	<0.01	0.16	0.19
Fat	1.58	1.60	1.58	0.018	0.41	0.90	0.34
True protein	1.36	1.34	1.34	0.010	0.0502	0.11	0.71
Lactose	2.21	2.16	2.18	0.018	0.02	0.12	0.43
Energy (MJ/d)	132.1	131.4	131.0	1.13	0.60	0.40	0.75
Composition (g/kg)							
Fat	34.3	35.6	34.7	0.31	<0.01	0.29	0.013
True protein	29.5	29.7	29.5	0.10	0.07	0.81	0.04
Fat:protein ratio	1.165	1.199	1.177	0.0098	<0.01	0.29	0.07
Lactose	47.73	47.85	47.66	0.070	0.105	0.34	0.0098
Energy (MJ/kg)	2.86	2.92	2.88	0.013	<0.01	0.39	<0.01
SCC (x 1000 cells/ml)	210	223	224	27.7	0.69	0.65	0.96
Body Condition Score							
BCS, units	2.31	2.33	2.33	0.034	0.37	0.29	0.88
BCS change, units/30 d	-0.07	-0.09	-0.03	0.023	0.62	0.21	0.08
Energetics							
Total NE output (MJ/d)	172.4	170.9	173.2	1.50	0.68	0.81	0.53
Diet NE concentration (MJ/kg)	6.12	6.00	6.06	0.023	0.12	0.38	0.28

[a] Standard error of the mean

Table 4.10
Effects of sodium bicarbonate (SB) and calcium magnesium carbonate (CMC) supplementation on digestible intakes and milk concentration of Na, Ca and Mg of high producing dairy cows.

	Treatment				*P*		
	C	SB	CMC	SEM[a]	C *vs.* SB	C *vs.* CMC	SB *vs.* CMC
Digestible intakes (g/d)							
Ca	69.2	61.5	80.1	5.63	0.56	0.44	0.24
Mg	13.9	9.6	20.0	2.86	0.53	0.40	0.21
Na	61.6	110.7	70.7	3.17	0.02	0.29	0.02
Milk concentration (mg/L)							
Ca	1190	1212	1200	24.0	0.51	0.76	0.72
Mg	104	97	100	2.5	0.05	0.29	0.34
Na	323	330	323	12.3	0.62	0.99	0.60

[a] Standard error of the mean

Chapter 5: Discussion

5.1 Product and ration evaluation

The nutrient profile of the feeds (Table 4.2 and 4.3) indicates that these were generally similar to those reported by Swanepoel et al. (2010) in a survey of typical California dairies. However, there was a relatively low DM and high ash level for alfalfa fresh chop, while fresh chop NDFom, canola pellet aNDF and almond hulls aNDF and aNDFom also differed slightly, compared to studies reported by Swanepoel et al. (2010). The ingredient composition of the diets (Table 4.4) was also similar to that of the dairies reported by Swanepoel et al. (2010). Notable exceptions were the lower relative incorporation of corn silage, alfalfa hay and alfalfa fresh chop. However, our ration contained 18 ingredients, excluding the buffers, which was higher than the average of 10 and a maximum of 14 ingredients/dairy reported by Swanepoel et al. (2010) and explains, at least in part, why some ingredients had lower incorporation levels. Overall, these comparisons indicate that our experimental diets were reasonably representative of typical contemporary California dairies.

Similar *in vivo* aNDF digestion and $dNDF_{30}$ values for the three experimental TMR indicate that aNDF digestion was not affected by treatment. The increase in 24 h *in vitro* gas production was likely due to dissociation of SB which resulted in an increase in gas volume due to CO_2 liberation, which is further supported by the low apparent DM for SB of 725 g/kg, which likely represents decomposition of SB at high temperatures and the subsequent release of CO_2 gas rather than true DM content. This is evidence to support use of $dNDF_{30}$ rather than *in vitro* gas production, to calculate dietary NE_l concentration. Increased *in vitro* gas production of CMC supplemented *vs.* C diets is difficult to explain, as CMC is not considered soluble at normal rumen pH, and does not buffer rumen fluid as demonstrated by a lack of change in *in vitro* pH between C and CMC supplemented diets. A low pH of the rumen fluid of the donor cow used for *in vitro* gas production may have led to partial dissociation of CMC and an associated increase in CO_2 production. However, although rumen fluid pH was not recorded, a low pH of the rumen fluid was unlikely as gas production of the incubated standard sample was normal and the donor cow was fed an all hay diet. A more likely explanation is

that a small fraction of CMC is soluble at normal rumen pH, which resulted in a slight increase in gas production and associated release of Ca and Mg. It has been reported that Mg has a positive effect on rumen microbial growth (Galbraith et al. 1971) and OM digestibility in sheep (Wilson, 1980), and that Ca increases cellulose degradation when rumen bacteria are exposed to increasing levels of Ca (Bryant et al., 1959; Morales Silva, 2005). An increase in cellulose digestion would further result in an elevated acetate to propionate ratio and increased gas volume, as propionate contains an extra carbon atom that would otherwise have formed a carbon dioxide molecule (Wolin, 1960), and it has therefore been recommended that differences in molar proportions of VFA are accounted for when reporting gas production values (Schofield and Pell, 1995). It is thus possible that slight increases in rumen liquor Ca and Mg concentrations with the CMC supplemented diet stimulated microbial activity and VFA production, or that differences in VFA proportions lead to an increase in gas volume *in vitro*. However, similar *in vivo* aNDF digestibilities between C and CMC supplemented diets indicates that potential increases in Ca and Mg concentrations in rumen fluid did not occur *in vivo*, or that differences occurred without affecting microbial fermentation and fibre degradation in the animal due to a more complex interaction between feed, animal and microbial population *in vivo*. This is further supported by the fact that there were no differences in milk Mg concentrations or Mg digestibility between C and CMC supplemented diets, considering that the rumen is the main or only site of Mg absorption in adult ruminants (Pfeffer et al., 1970; Martens and Rayssiguier, 1980; Martens and Gabel, 1986). Milk Ca and Mg concentrations of our study are consistent with previous research, where average Ca and Mg concentrations were 1201 and 100 in our study *vs.* a range of 1040 to 1280 and 100 to 150 mg/L, respectively, as reported by Lucey and Horne (2009). However, milk Na concentrations were marginally lower in our study compared to that reported by Lucey and Horne (2009; 325 *vs.* 350 to 600 mg/L).

While the lack of treatment differences in estimated NE using $dNDF_{30}$ is consistent with the similar calculated *in vivo* NE among treatments, there is a disagreement in absolute NE values between the two methods as NE predicted by $dNDF_{30}$ is substantially higher than the *in vivo* calculated NE value (Tables 4.6; 4.9). Since it is generally considered that *in vivo* NE prediction is the method of choice as it measures energy output in the animal, it is likely that the $dNDF_{30}$ prediction is

an overestimation of actual NE value of the diet in our study. Indeed, average $dNDF_{30}$ was 496 g/kg, compared to an average *in vivo* aNDF digestibility of 402 g/kg. Furthermore, Robinson and McQueen (1992) reported that approximately 0.86 of NDF whole tract digestion occurs in the rumen, and if this factor is used, predicted rumen aNDF digestion in our study would be 347 g/kg, which represents a 30% lower prediction compared to $dNDF_{30}$. Using this 'corrected' *in vivo* digestibility, predicted NE of the diet, using the $dNDF_{30}$ formula, would fall to 6.63 MJ/kg, which is much closer to the calculated *in vivo* NE value of 6.06 MJ/kg.

It is therefore likely that the high DM intakes of our cows resulted in a relatively lower rumen retention time of aNDF, and that the $dNDF_{30}$ incubation period was too long. This indicates that it may be necessary to reduce the dNDF incubation time for studies with cows that have high DM intakes, and use a 24 h, or even shorter, incubation period. Further support for this would come from studies which have reported mean rumen retention times of specific ingredients to be as low as 11 h for grains (Ayala-Burgos et al., 2003) and 23 h for forages (Ayala-Burgos, 1997).

5.2. Effects of sodium bicarbonate supplementation

5.2.1 Gastrointestinal effects

That DM intake was not affected by SB in our study, is consistent with a meta-analysis by Hu and Murphy (2005), who found that SB supplementation increased intake by 1.24 kg/d for corn silage based diets, but did not affect intake in non corn silage-based diets. Based on our experimental diet's low corn silage and starch levels, the acid production potential of the ration was likely low, while relatively high alfalfa and fibre levels resulted in increased dietary buffering capacity and rumination, respectively. This contrasts with numerous previous studies which had corn silage-based diets with high dietary proportions of starch, in which SB supplementation resulted in substantial productive benefits (e.g., Snyder et al., 1983; Erdman et al., 1980a; Rogers et al., 1985).

One of the typical responses to supplementing SB is an increase in rumen pH, but there have been reports of no effects (e.g., Hu and Murphy (2005) in non corn silage based diets; Kennelly et al.,

1999) or even a decrease (Rogers et al., 1985) in rumen pH. While it is important to remember that tendencies and numerical differences do not represent statistically significant differences and should therefore be treated with caution, it is interesting to note several statistically significant and non-significant parameters, which in combination, may point to a rumen buffering effect with SB supplementation in our study. There was a tendency for an elevated *in vitro* pH of the SB supplemented diet, while there was also a substantially lower numerical Mg digestibility in SB compared to C and CMC supplemented cows (111 *vs.* 170 and 157 g/kg). . The reticulorumen is the main site of Mg absorption, and rate of absorption is dependent on concentration, which increases with a decrease in rumen pH. Therefore, a numerical decrease in Mg digestibility for SB supplemented cows may have been due to a rumen buffering effect of SB and subsequent reduction in rumen Mg concentration and digestibility. A large variation in Mg digestibility is consistent with results of a study by Lomba et al. (1968), in which it was found that endogenous faecal Mg losses are highly variable, and large variations in Mg digestibility may have masked detection of statistical significance between treatment means in our study. A tendency for lower milk Mg concentration of SB supplemented cows may serve as further support for this theory. In addition, a large numerical difference in intake during the first 110 min of the morning between SB supplemented *vs.* C and CMC supplemented cows (50 and 24% higher, respectively), may indicate that rumen pH did not decrease to the same extent as in C or CMC supplemented cows, resulting in a delay of feed intake inhibition normally associated with a decrease in rumen pH. Furthermore, a trend to an elevated faecal pH with SB supplemented cows indicates that there was likely a buffering effect on the GIT. However, if there was indeed a buffering effect with SB supplementation, it would be difficult to know if this occurred ruminally or post ruminally. Nevertheless, the findings above in combination with previous research in which SB supplementation was found to result in rumen buffering in dairy cows (e.g., Rogers et al., 1982; Staples et al., 1986; Wiedmeier et al., 1987), supports the possibility that there was a rumen buffering effect with SB supplementation in our study, which may have further resulted in residual hindgut buffering.

Rumen buffering is often associated with a shift in VFA ratios to an increased acetate: propionate ratio, and to a change in microbial activity, where fibrolytic activity is progressively reduced as pH

values fall below 6.0 (Simpson et al., 1977; Mould et al., 1983; Hoover et al., 1984). This has been attributed to inhibition of growth of several bacterial species (Russell et al., 1979), washout of cellulolytic bacteria (Russell and Dombrowski, 1980), and a reduction in number of cellulolytic microbes *(*Mould et al., 1983; Mould and Orskov, 1983; Hoover et al., 1984). A shift in VFA ratios has been linked to changes in milk fat proportion and yield due to propionate's lipogenic properties (as propionate stimulates insulin secretion which increases body fat deposition, thus reducing milk fat synthesis). However, in the current study, an increase in milk fat proportion without affecting milk fat yield indicates that the rumen buffering effect may have occurred without a shift in VFA ratios. This is consistent with the meta analysis of Hu and Murphy (2005), who reported that SB supplementation decreased molar proportions of propionate in SB supplemented cows fed a corn silage based diet, but did not affect acetate or propionate concentrations or their ratios in cows fed non corn silage based diets, while rumen pH was not altered. If there was in fact a rumen buffering effect with SB supplementation in our study, this discrepancy with Hu and Murphy (2005) may be due to SB supplementation providing only a minimal buffering effect *in vivo*, which is reasonable considering the tendency for a small *in vitro* pH difference between C and SB supplemented diets, thus not appreciably affecting VFA production. It has been suggested that rumen pH should be maintained above 6.0 to 6.1 to avoid inhibition of cellulolysis (Mould et al., 1983) and, as *in vivo* NDF digestibility did not differ between C and SB supplemented diets, it may be speculated that rumen pH was predominantly above this level. Furthermore, a relatively high and stable rumen pH would imply that rumen buffering was not physiologically required for the conditions and type of diet fed in this experiment, i.e., a diet with normal fibre levels (aNDF levels of 334 for this diet *vs.* NRC (2001) minimum recommendation of 250 g/kg), relatively low corn silage and readily fermentable carbohydrates levels, and very high producing cows. While high DM intakes of cows may increase their risk of acidosis due to a relatively higher intake of fermentable carbohydrates, fibre intakes also increased and would be expected to stimulate rumination thereby enhancing saliva flow and buffering capacity. Furthermore, a high milk production level would sustain a large diffusion gradient of substrates (i.e., VFA) between the rumen, blood and mammary gland, therefore maintaining high rates

of removal of VFA from the rumen into the blood to ensure relatively high and stable rumen pH levels.

An alternative theory explaining the effects of SB on productivity, is an increased water intake, increased rumen fluid dilution rate and increased starch flow out of the rumen resulting in decreased propionate production (Russell and Chow, 1993). The tendency for reduced DM digestibility with SB supplementation and numerically lower digestibility of aNDFom, CP, and fat, without a change in $dNDF_{30}$, may be indicative of an increased rate of passage. While increased rate of passage supports an increase in feed intake capacity, this could have been counteracted by an increase in water intake, being consistent with the equal DM intakes among C and SB supplemented cows. As discussed previously, a shift in propionate production, and/or VFA ratios, in this study was unlikely. However, an increased rate of passage is possible without affecting propionate production. The low starch levels of our diet (i.e., 160 g/kg DM) may have resulted in virtually complete starch fermentation in the rumen, resulting in little or no starch flow from the rumen, and an increased rate of passage would therefore not affect rumen propionate production. This hypothesis is supported by a study conducted by Wiedmeier et al. (1987), in which outflow of rumen fluid increased from 68 to 88 L/d without affecting the concentration of acetate or propionate, or their ratio. Although the cows used were "barren" (i.e., probably cows with a low DM intake), the estimated starch content according to ingredient composition of that diet was 257 g/kg DM, which may be comparable to our lower starch content (160 g/kg DM), assuming a lower intake and rate of passage in the cows used by Wiedmeier et al. (1987).

Recent research indicates that SB may elicit its physiological effects on milk fat synthesis by increasing biohydrogenation of fatty acids in the rumen due to the elevated pH (Bauman and Griinari, 2003; Oetzel, 2007; Fuentes et al., 2009). This reduces the amount of specific fatty acids that are absorbed from the small intestine which are known to directly inhibit milk fat synthesis in the mammary gland (i.e., predominantly *trans*-10 $C_{18:1}$ and *trans*-10, *cis*-12 conjugated linoleic acid (CLA)). While it is possible that the rate of fatty acid biohydrogenation was increased in this study due to a higher rumen pH in SB supplemented cows, an increased rate of passage may have negated this effect, resulting in absorption of similar amounts of inhibitory fatty acids from the small intestine

and equivalent milk fat yields between C and SB supplemented cows. However, based on this diet's normal fibre and, probably, low unsaturated fat levels, it is unlikely that the microbial capacity for biohydrogenation of unsaturated fatty acids was overwhelmed, and that amounts of inhibitory fatty acids absorbed, regardless of changes in rate of passage, were not large enough to lead to treatment differences in milk fat yield. Based on these hypotheses, it may be speculated that the increase in milk fat proportion was largely due to a concentration of milk fat due to the reduced milk yield of SB supplemented cows.

An increase in the fat to protein ratio (FPR) of SB supplemented cows primarily reflects the increased proportion of fat, and similar protein proportions. A FPR of about 1.18 among treatments would indicate that the cows in our study were in a positive energy balance, at least according to Hagert (1991) and Dirksen (1994), who found that a ratio of less than 1.4 was indicative of optimal or positive energy balance, and those above 1.4 of an energy deficit. When cows are in an energy deficit, fat mobilization from adipose tissue partially maintains milk fat synthesis, and a dietary deficiency of energy in the rumen results in reduced microbial protein synthesis and a subsequent reduction in milk protein. However, FPR is a moderately good indicator of energy status (Hagert, 1991; Dirksen, 1994) and as cows in the current study were in negative energy balance (based on a loss of body condition) despite having a FPR of less than 1.4, suggests that FPR should not be used as an indicator of energy status in isolation. Enemark (2009) reported that a normal FPR ratio is 1.0-1.5, with values below 1.0 being indicative of SARA. A ratio in our study much higher than the critical value of 1.0 demonstrates that the risk of SARA for our C cows was likely very low, and may partly explain the lack of benefit with SB feeding.

The tendency of SB supplemented cows to have lower milk protein yield may be due to a combination of interrelated factors. While an increase in yield and efficiency of microbial protein synthesis occurs with increased rate of ruminal passage and associated higher dilution rates (Hoover and Miller, 1992; Firkens et al., 1992; Bach et al., 2005a), microbial protein synthesis is also correlated with digestibility where, on average, 16.9 g microbial crude protein is synthesized per 100 g apparently digested OM (Stern and Hoover, 1979). Feeding SB may therefore have stimulated microbial protein synthesis due to a higher rate of passage but, to a larger degree, inhibited microbial

protein synthesis by reducing DM digestibility and energy available to microbes, thus reducing protein flow to the small intestine and decreasing milk protein synthesis. While it has been found that microbial protein flow is higher at lower rumen pH (Bach et al., 2005a), this is unlikely to have affected our results as this relationship is an indirect consequence of the inverse relationship between rumen pH and the dietary level of highly fermentable carbohydrates which increases the energy supply to, and thus protein synthesis by, rumen microbes (Stern et al., 2006).

5.2.2 Effects on blood acid base balance, mineral metabolism and fixed solids discharge

Apart from the potential buffering effect on the digestive tract, SB likely also affected blood acid base balance of the cows, due to the change in DCAD. It has been reported in a meta-analysis by Hu and Murphy (2004) that DCAD has a quadratic effect on milk and milk fat yield. Based on DCAD values observed in our study, the DM intake and milk and milk fat yields are consistent with results of of Hu and Murphy (2004). Using DCAD equations developed during this meta analysis, DM intake of the C and SB supplemented diets would both be predicted at 19.6 kg/d (Fig 5.1), while milk yield of the C and SB supplemented diets would be predicted at 24.3 and 24.1 kg/d, respectively, at a corresponding increase in DCAD of 37.5 to 45.6 mEq (Na+K-Cl)/100 g (Fig 5.2). It is possible that the small difference in predicted milk yield between C and SB supplemented diets would be amplified at higher rates of production, which would be consistent with our results. Furthermore, 40 g/kg FCM would be predicted at 22.9 *vs.* 23.0 kg/d, respectively, which is consistent with our results of almost identical milk fat yield between C and SB supplemented cows.

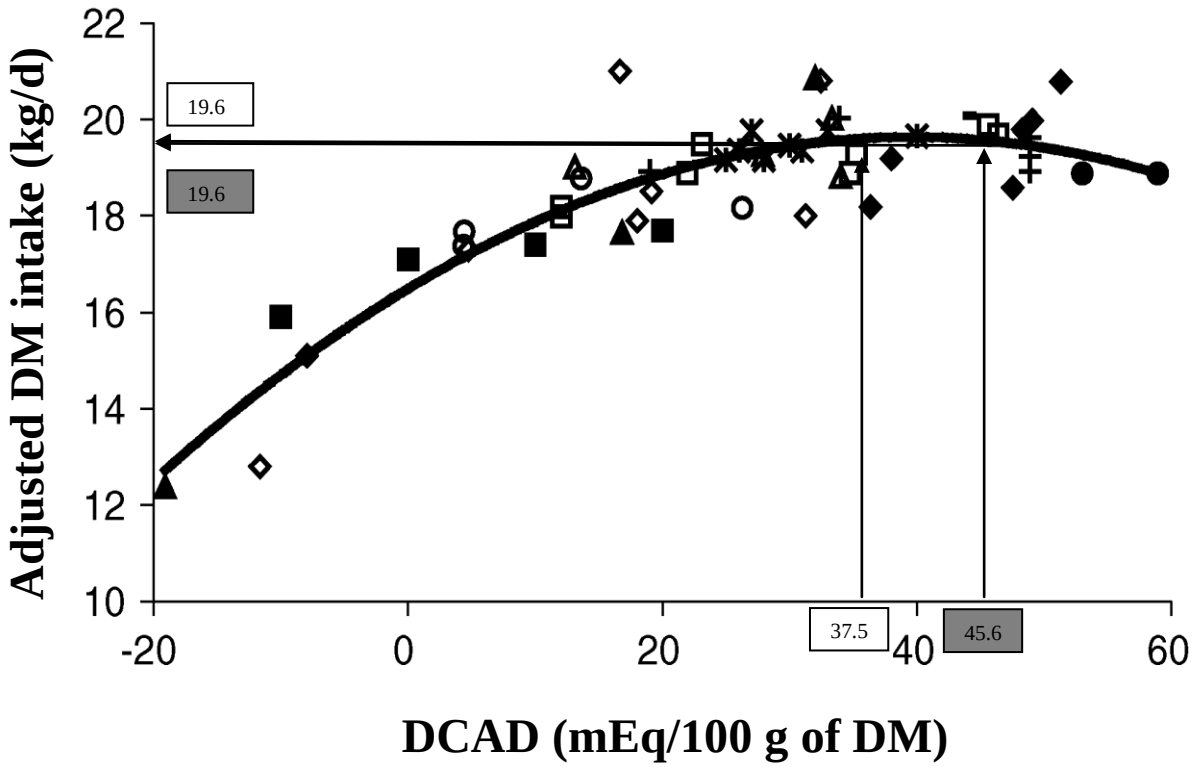

Fig 5.1: Effects of dietary cation-anion difference (DCAD) on DM intake (adjusted for random effect of study) for studies reported in a meta-analysis conducted by Hu and Murphy (2004), and predicted adjusted DM intake according to DCAD levels of the C (▭) and SB (▬) supplemented diets in our study.

The NRC (2001) recommendations for Na, K and Cl at milk yields of 45 kg/d are 2.2, 10.6 and 2.8 g/kg, respectively. This results in a DCAD of 28.8 mEq/100 g, compared to a DCAD of 34, 40 and 49 that has been found to maximise milk yield, DM intake and 40 g/kg FCM, respectively, in a meta-analysis conducted by Hu and Murphy (2004). This suggests that current NRC (2001) recommendations are unlikely to maximise milk yield, DM intake and milk fat yield (Hu and Murphy, 2004), which may also have indirect consequences on health and productivity of cows as peak milk yield is related to total lactation milk yield, while maximizing DM intake is crucial during early lactation to minimize energy deficit and related problems such as ketosis.

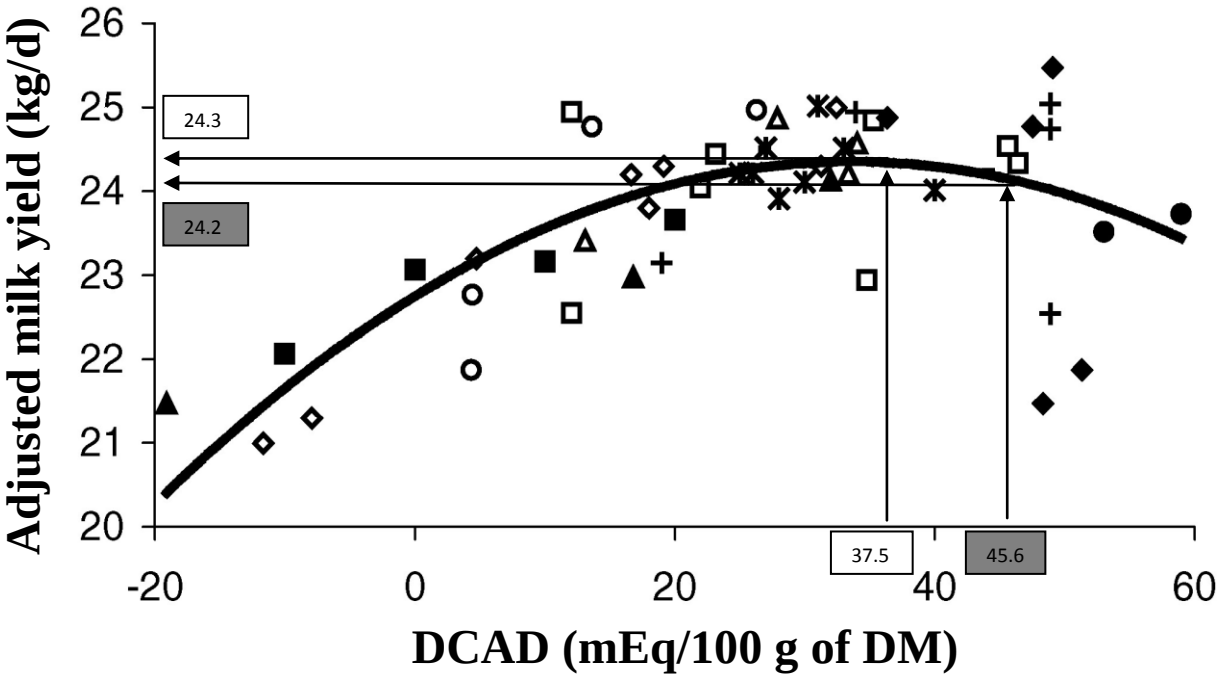

Fig 5.2: Effects of dietary cation-anion difference (DCAD) on milk yield (adjusted for random effect of study) for studies reported in a meta-analysis conducted by Hu and Murphy (2004), and predicted adjusted milk yield according to DCAD levels of the C (▭) and SB (▬) supplemented diets in our study.

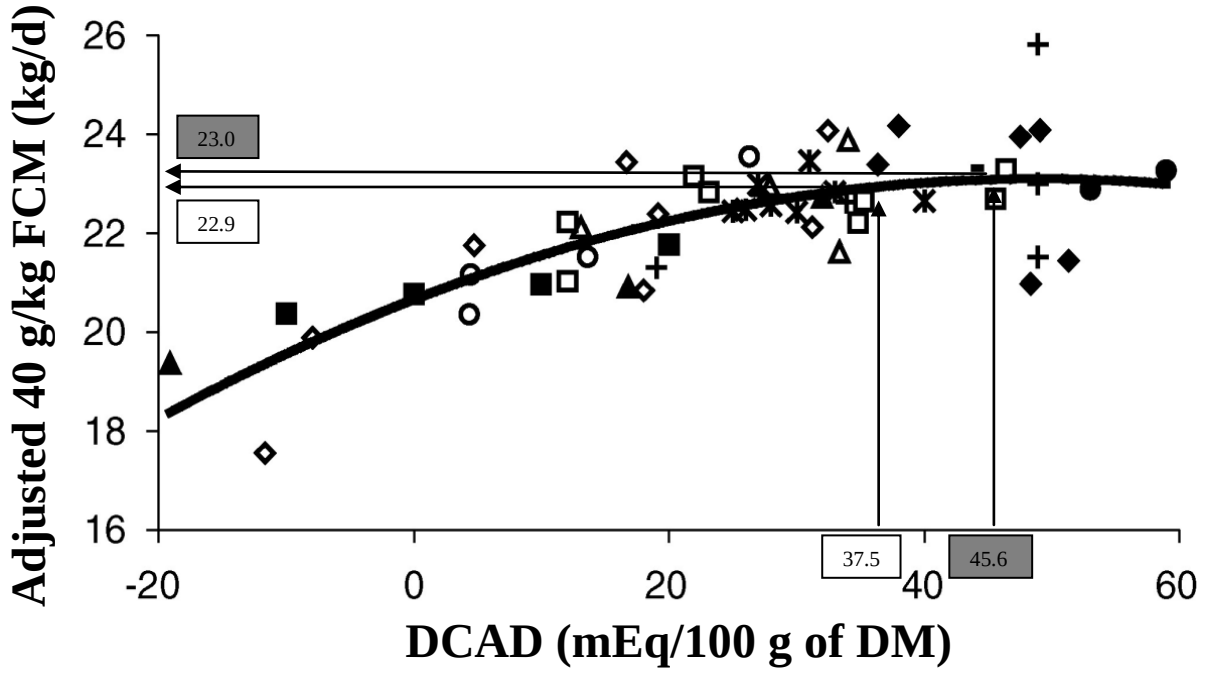

Fig 5.3: Effects of dietary cation-anion difference (DCAD) on 40 g/kg FCM yield (adjusted for random effect of study) for studies reported in a meta-analysis conducted by Hu and Murphy (2004), and predicted adjusted 40 g/kg FCM yield according to DCAD levels of the C (▭) and SB (▬) supplemented diets in our study.

Increased Na digestibility, and higher levels of Na in the SB supplemented diet, resulted in substantially higher intakes of absorbable Na compared to the C diet (Table 4.10). As blood Na levels are tightly regulated (Hu and Murphy, 2004), excess Na must leave the blood via one or more of several pathways. One involves Na loss in saliva and sweat associated with heat stress, but as this study was conducted in the spring, with average minimum and maximum temperatures of 6.7 +/- 3.46 and 21.0 +/- 5.09◦C, respectively, it is unlikely that cows experienced sweating or excess saliva production leading to increased Na excretion.

A second output for excess Na is bone deposition. While soft tissue contains Na at ~600 mg/kg wet mass, these levels are closely regulated by reabsorption and excretion in the kidneys via endocrine control to regulate blood pressure and fluid volume (Williams et al., 1983; NRC, 2001; Leheska et al., 2008). Although bone Na represents ~350 g/kg total body Na, only about half of this Na is bound to the surface of bone and part of the dynamic Na pool (Ammerman and Goodrich, 1983; Greene and Kleeman, 1991). This amounts to 10 mmol/kg BW and, in a 650 kg cow, this available Na equals about 149.5 g. Even in the unlikely scenario of this Na reservoir being completely depleted at the onset of SB feeding, it could have been replenished within about 3 days of SB feeding, at least based on differences in digestible Na intakes between C and SB supplemented cows, due to the relatively low Na requirements for bone deposition in relation to amounts supplied (Table 5.1). Any additional Na would have likely been shunted to the final and most important route of Na excretion, which is urine.

Digestible intakes of Na for C and SB supplemented cows were 61.6 and 110.7 g/d, which is equivalent to a 79.7% increase over C cows, while milk Na concentrations were not affected. Therefore, dietary SB supplementation likely substantially increased Na) discharge from the cows resulting in an increase in soil and water sodicity and the associated deterioration in soil and water quality.

Reduced milk yield of SB supplemented cows may have been due to increased intake of Na, consistent with previous studies which have found decreases in milk yield with an increase in Na intake. For example, Solomon et al. (1995) reported a reduction in milk yield from 35.2 to 33.1 kg/d when Na intake from water and salt supplementation was 69.3 and 46.0 g/d in saline and desalinated

water treatments, respectively, despite similar DM intakes (22.6 and 23.0 kg/d, respectively). Jaster et al. (1978) reported a decrease in milk yield from 34.8 kg/d in cows receiving normal tap water (196 ppm dissolved salts) to 32.9 kg/d in cows receiving saline water (tap water plus 2500 mg/L NaCl). Subclinical Na toxicity, resulting in symptoms like temporary diarrhea and reduced milk production, may occur in beef cattle consuming water or a diet with a NaCl concentration above 1 g/kg on DM basis (i.e., 0.393 Na/kg; Van Leeuwen, 1999), but caution must be exercised when extrapolating from beef to dairy cows due to differences in physiological requirements. Nevertheless, our C and SB supplemented diets contained 3.0 and 5.0 g Na/kg DM, respectively, compared to the NRC (2001) recommendation of 2.2 g Na/kg DM for cows with a daily milk yield of 45 kg. A reduction in milk yield may have occurred due to increased water loss in urine without an equivalent increase in water intake to compensate for increased water loss. Urine volume is a function of Na intake (Bannink et al., 1999), and therefore SB supplemented cows likely produced more urine than C cows. Using the equation proposed by Bannink et al. (1999), urine volumes of C and SB supplemented cows were estimated to be 37.2 and 43.7 kg/d, respectively. If cows were not consuming enough water to compensate for this additional loss, or if there was a physiological limitation in absorption and metabolism of additional water required (e.g., limitations in renal capacity), there may have been a physiological shortage of fluid in the body resulting in the reduction in milk yield. While milk lactose concentrations did not differ between C and SB supplemented cows, it may be hypothesized that in an attempt to regulate fluid homeostasis, milk yield was directly limited via a reduction in lactose synthesis which resulted in a decreased milk yield, as lactose is the primary osmotically active component of milk (Capuko and Akers, 2010).

5.2.3 Concluding remarks

Based on a lack of improvement in productivity or efficiency and a likely increase in Na discharge, use of SB in similar diets and conditions is not supported. Considering our results and previous research, SB supplementation is not supported for high producing cows consuming diets with ‘normal’ fibre levels, relatively low starch levels and not based solely, or mainly, on corn silage.

As our study was similar in production level and diet to many modern California dairies (Swanepoel et al., 2010) and because Kellogg et al. (2001) reported SB use in the Western USA (i.e., California, Colorado, Idaho, Oregon, Utah, Washington) at 0.85 of producers surveyed, it appears likely that SB supplementation frequently occurs without improving animal productivity or efficiency, while likely increasing Na output by the cows and the dairies.

5.3 Effects of CMC supplementation

5.3.1 Effects on the gastrointestinal tract and dairy cow productivity

The elevated faecal pH of CMC-supplemented cows without a change in *in vitro* ruminal fluid pH (Table 4.6) suggests that its buffering effect occurred post ruminally. The low pH of the abomasum is most conducive to CMC dissociation, and therefore it is likely that the GIT buffering occurred in the abomasum as well as the small and large intestine. However, buffering may also have occurred indirectly as a result of the Ca in CMC, which would be consistent with Noel et al. (1981), who reported that elevated dietary Ca increased pancreatic bicarbonate secretion. However, despite a buffering effect of CMC in the GIT based on an elevated faecal pH, there were no changes in digestibility, productivity (i.e., milk yield, milk composition and BCS) or efficiency of the cows as assessed by similar NE output and NE concentration of C and CMC supplemented diets. . Overall, this indicates that the buffering effect of CMC probably was not physiologically required, which may be due to the low dietary starch level. Limestone supplementation to diets with high starch levels (i.e., 518 g/kg DM) has previously resulted in lower faecal starch levels (89 *vs.* 221 g/kg DM) and a substantially higher faecal pH (8.21 *vs.* 5.67; Rogers et al., 1982). In this context, the relatively high faecal pH of 6.60 in our C cows indicates that hindgut fermentation and acid production was limited, which is supported by the virtually complete starch digestion *in vivo*. This lack of difference in animal productivity is consistent with Crawford et al. (2008), who reported no differences in average daily gain, DM intake, gain to feed ratio, water intake or rumen pH of beef steers supplemented with 75 or 150 g/kg DM of CMC. However, if the numerically smaller loss in BCS with CMC supplementation

was real, this could indicate a positive response since a key management objective during early lactation is to minimise loss in BCS in order to reduce associated depressions in productivity and incidence of related diseases such as ketosis. Control and CMC supplemented diets did not differ in DCAD, and acid-base mediated effects on performance were not expected. This lack of difference in milk yield, fat yield and DM intake between C and CMC supplemented diets is consistent with expectations according to the respective DCAD of our diets (Hu and Murphy, 2004).

5.3.2 Effects on mineral metabolism and dairy fixed solids discharge

Control and CMC-supplemented cows had similar intakes of digestible Na without differences in milk Na concentration, and therefore CMC feeding likely did not increase Na discharge of the dairy and prevented an increase in soil and water sodicity and related deterioration of water and soil quality. However, considering that productivity and efficiency of CMC supplemented cows was not improved, use of CMC in similar diets and conditions is not supported. Due to limited CMC research with lactating cows, future studies should examine effects of CMC supplementation on dairy cows under different conditions, especially those known to result in lower rumen pH (e.g., corn silage based diets) in order to determine if rumen solubility of CMC is increased and, if so, whether responses in productivity occur.

Chapter 6: Conclusions

Many published experiments have examined effects of SB supplementation on dairy cows fed rations very different from those typically used on contemporary California commercial dairies. Not only did ingredients often differ, but milk yields were often much lower. Furthermore, the low number of cows used in most university-based experiments limits the potential to detect differences in productivity. Thus, results of such studies have limited applicability to current practical conditions.

In this study, SB supplementation resulted in an increase in milk fat proportion, but reduced milk yield. As a result, there were no differences in milk fat yield or in efficiency of energy use of diets between C and SB supplemented cows. Changes in milk fat proportion and milk yield were likely due to an increase in DCAD and/or a rumen buffering effect. While there were no productive benefits of SB use, it likely substantially increased Na discharge resulting in an increase in soil and water sodicity and the associated deterioration in soil and water quality As the diet and experimental parameters, such as level of milk yield, of this study are typical of many modern California dairies, it is likely that a substantial proportion of current SB supplementation occurs without meaningful benefits to productivity, while increasing Na discharge. For diet and animal conditions comparable to those of this study, including typical California dairies with high yielding cows consuming diets with ‘normal’ fibre levels and a relatively low proportion of starch and corn silage, use of SB in the diet is not supported, based upon results of this study.

While CMC supplementation did not improve productivity or efficiency of dietary energy use of cows, Na discharge from the dairy was not increased. Therefore, use of CMC likely prevented an increase in soil and water sodicity and related deterioration of soil and water quality when compared to SB supplementation. However, for conditions comparable to those of this study, including typical California dairies, use of CMC in the diet is not supported due to a lack of improvement in animal performance.

Chapter 7: Critical evaluation

7.1 Review of critical factors

Conducting an experiment can be difficult, and it is therefore essential that it is meticulously designed and conducted. However, even when all schedules and protocols are thoroughly debated and planned, problems can still occur. In hindsight, a few aspects of this study could have been done more effectively.

7.1.1 Feed push up

The general philosophy for conducting this experiment was that there should be as little interference to normal operations as possible due to the study. However, it was noted during feed weighing that the high variability in hourly intake was partly due to the nature of feed push up which involved a perpendicular and horizontal action (i.e., some feed was pushed in and out of premarked sections at random). It may have been beneficial to prevent mechanical feed push-up on feed weigh days in favour of a manual push up. However, this could have influenced normal feeding behaviour of cows due to the activity of people working at the feedbunk and it might not have resulted in any benefit. Alternatively, electronic scales built in under the feedbunk could have given accurate feed intake data throughout a 24 h period, although the very high costs of such a system limits their use to university farms or research institutions.

7.1.2 Communication

Since most of the dairy farm workers only spoke Spanish, communication was a challenge at times, and it would have been beneficial to have had some Spanish language skills. In particular, the second feeding of one of the pens occurred earlier than usual during the final scheduled weighing of TMR for DM intake pattern determination, mainly because of a miscommunication due to the language barrier. There was little that could have been done at that point, which resulted in a loss of intake data for one pen during T110 – T 240 of the final period. This may partly explain why there

was a large numerical difference in intake between treatments without significance in the time*treatment interaction.

7.1.3 Pen counts

The DM intake initially relied on pen counts from the DairyComp 305 system. However, during the initial stages of the study, it was noticed that one of the pens had an unusually high number of cows, according to the DairyComp 305 system. Subsequent physical pen counts confirmed that this particular DairyComp 305 pen count was not accurate and from then on weekly physical pen counts were used for DM intake calculations. If this error would have remained undetected, it is likely that DM intake values would have been inaccurate. Initial DairyComp 305 pen counts (excluding the one outlier that was detected and corrected) remained very close to the average and therefore the DM intake values as reported accurately represent actual values. Nevertheless, a good philosophy for any study is to meticulously record anything out of the norm that may or may not seem relevant at the time, and to verify data by another approach wherever possible.

7.2 Benefits of conducting studies on commercial farms

While there are clear practical limitations to conducting a study on a commercial dairy, such as the inability to access and measure rumen parameters, there are several substantial benefits to be gained. Many scientific studies are conducted with small numbers of animals and therefore have limited replication due to obvious financial and time constraints, and this often results in difficulty to establish statistical significance in vital measurement parameters, especially in those that have a large standard error such as milk yield. Furthermore, diets, conditions, management and animals used in these university studies may not be fully representative of those used in commercial situations, which can limit potential application of results. Studies such as this one, on the other hand, highlight the importance of the practical applicability of the findings, which in turn can simplify important on-farm decisions and result in improvements in productivity, health, efficiency, environmental sustainability and/or economic viability of a farm and an industry.

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