Understanding and mitigating the impacts of major dietary changes on dairy cows

Victoria Marie Russo

Bachelor of Animal Science, Master of Agricultural Science

ORCID 0000-0002-5361-6015

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Department of Agriculture and Food Systems
Faculty of Veterinary and Agricultural Sciences
The University of Melbourne

Abstract

Four experiments were conducted to investigate the effects of major dietary changes on ruminal pH, ruminal fluid composition, eating behaviour, feed intake and milk production of dairy cows. The impacts of both diet composition and management strategies were evaluated. The initial experiment investigated the impact of early adaptation when instigating a complete dietary change from one forage to another at calving, as is common practice in Irish dairy farming. Three weeks prior to their expected calving date, 14 spring calving dairy cows were assigned to one of two treatments: pasture silage pre-partum followed by fresh cut perennial ryegrass (PRG) post-partum, or fresh PRG both pre and post-partum. There were no differences in dry matter intake (DMI), body condition score, energy balance or milk yield and composition between the treatments. The results of the initial experiment suggested that early adaptation to avoid a major dietary change at calving did not result in health or production benefits. This was speculated to be due to the similarities of the two diets, creating little challenge for the rumen to adapt.

The second experiment focused on a more challenging dietary change, incorporating a large amount of concentrate into a forage-only diet. Thirty-two lactating dairy cows were initially fed 100% lucerne hay cubes, wheat was then gradually substituted in until it comprised 40% of total dry matter (DM) and lucerne hay cubes, the remainder. Wheat was substituted for lucerne cubes via one of four strategies, (1) in six small increments (each 6.7% of total DM) over 6 days; (2) in six small increments (each 6.7% of total DM) over 11 days; (3) in three large increments (each 13.3% of total DM) over 6 days; or (4) in three large increments (each 13.3% of total DM) over 11 days. The 6-day strategies are considered rapid for the dairy industry yet none of the treatments resulted in ruminal fluid pH values that would have compromised ruminal function, nor were there differences in DMI or energy corrected milk (ECM) yields. Furthermore, there were no differences between ruminal fluid volatile fatty acid (VFA), lactate or ammonia concentrations. It is speculated that the properties of the lucerne cubes helped the ruminal contents resist the pronounced declines in pH often seen with the fermentation of large amounts of wheat. These results suggested that changes to rumen function are driven not only by the characteristics of the concentrate being introduced but also by those of the forage.

The third experiment aimed to investigate the role of forages in grain adaptation. Twenty-eight lactating dairy cows were fed either PRG hay or lucerne hay and wheat was gradually substituted for forage in three equal increments, over 6 or 11 days, until wheat made up 40% of DM (~ 8 kg DM/cow per day). The results varied significantly with forage type. Cows fed lucerne hay ate more, produced more ECM and had lower ruminal pH values. Furthermore, of the cows fed lucerne hay, those adapted to wheat in the shorter time frame (6 days) exhibited significantly lower mean ruminal pH values. Despite the ruminal pH of these cows declining to levels typically considered low, none of their other

measured parameters indicated compromised fermentation or acidosis. Rather, it was these same cows that had the greatest ECM yields, producing an average of 1.5 kg ECM/cow per day more than their 11-day counterparts. The 6-day adaptation strategy allowed for a rapid increase in metabolisable energy, while the hay promoted adequate buffering within the rumen. No difference was seen between adaptation strategies when PRG hay was fed. This was due to the higher metabolisable energy concentration and lower intake of the PRG hay resulting in a less pronounced increase in metabolisable energy intake with the wheat substitution. The greater intakes of cows fed the lucerne hay likely contributed to their greater ECM yields and lower ruminal pH values. However, both forages allowed the rumen contents to resist the large declines in ruminal pH that are typically seen during rapid grain adaptation.

The final experiment aimed to further evaluate the role that forage plays in ruminal, behavioural and production responses to the incorporation of large amounts of wheat grain into the diet. Sixteen dairy cows in early lactation were fed a forage only diet of either lucerne hay, PRG hay or one of two cultivars of fresh PRG pasture (cultivar Bealey or Base) for three weeks. The forage-only diet was then supplemented with crushed wheat grain at a rate of 8 kg DM/cow per day, with no adaptation period. Wheat comprised between 32 and 43% of total DMI and was fed over two meals, followed by forage, for one day only. Feeding fresh pasture resulted in lower ruminal pH values, with pH remaining below 6.0 for longer each day. Following supplementation of wheat, cows fed pasture exhibited ruminal fluid pH levels associated with sub-acute ruminal acidosis. Hay created a ruminal environment that was better able to cope with the influx of acid produced as wheat was digested. A combination of increased ruminating time and a decreased rate of fermentation are likely responsible for the higher ruminal fluid pH values. The ruminal environment of cows fed lucerne hay remained most stable throughout the grain challenge, with ruminal fluid spending the least amount of time below pH 6.0.

Reducing the introductory time for concentrates into a dairy cow's diet means an ability to rapidly increase the energy content of a diet, resulting in milk production benefits. However, this thesis highlights the importance of forage choice when determining introduction strategies. Traditional, gradual adaptation strategies must still be employed when feeding highly digestible fresh forages. However, more aggressive adaptation strategies can be implemented when hays are used as the base forage. In situations where high energy grains are substituted for a low energy, high fibre basal forage, rapid introduction can have milk production benefits over gradual strategies.

Declaration

This is to certify that:

i. The thesis comprises only my original work towards the PhD except where indicated in the preface,

ii. Due acknowledgement has been made in the text to all other material used,

iii. The thesis is fewer than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Victoria Marie Russo

26/07/2018

In loving memory of my Dad and Murphy.

Preface

This thesis contains the following original manuscripts by V.M. Russo as the primary researcher and author:

1.	Full title:	**Manipulation of the pre-partum diet of dairy cows to promote early adaptation to fresh perennial ryegrass**
	Authors:	V. M. Russo, W. J. Wales, B. J. Leury, M. C. Hannah and E. Kennedy
	Candidates contribution:	80%
	Status:	Unpublished material not submitted for publication
2.	Full title:	**Effect of wheat adaptation strategies on rumen parameters and dry matter intake of late lactation dairy cows**
	Authors:	V. M. Russo, B. J. Leury, E. Kennedy, M. C. Hannah, M. J. Auldist and W. J. Wales
	Candidates contribution:	80%
	Status:	Published
	Journal name:	Animal Production Science
3.	Full title:	**Forage type influences milk yield and ruminal responses to wheat adaptation in late lactation dairy cows**
	Authors:	V. M. Russo, B. J. Leury, E. Kennedy, M. C. Hannah, M. J. Auldist and W. J. Wales
	Candidates contribution:	80%
	Status:	Published
	Journal:	Journal of Dairy Science
4.	Full title:	**Forage type influences ruminal responses to a wheat grain challenge in early lactation dairy cows**
	Authors:	V. M. Russo, B. J. Leury, E. Kennedy, M. C. Hannah, M. J. Auldist, G. L. Morris and W. J. Wales
	Candidates contribution:	80%
	Status:	Unpublished material not submitted for publication

For all publications included in this thesis, co-authors of published work have completed The University of Melbourne's co-author authorisation form. All co-authors have certified that the contribution of the candidate is greater than 50% and is the primary author of these publications.

The candidate's advisory panel has approved inclusion of the published work listed in this thesis. The principal supervisor of this work, Professor B. J. Leury, has signed The University of Melbourne's declaration for a thesis with publication.

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List of abbreviations and symbols

%	Percent
~	Approximately
<	Greater than
>	Less than
≥	Greater than or equal to
°C	Degrees Celsius
ADF	Acid detergent fibre
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BCS	Body condition score
BHBA	Beta-hydroxybutyric acid
BW	Body weight
CF	Crude fat
CH_4	Methane
Cl	Chloride
CO_2	Carbon dioxide
CP	Crude protein
d	Day
DCAD	Dietary cation-anion difference
DEDJTR	Department of Economic Development, Jobs, Transport and Resources
DIM	Days in milk
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
ECM	Energy corrected milk
FCE	Feed conversion efficiency
g	Gram
GC	Gas chromatography
h	Hour
K	Potassium
kg	Kilogram
ME	Metabolisable energy
MJ	Megajoule
mL	Millilitre
MLW	Metabolic live weight
MY	Milk yield
Na	Sodium
NDF	Neutral detergent fibre
NEB	Negative energy balance
NEFA	Non-esterified fatty acids
NFC	Non-fibrous carbohydrates
OD	Outer diameter
PMR	Partial mixed ration
PRG	Perennial ryegrass
S	Sulfur

SARA	Subacute ruminal acidosis
SD	Standard deviation
SED	Standard error of the difference
TDN	Total digestible nutrients
TMR	Total mixed ration
VFA	Volatile fatty acids

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Chapter 1

Introduction

1.1 General introduction

A major change to the diet of a dairy cow can influence numerous physiological and behavioural processes including intake, milk production, eating behaviour, ruminal fluid pH and fermentative end products. The magnitude of the dietary change, as well as feeding management during the process are critical factors in determining successful adaptation to the new diet. The pasture-based dairy systems of Victoria (Australia) and Ireland both implement major dietary changes at various times throughout the year. These changes pose unique challenges that must be overcome to ensure cows adequately adapt to the new diet and milk production is optimised.

Dairy farming systems throughout Australia can generally be categorised into one of five systems based on their key feed inputs. Of these five systems, four are pasture based and are as follows:
System 1: low bail system, including grazed pasture, other forages and less than 1.0 t/cow.year of concentrates fed in the dairy shed during milking;
System 2: moderate-high bail system, including grazed pasture, other forages and more than 1 t/cow.year concentrates fed in the dairy shed during milking;
System 3: partial mixed ration (PMR) system, including pasture grazed all year, PMR on a feed pad and concentrates fed in the dairy shed;
System 4: hybrid system, including pasture grazed for a period of less than 9 months per year, PMR on a feed pad and concentrates fed in the dairy shed.
All four of these systems use both pasture and concentrates as major feed components. Hence, changes incorporating more or less of either component must be implemented at some point throughout the lactation.

In Ireland throughout winter, when pasture is limited due to poor growth, cows are housed indoors and fed predominantly pasture silage. Immediately post calving, in spring, they are switched to a diet of grazed pasture and concentrates in the dairy. As the weather and the pasture availability begin to decline later in the year, they are once again returned indoors and fed pasture silage. In Victoria, most dairy cows receive a well formulated ration and pasture throughout early and mid-lactation, resulting in very stable milk production. Later in lactation, as spring gives way to summer, pasture availability declines and they are moved to a diet largely consisting of conserved forages and cereal grains. In autumn, when the pasture growth returns, the late lactation cows are moved back to large amounts of grazed pasture.

As the main source of feed for dairy cows in both Victoria and Ireland, forages influence how the rumen functions. The contribution of forages to maintaining a stable rumen environment derives largely from their impact on fermentation and rumination. The microbial digestion of forages typically occurs slowly and results in a gradual release of energy, in the form of organic acids. However, the rate and

extent of fermentation varies considerably both between and within forage types. Their impact on ruminal pH, end products of digestion and eating behaviour varies greatly and impacts heavily on animal production. Furthermore, the fermentation of forages may influence the adaptation process to new diets, particularly the introduction of concentrates. Forages alone cannot meet the energy demands of a high producing dairy cow. Hence, in pasture-based systems, the modern dairy cow requires large amounts of concentrates in order to approach her genetic potential. Concentrates, such as barley, wheat and maize grain, ferment rapidly compared to forages and large amounts of organic acids are produced, potentially resulting in acidotic conditions. The period of greatest risk for the cow is generally when concentrates are first offered, as the microbial populations are not yet adapted to cope with the extra acid load. Therefore, an adequate adaptation process needs to be implemented. This typically involves gradually increasing the amount of grain over a number of weeks but varies with the type of concentrate being introduced and the target quantity. For pasture-based dairy systems there are no detailed guidelines on the most efficient management techniques with which to implement major dietary changes. The use of a gradual adaptation process can sacrifice convenience and feed efficiency (energy corrected milk yield/feed dry matter intake). Insight into the quickest yet most optimal form of adaptation, in terms of feeding behaviour, rumen function, dry matter intake and milk production, would improve nutritional management decisions for pasture based dairy systems.

1.2 Objective of this thesis

The objective of this thesis is to investigate factors that influence the adaptation of the rumen following major dietary changes in pasture-based systems by establishing the effects on ruminal pH, dry matter and energy intake and milk production, and where possible propose strategies to alleviate the problems encountered. A further objective is to determine the role of both the initial diet and the diet being introduced and how management decisions influence the adaptation process.

1.3 Specific aims of this research

- To determine the role of both the initial diet and the new diet in an adequate adaptation.
- To determine how management strategies can affect adaptation to a new diet, specifically the stability of the rumen environment and consequently, dry matter intake, eating behaviour and milk production of dairy cows.
- To determine how the choice of forage affects the stability of the rumen environment, specifically ruminal fluid pH.

1.4 Scope of this thesis

This thesis will investigate the effects of major dietary changes on ruminal pH, ruminal fluid composition, eating behaviour, feed intake and milk production of dairy cows. This thesis will focus on dairy production systems that offer forage and concentrate separately, as is most common in both the

Victorian and Irish dairy industries. Investigating the effects on the rumen microbiome, rates of feed degradation and post ruminal digestion are outside the scope of this thesis.

1.5 Description of the research within this thesis

The research contributing towards this thesis investigates major dietary changes to forage fed dairy cows. The initial experiment, detailed in Chapter 3, investigated how an abrupt change between two forages affected intake, eating behaviour and subsequent milk production. The experiment was conducted in Ireland where the diet of dairy cows is changed abruptly from pasture silage to fresh grazed pasture at calving. Chapter 4 describes an experiment investigating the effects different wheat introduction strategies have on the adaptation process. Four different strategies were used to introduce large amounts of wheat into the diet of previously non-adapted cows. Chapter 5 then describes how responses to the wheat adaptation strategies differ with basal forage, using two types of conserved forage and two wheat introduction methods. Chapter 6 reports on the final experiment of the thesis which investigated the effects of an extreme wheat challenge on non-adapted cows consuming one of four forage types. The concluding chapter, discusses the impact of the research within the dairy industry and its implications for mitigating the effects of major dietary changes on dairy cows.

Chapter 2

Review of scientific literature

2.1 The Victorian dairy industry

The majority of Australian dairy farms are located in the south-eastern state of Victoria. Victoria produced 5.77 billion litres of milk in 2017-18, 64% of the nation's milk production (DEDJTR 2018). There are currently 3,881 dairy farms in Victoria with an average herd size of 259 cows and an average annual milk production of 6,072 L/cow (Dairy Australia 2018). Due to favourable climatic conditions, the Victorian dairy industry is primarily pasture based, with grazed pasture accounting for ~47% of total metabolisable energy (ME) fed (DEDJTR 2018). Based on feed inputs, dairy farms within Victoria can be categorised into five main systems, these are:

System 1: low bail system, including grazed pasture, other forages and less than 1.0 t/cow.year of concentrates fed in the dairy shed during milking;

System 2: moderate-high bail system, including grazed pasture, other forages and more than 1 t/cow.year concentrates fed in the dairy shed during milking;

System 3: partial mixed ration (PMR) system, including pasture grazed all year, PMR on a feed pad and concentrates fed in the dairy shed;

System 4: hybrid system, including pasture grazed for a period of less than 9 months per year, PMR on a feed pad and concentrates fed in the dairy shed;

System 5: total mixed ration (TMR) system, i.e. zero grazing, cows are housed and fed a total mixed ration all year.

According to the most recent survey by Dairy Australia (Dairy Australia 2015) grazed pasture is used by 99% of Victorian dairy farmers (systems 1 through 4) and of those, 96% incorporate some type of supplementary feeding such as concentrates, hay or silage. In the current survey, of the 99% of Victorian farms utilising grazed pasture 23% are categorised as system 1, 65% as system 2, 6% as system 3 and 4% as system 4.

Most Australian pasture based dairy systems manage their herds to optimise the utilisation of the seasonal growth of pasture. This is done through calving in late winter/early spring followed by 10 months of lactation and an 8-week drying off period. This strategy leads to Victoria's peak milk production occurring in October (spring), approximately two months after the peak in calving (DEDJTR 2018). Ryegrass dominant pasture is the most common forage utilised. However, during summer, it is of poor quality (high neutral detergent fibre (NDF) concentration, low dry mater digestibility (DMD) and low crude protein (CP) concentration) (Walsh and Birrell 1987; Smith *et al.* 1998; Doyle and Stockdale 2011) due to its poor tolerance of high temperatures and water deficits. This variability in pasture nutritive characteristics means that the utilisation of supplements, both concentrates and conserved forage, varies with season. In 2017/18 concentrates comprised ~31% of total ME fed to Victorian dairy cows, and conserved forage (hay and silage) made up ~21% (DEDJTR 2018). The most commonly fed concentrates are wheat, maize and barley grains. The majority of Victorian dairy farmers (65%) offer >1.0 tonne/cow.year of concentrates through the parlour during milking (system 2) (Dairy

Australia 2015). Concentrates may be fed at a single rate throughout the supplement feeding period or further variability could be included by stepping the amount up or down depending on characteristics such as stage of lactation.

2.2 The Irish dairy industry

Ireland has a total of 18,000 dairy farms and an average herd size of 80 cows (Irish Food Authority 2017). Like Victoria, the Irish dairy industry is reliant on pasture for most of its feed, allowing it to remain highly cost efficient. Irish dairy farmers typically use a combination of grazed pasture, pasture silage and concentrates to meet the energy needs of their herds. Similar to the pasture based systems of Victoria, the majority of Irish dairy farms implement seasonal based calving systems in order to match pasture supply with feed demands (Dillon *et al.* 1995; Horan *et al.* 2005). Based on this premise, a compact calving period occurs at the end of winter aiming to match peak milk production with the peak pasture growing season. The grazing season runs from February to November, after which an indoor period begins, and silage is fed. During this period of silage feeding, cows are supplemented with concentrates in order to meet milk production potentials (Dillon *et al.* 1995; Kennedy *et al.* 2005). Typically, during the grazing season low amounts of high energy concentrates (~3 kg/cow per day) are fed to maximise ME intake or when pasture supply or nutritive characteristics are limiting. Concentrates in Ireland are normally fed as a pellet comprised of a cereal such as wheat grain, a protein source and additional energy sources such as molasses and citrus pulp. The results of a high pasture and low concentrate system is reflected in a relatively low yield per cow, averaging 5,000 L in 2017 (Irish Food Authority 2017). However, the national herd size has increased by 300,000 cows in the last 4 years, now reaching 1.4 million cows (Irish Food Authority 2017). With a growing herd and increasing cow productivity total milk production reached 7.1 billion L in 2017 and is forecast to increase a further 30% by 2020 (Bord Bia 2017).

2.3 The rumen

This section has been written to provide a basic overview of the rumen and its function. A detailed review of the anatomy, histology and physiological regulation of rumen function, including absorption mechanisms, is beyond the scope of this thesis and has been covered in the following publications: Baker *et al.* (1984); Dijkstra *et al.* (1993); Abdoun *et al.* (2006).

The digestive system of ruminants is unique in that it has four stomach compartments, the reticulum, rumen, omasum and abomasum. Despite anatomical differences, the reticulum and rumen are only partially separated by a fold of tissue, and the two compartments operate in combination. The reticulo-rumen, the first and largest of the compartments, contains 65 to 80 % of total ingesta in the digestive tract of a cow (Holmes *et al.* 2007). The anaerobic microorganisms within the reticulo-rumen (bacteria, protozoa and fungi) are responsible for microbial digestion of feed to provide energy, protein, minerals

and vitamins to the host. Microbial digestion can only occur once feed has been adequately masticated to allow the microbes access to the interior of the plant material. Feed within the rumen is organised in a stratified layer according to particle size. The smallest particles occupy the ventral part of the rumen and particles of increasing size above, with the largest particles floating on top. The dorsal part of the rumen is occupied by gases produced during fermentation. Feed is mixed and moved around via contractions of the rumen wall which are covered by highly vascularised papillae designed for the absorption of fermentative end products. Largest particles are regurgitated back up the oesophagus and re-masticated, through the process of rumination. The rate at which feed is digested and which end products are produced is determined largely by feed composition and particle size. The primary end products of rumen fermentation are volatile fatty acids (VFA), ammonia, CO_2 and CH_4. The fermentation pathways leading to these can be seen in Figure 2.1.

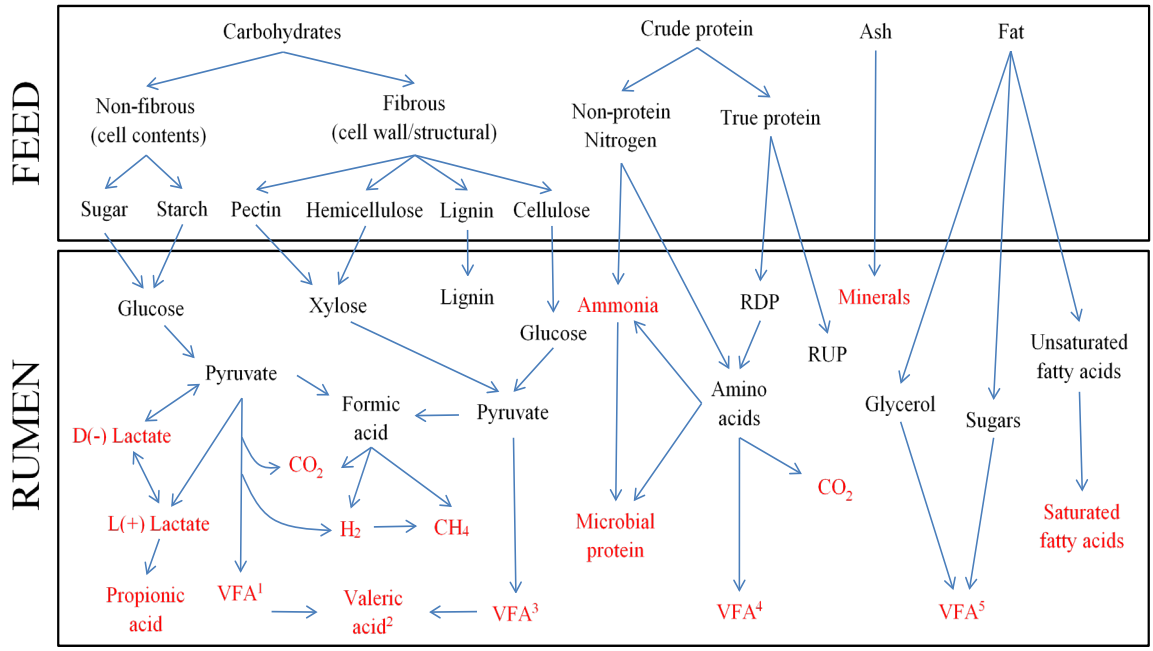

Figure 2.1. The fermentation of feed within the rumen, highlighting end products (items written in red). Adapted from: Bergman (1990); Leek (1993); Harfoot and Hazlewood (1997); Holmes *et al.* (2007); Dijkstra *et al.* (2012); Owens and Basalan (2016).

[1]Volatile fatty acids; mainly propionic and butyric acids.
[2]Valeric acid is produced through the combination of acetate and propionate.
[3]Volatile fatty acids; mainly acetic acid.
[4]Volatile fatty acids; acetic, propionic, butyric, isobutyric, isovaleric and 2-methylbutyric acids.
[5]Volatile fatty acids; mainly propionic and butyric, but also caproic acid.

Primary bacteria degrade feed and are categorised as either cellulolytic or amylolytic, depending on whether they degrade cellulose or starch. Secondary bacteria utilise the end products of primary bacteria digestion and include the bacteria like microbes (Archaea) responsible for CH_4 formation and those bacteria that convert lactic acid to propionate. While not essential for a ruminant to survive, the

protozoa fulfil roles that contribute to optimising rumen function (Williams and Coleman 2012). The ruminal protozoa engulf starch particles and ruminal bacteria and use lactate as an energy source. They are responsible for bacterial protein breakdown and feed protein degradation, contributing to ruminal ammonia (Newbold *et al.* 2015). Additionally, the protozoa contribute to fibre digestion (Williams and Coleman 2012). The protozoa are highly sensitive to changes in the ruminal environment, particularly low pH. Fungi are present in the smallest proportions of ruminal microbes and they have an active role in fibre degradation (Russell and Rychlik 2001). The microorganisms also act as a major source of protein for the host. They continuously flow out of the reticulo-rumen to the abomasum where they are digested to amino acids, peptides and ammonia. Their loss in population due to outflow is offset by their rapid proliferation within the ruminal fluid. To maintain active fermentation and microbial growth, the microorganisms within the rumen require nutrients and a stable environment. The temperature, pH and anaerobic conditions must all be finely maintained to optimise function. A detailed description of the species compiling the rumen microbiome and their functions can be found in the following review articles: Hobson *et al.* (1982a, 1982b); Jouany and Ushida (1999); Wang and McAllister (2002); Krause *et al.* (2013).

2.4 Ruminal fluid pH

The regulation of ruminal fluid pH is vital for optimising both production and animal health (Kaufmann 1976; Erdman *et al.* 1982; Owens *et al.* 1998; Kolver and de Veth 2002). Hence, understanding and knowing how to control the pH of the rumen is critical in dairy cow management (Humer *et al.* 2018). Ruminal fluid pH, is continually regulated and challenged by changing volumes of saliva, acid production and absorption, water flow and feed composition (Turner and Hodgetts 1955; Bailey and Balch 1961; Rumsey *et al.* 1970; Allen 1997). The type and amount of forage and supplements consumed all effect the ruminal pH of a dairy cow (Appendix I).

Feeds high in carbohydrates, such as wheat grain, are often supplemented to grazing dairy cows to increase dry matter intake (DMI) and meet the energy demands of high milk production. The fermentation of carbohydrates within the rumen by anaerobic microbes results in the production of VFA and lactate (Owens *et al.* 1998), see Figure 2.1. Volatile fatty acids are the main source of energy for dairy cows and are the main source of H^+ within the ruminal fluid and hence acidity (Briggs *et al.* 1957). The major VFA produced are acetate, propionate and butyrate, with the proportion of these varying depending on the diet. Typically, the acetate to propionate ratio will decrease as the proportion of concentrates in the diet increase in relation to forage (Murphy *et al*. 1982; Sutton 1985).

The products of fermentation are continuously being removed from the ruminal fluid, via absorption across the rumen epithelium, eructation or passage to the omasum. The majority of VFA are passively absorbed through the rumen wall for metabolism by body tissues. However, if the amount of readily

fermented carbohydrates entering the ruminal fluid is increased suddenly the amount of acid produced within the rumen also increases. If the amount of acid is in excess of what can be absorbed by the body or buffered by the rumen contents, this can cause the cow to become acidotic, resulting in both production and health issues (Tremere *et al.* 1968; Owens *et al.* 1998). The absorption and removal of acids from the rumen helps maintain a stable pH. Ruminal pH is also important in influencing this rate of absorption. As the proportion of acids in the rumen increases, the rate of absorption also increases but not at a rate sufficient to counter a decline in ruminal fluid pH. While it is acknowledged that in general a negative correlation exists between VFA and ruminal pH (Kolver and de Veth 2002), individual VFA affect ruminal pH differently, due to different rates of absorption. Acetate has the greatest reduction on ruminal pH, followed by propionate, then butyrate (France and Dijkstra 2005).

Bacteria, protozoa and fungi produce lactate during carbohydrate fermentation, which can be absorbed across the rumen epithelium or converted by some bacteria and protozoa within the rumen to acetate, propionate and long chain fatty acids (Goff and Horst 1997). Lactate exists in two isomers, D (-) lactate and L (+) lactate, of which the latter is more dominant in the rumen, while D (-) lactate tends to increase with lower pH (Giesecke and Stangassinger 1980). The contribution of lactate to ruminal acidity is approximately 10 times stronger than the major VFA (acetate, propionate or butyrate), but a constant interaction between the microbes that produce lactate (*Streptococcus bovis*) and those that utilise it (mainly *Megasphaera elsdenii* and *Selenomonas ruminantium*) mean it is normally present in relatively low concentrations within ruminal fluid (Briggs *et al.* 1957; Goff and Horst 1997).

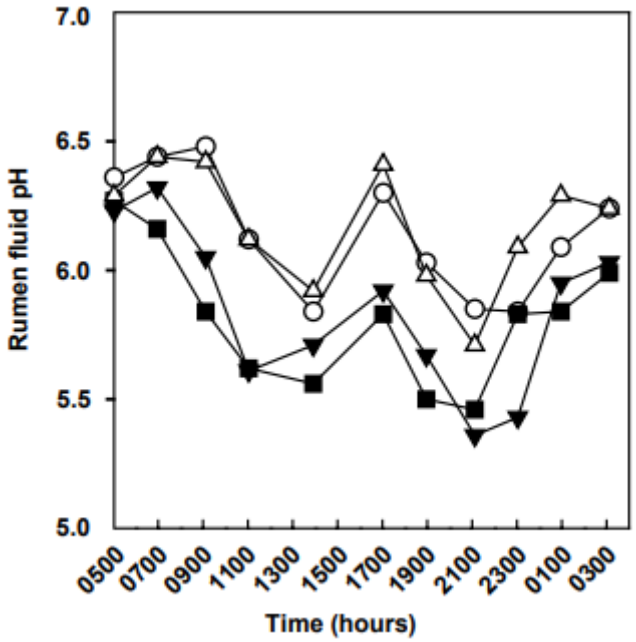

Figure 2.2. Daily ruminal fluid pH pattern of cows fed either pasture only (○), pasture plus 4.7 kg DM cereal grain pellets (■), pasture plus 1.6 kg DM straw (△) or pasture plus 5.0 kg DM cereal grain pellets (▼). Source: Wales and Doyle (2003).

As ruminal pH is affected by numerous factors, both the value and the range can vary greatly throughout the course of a day (Figure 2.2). Variation in feed intake has the major influence on diurnal variation of pH, with pH declining immediately after a meal and then gradually increasing until the next meal (Allen 1997). The pH does not remain constant because the level of acid production and absorption or removal are not equal. Variation in diet also has a major influence on ruminal pH, with readily fermentable concentrates causing greater reductions in pH relative to more slowly fermentable substrates such as conserved forages which are higher in NDF (Allen 1997).

2.4.1 The importance of regulating ruminal fluid pH

The regulation of ruminal pH is of high homeostatic importance, because both alkalotic and acidic fluctuations cause health problems and reductions in milk production. Diets high in protein can result is ruminal alkalosis, due to high concentrations of ammonia. However, issues with high ruminal pH are far less common and consist of far fewer reports (Cordes *et al.* 1969; Loste *et al.* 2005) due to the feeding of energy-rich diets. A low ruminal fluid pH for extended periods of time or on repeated occasions can lead to reduced or variable DMI, impeded nutrient digestion, reduced concentration of milk fat, liver abscesses, laminitis, inflammation and diarrhoea (Owens *et al.* 1998; Dijkstra *et al.* 2012).

Ruminal pH below 6.0 can inhibit the activity of cellulolytic bacteria, causing a reduction in fibre digestion (Erdman 1988; Russell and Wilson 1996). Ruminal pH below 5.0 causes a significant decrease in ruminal activity overall (Dougherty 1976). Therefore, the digestion of high energy feeds that produce large quantities of VFA, can lead to poor fibre digestion, limiting nutrient absorption and impacting both health and production. Due to the reduction in fibre digestion below pH 6.0, this value is often used as a minimum acceptable threshold in systems where total mixed rations are consumed. However, a mean daily ruminal pH between 5.8 and 6.2 has been associated with high producing grazing dairy cows, indicating this as the desired range for achieving peak production and an adequately adapted animal (Kolver and de Veth 2002). Hence, within the literature, the lower limit of ideal ruminal fluid pH is somewhat contentious with arguments for both 5.8 and 6.0.

The regulation of ruminal pH, through minimising peaks and troughs, is also important for optimising cellulose and DM digestion; a response that has been demonstrated both *in vivo* and *in vitro* (Terry *et al.* 1969; Mould and Ørskov 1983; Mould *et al.* 1983; Wales *et al.* 2004). Strategies for minimising the variability and creating an optimum ruminal pH include feeding smaller meals more frequently and feeding concentrates and forage as a single ration (Auldist *et al.* 2013). However, throughout Victoria and Ireland the most common system employed is to feed concentrates twice daily in the parlour during milking. Feeding large amounts of readily fermentable starch in this way can result in huge fluctuations in ruminal pH and extended periods during which ruminal pH is below 6.0 (Wales and Doyle 2003).

2.4.2 Acidosis

The rumen and its microbiome have evolved to efficiently digest forages. However, the nature of dairy systems today means that cows are now consuming large amounts of concentrates in addition to forages. The rapid fermentation of large amounts of high-starch concentrates can lead to ruminal acidosis, a metabolic condition resulting from the accumulation of excess acid within the ruminal fluid. During this process large quantities of VFA, and sometimes lactate, are produced, and cannot be absorbed as quickly as required. This results in a decrease in ruminal fluid pH and reduces the efficacy of rumen flora, impeding digestion (Owens *et al.* 1998). The digestion of starch and decline in ruminal pH to levels below 6.0 favours the proliferation of amylolytic bacteria (Mackie and Gilchrist 1979) while cellulolytic bacteria and associated fibre digestion are inhibited (Russell and Wilson 1996) (Figure 2.3).

Acidosis is categorised as either subacute or acute based on ruminal fluid pH. Subacute ruminal acidosis (SARA) is characterised by a drop in pH primarily due to the accumulation of VFA and the threshold used to define SARA ranges from 5.5 to 5.8 (Krause and Oetzel 2006; Penner *et al.* 2007). Acute ruminal acidosis is often characterised by the accumulation of lactate, particularly D (-) lactate as its prevalence increases at low pH and is the slower of the two isomers to be absorbed and metabolised (Giesecke and Stangassinger 1980; Harmon *et al.* 1985). The threshold used to define acute ruminal acidosis ranges from 5.0 to 5.2 (Nocek 1997; Penner *et al.* 2007). Acute acidosis generally occurs in situations where large amounts of grain are fed to cows that have not been adequately adapted. Inadequate adaptation means that the population of acid utilising bacteria is insufficient to cope with the acid production. Within the pH range 6.0 to 6.5 utilisation of lactate is optimised and so any accumulation is avoided. However, accumulation can occur when adapting cows to a high concentrate diet, as the rate of production exceeds the rate of utilisation (Counotte and Prins 1981; Allen 1997). The microbes that produce lactate are able to respond quickly to the influx of starch by multiplying rapidly and using the available carbohydrate to produce lactate. However, the population of microbes that utilise lactate may take 3 to 4 weeks to increase to numbers great enough to minimise lactate accumulation (Goff and Horst 1997). When the ruminal pH drops below 5.5, lactate is produced at a rate greater than its removal and it begins to accumulate within the rumen (Counotte and Prins 1981; Allen 1997), causing an even greater decline in pH (Figure 2.3). Additionally, as the rumen epithelia are not yet adapted to a high starch diet, absorption of organic acids cannot occur rapidly enough to help alleviate the problem. Furthermore, lactate is absorbed much slower from the rumen than VFA. Hence, acute acidosis is often caused by sudden dietary change (Lean *et al.* 2007). Once the animal has adapted to the diet and the microbiome has successfully shifted, the concentration of lactate will decrease. While an animal is suffering from acidosis, ruminal pH can fall to levels that cause the protozoa and bacteria to die or become completely inactive (Goff and Horst 1997). Clinical signs of acidosis include anorexia, abdominal pain, reduced milk production, scouring, lethargy, staggering, recumbency and death (Krause and Oetzel 2006).

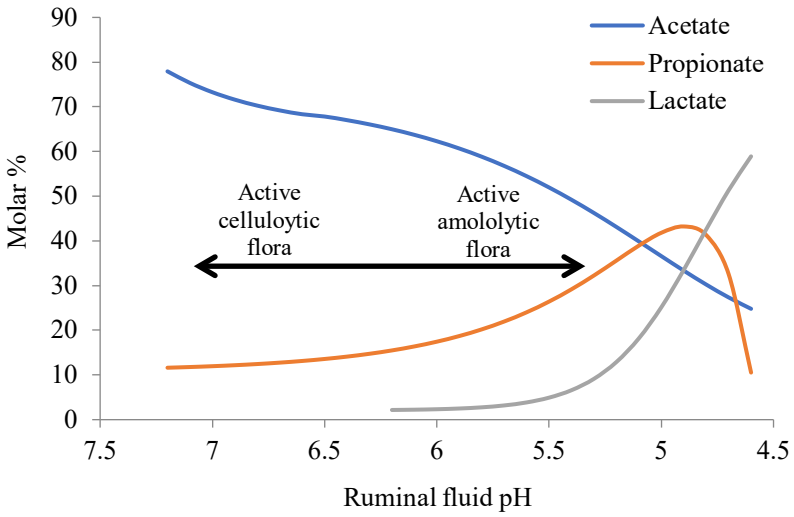

Figure 2.3. The relationship between pH and the major organic acids within the rumen. Adapted from Kaufmann *et al.* (1980).

2.4.3 Measuring ruminal fluid pH

If done correctly, measuring ruminal fluid pH can be a reliable method for diagnosing acidosis as well as identifying optimum rumen adaptation. As ruminal pH varies throughout the day and throughout the rumen, both the timing and the sampling location are critical. There are several different methods for measuring ruminal fluid pH but most, however, have limitations.

The use of a vacuum pump and an oral stomach tube (oro-ruminal probe) to obtain ruminal fluid is commonly used in research settings (Geishauser 1993; Bramley *et al.* 2008; Moate *et al.* 2014). However, this method of collection routinely involves some level of saliva (which has a high pH) contamination, leading to pH readings that are typically higher than other methods (Duffield *et al.* 2004). Rumenocentesis involves inserting a stainless steel needle (e.g. 1.6 mm (OD) × 130 mm) into the ventral rumen, and aspirating ruminal fluid with a syringe (Garrett *et al.* 1999). As this is an invasive surgical procedure, it is more complex and typically involves the use of local anaesthetic and sterile equipment. Ruminal fluid can easily be sampled through a rumen cannula that has been surgically fitted into a cow allowing direct access to the rumen (Duffield 1999; Martineau *et al.* 2015). However, spot sampling has limitations in that to adequately represent daily pH patterns it must be performed frequently throughout the course of a day. This method of sampling, while laborious and faced with limitations, is common in research settings (Greenwood *et al.* 2014).

The methods described previously require the ruminal fluid to be removed from the cow, thereby potentially exposing it to an aerobic environment. The pH of these samples will then vary with exposure to air (Marden *et al.* 2005). The first *in vivo* ruminal pH measurements on cows were taken using a glass electrode inserted through a rumen cannula (Smith 1941). This method still had limitations in that the

measurements were spot samples and did not truly represent daily pH patterns. The first continuous *in vivo* measurements of ruminal pH were reported by Johnson and Sutton (1968) and McArthur and Miltimore (1968), describing measurements over a month with probes robust enough to withstand the ruminal environment. The first technology for continuously measuring ruminal pH meant that any indwelling probes had to be wired to a receiver located outside of the rumen. This then meant cows had to be tethered and confined for measurements. Over the years many researchers have contributed to improving the technology (Maekawa *et al.* 2002a; Beauchemin and Yang 2005; Gasteiner *et al.* 2009; Phillips *et al.* 2010), such that several wireless pH sensors now exist with remote downloading. In order to be delivered into the rumen, the sensors can be dosed orally or inserted via a rumen cannula. It is preferable for sensors to be weighted to ensure they remain on the floor of the rumen and readings are consistently measured from the same location. Sensors typically remain in the rumen for ≥24 h, continually taking measurements and either storing them internally or transmitting them wirelessly. Numerous different sensors have been used and reported in the literature (Penner *et al.* 2006; Kaur *et al.* 2010; Falk *et al.* 2016).

A comparison of techniques (rumenocentesis, oro-ruminal probe, indwelling pH meter and cranial-ventral samples through a cannula) showed significant variability in pH values (Duffield *et al.* 2004). The lowest pH values were reported in samples collected via rumenocentesis, 0.28 to 0.33 units lower than equivalent samples collected via cannula (Garrett *et al.* 1999; Duffield *et al.* 2004). The oro-ruminal probe samples recorded the highest pH values, due to saliva contamination. The strongest correlation was reported between the pH of the rumen cannulation samples and the pH recorded by the indwelling pH meter (Duffield *et al.* 2004). Later research then compared the pH of samples collected via cannula to those recorded by an indwelling pH meter and found a significant difference between the two methods (Marden *et al.* 2005). The pH values obtained via cannula were higher than those recorded by the indwelling probe. Furthermore, there was no correlation between the two sampling techniques, with the difference varying throughout the sampling period. Exposure to atmospheric oxygen was deemed responsible for the variation and therefore only methods that use indwelling probes can provide accurate measurements of ruminal fluid pH.

2.5 Ruminal fluid buffering

The magnitude of ruminal fluid pH changes following feed consumption is dependent not only on the type and amount of feed that is being consumed, but also the buffering capacity of the ruminal contents. The concentration of VFA that accumulates within the rumen at any given point could yield a pH value between 2.78 and 3.03, if dissolved in water (Turner and Hodgetts 1955). However, the pH range within the rumen is typically between 5.50 and 7.80, indicating a highly successful buffering process. Buffering within the rumen is controlled through a combination of feed, saliva and acids. To prevent a drop in pH, the acids that are produced within the rumen need to be eliminated either by absorption

through the ruminal epithelium, passage from the rumen to the abomasum and lower gut or neutralisation by buffers. Absorption is responsible for the removal of approximately 53% of the acids in the rumen, buffered ruminal contents neutralise about 30% and the remainder are washed out continuing further down the digestive tract (Allen 1997; González *et al.* 2012). Both alkalising agents and buffers assist in the neutralising of additional acids within the rumen. A buffer is a material that, when present in a solution, successfully increases its ability to resist changes in pH if an acid or base is added, while an alkalising agent acts by increasing the pH of a solution.

The buffering capacity of the rumen changes depending on the pH. Emmanuel *et al.* (1969) studied the ruminal contents of sheep fed a pelleted roughage-concentrate ration within several pH ranges. The buffering capacity was greatest in the pH range 4 to 6, followed by 5 to 7 and lowest in the 6 to 8 range. In the lowest pH range (4 to 6), a strong correlation between VFA concentrations and buffering capacity was reported, indicating that VFA were contributing significantly to buffering capacity. This was supported by Counotte *et al.* (1979) showing the same pattern in the ruminal fluid of dairy cows. When in their acid form and when partly neutralised, VFA are effective buffers within the rumen and play an important role in preventing further reductions in pH at low levels (Turner and Hodgetts 1955; Briggs *et al.* 1957; Emmanuel *et al.* 1969; Aschenbach *et al.* 2011). Furthermore, as the pH within the rumen decreases, short-chain fatty acids that are absorbed through the rumen wall begin to draw hydrogen ions with them, reducing the acidity within the rumen. However, because the buffering action of VFA is optimised at a low pH, they would potentially stabilise the pH around 4.8 (Counotte *et al.* 1979).

2.5.1 Saliva

The production of saliva is regulated by the animal's diet and is critical for food lubrication as well as pH regulation (Maekawa *et al.* 2002b). Saliva is the greatest contributor to buffering within the rumen; although it is a weak buffer below pH 5.5 and above 7.5 (Bartley 1975). The buffering ability of saliva is due to the high concentrations of bicarbonate and phosphate (Bailey and Balch 1961). A detailed description of saliva composition can be seen in Table 2.1. Bicarbonate and phosphate work in unison to increase ruminal pH through a combination of alkalisation and buffering. Phosphate, while contributing very little to the buffering of ruminal fluid, is valuable in that it neutralises acids. Bicarbonate is identified as the most important buffer within the rumen at normal pH levels (i.e. 5.8 to 7.0) (Emmanuel *et al.* 1969; Counotte *et al.* 1979). When the pH falls below 5.8, bicarbonate no longer plays a significant role in buffering (Turner and Hodgetts 1955) but does act to neutralise acids at pH 6.25 and lower (Counotte and Prins 1978; Counotte *et al.* 1979). Additional to saliva, the rumen epithelium itself excretes bicarbonate into the rumen as well as absorbing acids, both actions contributing to the stabilisation of ruminal pH (Gäbel *et al.* 1991; Aschenbach *et al.* 2011).

Table 2.1. Composition of saliva collected as it descended into the rumen[1]. Values are means from five dry cows with samples collected at 2 and 8 h after the beginning of feeding. All cows were receiving hay with sodium chloride provided *ad libitum*

	Saliva
Sodium	161
Bicarbonate	126
Phosphate	26
Chloride	7.1
Potassium	6.2
Total nitrogen (mg/100 mL)	5.6
Urea nitrogen (mg/100 mL)	3.9
Sum of cations	167
Sum of anions	159
Dry matter (%)	1.02
Ash (%)	0.89
pH	8.4

[1]All values are milliequivalents/L unless otherwise indicated.
Source: Bailey and Balch (1961).

Early lactation dairy cows produce up to 308 L of saliva per day (Cassida and Stokes 1986), with significant diurnal variation in production (Meyer *et al.* 1964). Rate of saliva secretion varies with time after feeding, increasing with time post-feeding (Bailey and Balch 1961; Meyer *et al.* 1964). Production is lowest 1 h after the beginning of feeding and greatest 14 h post-feeding. The rate and amount of salivation is driven by chewing and rumination, which in turn is driven by the diet; namely DM concentration, fibre concentration, intake and particle length (Emery *et al.* 1960; Bailey and Balch 1961; Meyer *et al.* 1964). Saliva secretion increases as the proportion of forage within the diet increases (Maekawa *et al.* 2002b). Salivation is also largely influenced by the water concentration of feed, with increased water concentration resulting in reduced resting and chewing salivation rates (Meyer *et al.* 1964). Appendix II lists the insalivation rate and resting rate of cows on different diets as reported in published literature. Level of feeding can influence saliva composition and pH. For example both the phosphate level of saliva entering the rumen is greater at higher amounts of hay feeding (Bailey and Balch 1961) resulting in higher pH levels (Emery *et al.* 1960).

2.6 Intrinsic buffering capacity of feeds

Ingested feed influences ruminal pH through several factors including salivation, nutritive composition, rate of fermentation, fermentation products and intrinsic buffering capacity. The buffering capacity of feed refers to its ability to resist change in pH when an acid or base is added. Several studies have investigated the intrinsic buffering capacity of a variety of feedstuffs, with particular attention highlighting the high buffering capacity of legumes (Wilson 1935; McDonald and Henderson 1962;

Greenhill 1964; Wohlt *et al.* 1987). While exact figures are difficult to compare due to variations in methodologies, most studies found that grasses tend to have a low buffering capacity compared with legumes. Hays and sources of proteins have a high buffering capacity; approximately three to four times higher than that of cereal grains (Jasaitis *et al.* 1987; Moharrery 2007).

Using a continuous culture system, Crawford *et al.* (1983) investigated the amount of buffer required to maintain ruminal fluid at a pH of 6.5 when fermenting different feeds. They found that all concentrates had higher base requirements than forages, and high energy feeds such as maize, barley and wheat bran required the greatest amount of additional base. In contrast, high protein feeds including corn gluten meal, clover hay and dried brewers grain required significantly less base to be added. Furthermore, soybean meal and lucerne hay had such high buffering capacities that they required the addition of an acid to maintain the pH at 6.5. Later work by Jasaitis *et al.* (1987) further supported these findings identifying feeds high in energy (e.g. wheat) as having the lowest buffering capacity, low protein feeds sitting intermediate and high protein feeds have the greatest buffering capacity. Forages appear to have natural buffering or acid-consuming abilities (Playne and McDonald 1966). This, combined with their ability to increase salivation production, means that forages play an important role in controlling ruminal fluid pH.

Despite the apparent intrinsic buffering capacity of feeds, several studies claim it has little influence within the rumen. Turner and Hodgetts (1955) argued that the particulate matter within the ruminal fluid had very little effect on ruminal buffering capacity. Ruminal fluid with 3.5% particulate was compared to ruminal fluid with the particulate removed, and there was found to be little difference in buffering capacities. Counotte *et al.* (1979) used only the concentrations of bicarbonate, VFA and phosphate in ruminal fluid to determine buffering capacity and found only minor discrepancies with total ruminal fluid buffering capacity values. This work indicated little contribution of the intrinsic buffering capacity of feeds. Furthermore, when the buffering capacity of feed was compared to that of saliva within the normal physiological pH range, it was determined that feed buffering capacity was only small and of far less importance for maintaining ruminal fluid pH (Wohlt *et al.* 1987; Allen 1997). Allen (1997) stated that in the normal functioning pH range of a dairy cow's rumen (5.5 to 6.8), feeds have little buffering effect. Feeds have the greatest buffering effect when the pH drops below this range, which is when it is needed most, in order to prevent health and production issues associated with low pH.

2.6.1 Determining the buffering capacity of feed

The most common method of determining buffering capacity is to perform an acid-base titration. The initial pH of the feed must first be determined by suspending a known quantity of ground feed in a measured amount of water. The solution is stirred and allowed to equilibrate, generally for 1 to 3 minutes, before using a pH meter to determine the initial pH of the feedstuff. Titrations are then

performed through the addition of an acid or a base in known quantities to reach the target pH (Playne and McDonald 1966). Titration curves can then be plotted, with milliequivalents of acid or base versus pH. There is a huge amount of variability in not only titration methods, but also in the way buffering capacity is described. The majority of methods have been developed for determining the viability of a forage for ensilage, the most common of these is from Playne and McDonald (1966). The sample is first titrated to a pH of 3.0 and then back to pH 6.0. Buffering capacity is then expressed as the milliequivalents of alkali required to change pH from 4 to 6 per 100 g of DM. Others calculate the titratable acidity and alkalinity separately, and then calculate buffering capacity by dividing titratable acidity or alkalinity by the total change in pH units (e.g. from the initial pH to 4 or 9) (Jasaitis *et al.* 1987). While most papers assign a value for buffering capacity, Wohlt *et al.* (1987) presents only titration curves, indicating that a number does not accurately describe the buffering capacity, as the slope of the curve differs within and among feed types. Although the majority of studies calculate buffering capacity of different feed types using titrations, defining one specific protocol as the recognised method or performing a comparison of results between studies is difficult. This is due to variation in the amount of feed titrated, volume of water used for suspension, type of acid or base used, pH range and the way in which results are calculated and described.

2.6.2 Drivers of buffering capacity

Differences in the buffering capacities of feeds are thought largely to be due to chemical composition, however, no precise relationships have been identified. The use of feed composition parameters to predict buffering capacity would be ideal, as it would mean a relatively easy way to determine the buffering capacity of rations.

Dietary fibre influences the buffering ability of feeds; namely the cell wall concentration and the cation exchange capacity of plant material (McBurney *et al.* 1983). A feed's cation exchange capacity describes its ability to bind hydrogen ions within the rumen, a reduction in free hydrogen ions means decreased acidity within the ruminal fluid. Both high cation exchange capacity and high cell wall concentration, are thought to contribute to lucerne's high buffering capacity (McBurney *et al.* 1983). Furthermore, high fibre concentration increases salivary secretion neutralising acids produced during fermentation. The suggestion that fibre contributes in some way to the buffering capacity of feed is logical, as it is often high-producing dairy cows on a high concentrate, low-roughage diet that require additional dietary buffers. High energy feeds have a relatively low buffering capacity but have been found to vary. A possible reason for this may be differences in fibre concentration (Jasaitis *et al.* 1987). However, Crawford *et al.* (1983) found that fibre correlated well with buffering capacity in forages, but not in grains or grain by-products.

The highest buffering capacity has been found in high protein feeds (>35% crude protein (CP)) and lowest in high energy feeds, with low protein feeds (15 to 35% CP) sitting intermediate (Jasaitis *et al.* 1987), indicating protein concentration may be a driver of buffering capacity. Logically, protein fermentation produces ammonia, which is the main base within the rumen, contributing to an increase in pH (Crawford *et al.* 1983). Higher CP concentrations typically correlate with an increased initial pH and increased buffering capacity of feed, suggested to be a result of the buffering ability of the amino groups (Crawford *et al.* 1983; Jasaitis *et al.* 1987). A relationship has been noted between CP concentration and buffering capacity when ensiling grass and legume samples, although this relationship varied between species and CP did not appear to be entirely responsible for buffering capacity (McDonald and Henderson 1962). This was further supported by Wohlt *et al.* (1987) who found that buffering capacity was highest for high protein feeds, yet varied greatly and that factors other than protein concentration must be influential. Furthermore, at similar CP concentrations, legumes still showed a higher buffering capacity than grasses (McDonald and Henderson 1962). The theory that protein plays a major role in buffering capacity has been tested by manipulating the protein concentration of plant material. Playne and McDonald (1966) extracted protein from Italian ryegrass and red clover to test the buffering capacity of the extracted protein fraction between pH 4.0 and 6.0. They found that protein only made a small contribution (approximately 10 to 20%) to the high buffering capacity of these samples. These results supported previous findings by Playne (1963). McDonald and Henderson (1962) fertilised a number of species with ammonium sulphate, increasing their CP concentration and found that even when heavily fertilised, a low buffering capacity could still occur *in vitro*. Playne (1963) studied the impact of nitrogen fertilisation on sorghum buffering capacity, while McDonald and Henderson (1962) looked at the effects with grass. Both reported that fertilisation increased the CP concentration of the plant material but had no significant effect on buffering capacity.

Using a continuous culture system, Crawford *et al.* (1983) created a formula which used feed components to predict the *in vitro* base requirements for maintaining a 6.5 pH. The formula used both CP and acid detergent fibre (ADF) concentration to predict buffering capacity. A very strong correlation between predicted buffering capacity and actual ($R^2 = 0.84$, $P < 0.001$) was observed, indicating that both CP and ADF are critical components in buffering capacity. How these continuous culture results relate to actual requirements within a rumen were not established and are unlikely to correlate, as the predicted volume of base requirements were far higher than practical feeding of dietary buffers in current protocols. The results are likely to vary *in vivo* as the ADF concentrations influence DM intake, rumination and saliva flow. While this equation may not be transferrable to an actual animal *in situ* it does highlight the role that CP and ADF play in the buffering capacity of feeds.

When studying the ruminal ingesta of yearling steers, a strong positive correlation was found between the pH and the percentage of ash, suggesting it may play a buffering role (Cason *et al.* 1954).

Furthermore, Crawford *et al.* (1983) found that grains, grain by-products and forages had buffering capacities that correlated closely with ash. Numerous studies have explored the theory that buffering capacity is determined, in some way, by the mineral concentration of feed and while there is a correlation, the exact relationship has yet to be determined. Jasaitis *et al.* (1987) investigated the correlation between total ash and buffering capacity but could not find a direct relationship. Other studies have explored the involvement of the cation and anion fractions of the feed. When examining 24 feedstuffs, acid buffering capacity was found to correlate strongly with the dietary cation-anion difference (DCAD = Na + K − Cl − S), total cations and total ash (Jasaitis *et al.* 1987). Diets with higher DCAD concentrations, have been associated with higher ruminal pH and increased DMI (Tucker *et al.* 1988; Wildman *et al.* 2007). Playne and McDonald (1966) found the anion fraction of feed to be an important contributor to buffering capacity between pH 4 and 6, accounting for 68 to 80% of the total buffering capacity. So far, total ash and cation concentration appear to be the best tools for predicting buffering capacity (Jasaitis *et al.* 1987; Wohlt *et al.* 1987).

It has been identified that, as a plant matures, its buffering capacity decreases (Jasaitis *et al.* 1987; Wohlt *et al.* 1987). Playne (1963) examined the buffering capacity of fresh sorghum plant material at different stages of growth; 6, 9 and 12 weeks after planting. Buffering capacity was highest at the earliest growth stage and reduced thereafter. Furthermore, when comparing the buffering capacity of three growth stages of ryegrass, clover and lucerne, Greenhill (1964) found that in all species, the least mature sample appeared to be more highly buffered. The cause of this is suggested to be due to a decrease in organic acids as the plants age.

2.6.3 Feed preparation and buffering capacity

It is well known that it is difficult to ensile legumes because of their high buffering capacity (Wilson 1935) and to combat this, the wilting of lucerne prior to ensilage has resulted in a decreased buffering capacity. The buffering capacity of both fresh and ensiled red clover was also significantly lower for wilted samples compared to both ground and chopped samples (Playne and McDonald 1966). It was suggested that the wilting reduces the organic acid concentration within the lucerne. Contrary to this, Greenhill (1964) found that wilting resulted in a slight increase in buffering capacity, although the change was not significant. Most processing of plants influences their ion concentration and so, possibly, their buffering capacity (Jasaitis *et al.* 1987). During ensilage, buffering capacity increases significantly due to the production of lactate and acetate (McDonald and Henderson 1962; Playne 1963; Playne and McDonald 1966; Wohlt *et al.* 1987). Buffering capacity was found to be higher for fresh material when compared to dried, milled samples when tested *in vitro* (McDonald and Henderson 1962).

2.7 Dietary changes and adaptation strategies

Extensive research exists describing the ruminal environment of dairy cows fully adapted to a wide range of diets. The effects of excessive intakes of starch on the rumen have also been studied in detail (see review by Huntington (1997)). However, information relating to the changes occurring during the adaptation of a lactating dairy cow to a new diet are very limited, despite dietary changes being a routine part of the industry. Changes to the diet require adaptive responses from the microbial populations within the rumen as well as from the papillae on the rumen walls. The diet of the host and the type of VFA being produced determines the number, distribution and length of papillae. Diets high in starch result in greater quantities of butyrate and propionate, which rely on a greater quantity of blood for absorption. This in turn means longer and more numerous papillae. High fibre diets favouring acetate production result in smaller, less numerous papillae (Gaebel *et al*. 1987). The adaptive process of the ruminal mucosa to switch from one to the other can require up to 3 to 8 weeks to complete (Membrive 2016). A rapid change to a high starch diet can result in inadequate absorption of end products and acid accumulation in the rumen (Owens *et al*. 1998).

When using daily increments to introduce a concentrate mixture (50% ground wheat, 15% ground oats, 15% corn distillers dried grains, 8% soybean meal, 10% molasses and 2% minerals, on a DM basis) to 20 yearling dairy heifers, Tremere *et al.* (1968) reported that increments smaller than 7 g per unit of metabolic BW were required in order to prevent heifers reducing their DMI. The larger the daily increments in concentrates, the sooner the heifers began to refuse feed. Reduced ruminal pH values and elevated concentrations of lactate and VFA were found within the rumens of cows that had reduced DMI (Tremere *et al.* 1968). Using two ruminally fistulated, non-lactating cows, Van Vuuren *et al.* (1979) investigated strategies for changing from an all hay diet to a high concentrate diet. Three strategies were employed; 1) daily increments of 1 kg, 2) 4 kg on day 1 followed by 1 kg increments for 3 days and 0.5 kg increments for the remainder and 3) 4 kg on day 1 followed by 2 kg increments thereafter. These strategies continued until the refusal was greater than the daily increment. Strategy 2 in which increments were smallest after 8 kg, was found to have the greatest DMI. While strategy 3 was found to have the lowest DMI, but this did not correlate with the lowest ruminal pH or high lactate concentrations. There were no significant differences between ruminal pH or acid concentrations within the rumen.

As research in this area is limited, some information can be drawn from experiments that did not intend to focus on the adaptation period. For example, Pourazad *et al.* (2016) investigated the effects of transient feeding a high concentrate diet to non-lactating dairy cows on incidences of acidosis. Prior to the transient feeding, the cows consumed forage only (pasture silage and pasture hay) for up to 3 weeks and then underwent a 6-day adaptation period. During the adaptation period, the amount of concentrate mix (33% barley, 30% wheat, 17% rapeseed meal, 15% corn, 3% dried beet pulp and 2% minerals, on

a DM basis) was increased at a rate of 10% of the diet per day until it reached 60% (on a DM basis), with forage the remainder. During this 6-day adaptation period, ruminal pH was below 5.8 for an average of 204 min/cow per day and the average ruminal pH was 6.14. While the mean is marginally lower than recommended for avoiding SARA (Zebeli *et al.* 2008) the time spent below pH 5.8 is not concerning, indicative of an acceptable adaptation strategy.

Some of the research into dietary changes in dairy cows focuses on feeding mixed rations. Moseley *et al.* (1976) and Hernandez-Urdaneta *et al.* (1976) reported the effects of abruptly changing the energy concentration of a ration fed to dairy cows at different stages of lactation. All results reported, such as reduced pH and increased acid concentration within the rumen, corresponded with other reports of animals adapted to similar diets. Only minor disruption to rumen fermentation as a result of the abrupt diet changes was reported. This is likely because the cows were already adapted to high amounts of concentrate feeding, as the diet was increased from 40% concentrate to 60%.

Although still limited, the research into dietary changes for feedlot cattle is more extensive. The nature of feedlots mean that animals are fed mixed rations, in contrast to forage and concentrates separately. When adapting 12 feedlot heifers to a diet of 90% concentrate from one of 40% concentrate, Bevans *et al.* (2005) found no immediate benefits of a gradual adaptation strategy compared to a rapid strategy. However, the authors did report greater variation in many ruminal pH parameters such as daily mean, minimum and maximum when the rapid adaptation strategy was employed, indicating a lot of individual animal variability. It was concluded that this type of adaptation strategy could result in acidosis in the more susceptible animals. In an attempt to induce acidosis, Coe *et al.* (1999) rapidly transitioned four ruminally cannulated steers from a lucerne hay diet to 100% concentrate in 7 days using 3 incremental changes. The concentrate diet included 65% cracked corn and 25% cracked wheat on a DM basis. All cows were fed once daily and ruminal fluid was sampled before and after feeding. The ruminal pH and ammonia concentrations decreased with increasing energy concentration of the diet. Total VFA, as well as the molar proportions of propionate, butyrate and valerate were found to increase with greater concentrate proportions. However, these experimental conditions failed to induce acidosis. The lowest pH recorded was 5.68 and there was no accumulation of lactate. The authors did not provide any explanation as to why this rapid introduction of concentrates failed to induce acidosis.

Sheep have been used as a model for dairy cows in dietary changes. Mackie *et al.* (1978) imposed a stepwise adaptation from a forage and molasses diet to one containing 14, 34, 50, 61 and 70% maize grain on a DM basis, on eight ruminally cannulated merino wethers. The wethers were given 14 days to adapt to the 14% maize grain diet and 7 days for all other increments. As grain increased in the diet, ruminal pH declined. However, the lowest ruminal pH recorded was 5.3 and only small transient amounts of lactate accumulated. The ability of the rumen to resist any critical declines in ruminal pH

was attributed to the slow introduction of the maize allowing gradual changes in the microbial population. The microbiome shifted from acid sensitive populations to acid tolerant ones. Contrarily, Grubb and Dehority (1975) abruptly changed a ration being offered to three wethers from 100% orchard grass to 60% cracked corn and 40% orchard grass. It was reported that after 21 days, the shift in bacterial populations towards greater numbers of amylolytic bacteria, still had not stabilised. When making the same dietary change, Potter and Dehority (1973) indicated that the adaptation was actually a lot quicker and was complete after only 5 days, based on digestibility data. Warner (1962) reported that the rumen microbial population of sheep required 10 days to complete adapting to any major change in the diet.

The time required for the rumen to adapt will depend on several things, including how drastic the dietary change is, the adaptation strategy being employed, and the characteristics of the feeds being introduced and of those already being consumed. Furthermore, there are vast differences between animals undergoing the same adaptation process (Grubb and Dehority 1975; Tajima *et al.* 2001).

2.8 Conclusions of the literature review

Changes to the type and composition of a dairy cow's diet will involve some level of metabolic adaptation. How quickly or well an animal adapts is determined by numerous factors; composition of both the new diet and the previous diet, level of milk production, DMI, nutritive characteristics of the feed, stage of lactation and management strategies. It is specifically the microbes within the rumen that need to adapt and the successful adaptation to any diet is largely determined by the regulation of ruminal fluid pH. Very few studies have investigated major dietary changes in lactating dairy cows and those that have are not pasture based but focus on feeding mixed rations. A stepwise adaptation to a diet including high amounts of rapidly fermented starch is routinely practiced within the dairy industry and yet there are no studies that investigate adapting lactating dairy cows from an all forage diet to one incorporating high amounts of starch fed separately.

The role forages play, particularly their buffering capacity, in facilitating optimum adaptation have not been investigated. Exactly what determines buffering capacity in feedstuffs is yet to be determined. The majority of work investigating the buffering capacity of feeds has been conducted *in vitro* and currently there appears to be a lack of recent information around its influence *in vivo*. Of importance is the need to determine the effects during diet changeover and how the buffering capacity of one feed can influence the rate at which a new diet is introduced.

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Chapter 3

Manipulation of the pre-partum diet of dairy cows to promote early adaptation to fresh perennial ryegrass

V. M. Russo[A,B,C,D], W. J. Wales[B], B. J. Leury[C], M. C. Hannah[B] and E. Kennedy[A]

[A]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland.

[B]Agriculture Victoria Research, Ellinbank, Vic. 3821, Australia.

[C]Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Vic. 3010, Australia.

[D]Corresponding author. Email: victoria.russo@ecodev.vic.gov.au

Abstract

The diet of dairy cows in Ireland traditionally changes abruptly from predominantly pasture silage before calving to grazed perennial ryegrass (PRG) immediately after calving. This potentially leads to problems with adaptation of microbes in the rumen with consequences of reduced intake and ultimately lower milk production. The purpose of this experiment was to determine if introducing dairy cows to fresh PRG in the final weeks of pregnancy, thus eliminating a major dietary change at calving, could improve the adaptation process, potentially increasing DMI and milk production in early lactation. Three weeks prior to their expected calving date, 14 spring calving dairy cows were assigned to one of two treatments: pasture silage pre-partum followed by fresh cut PRG post-partum (control), or fresh PRG both pre and post-partum (GG). For both treatments, DMI increased post-partum, but there was no difference between treatments, pre or post-partum (5.9 and 8.8 kg DM/cow per day, respectively). There were no differences in milk yield or composition between the treatments. Body condition score declined following parturition but was not different between the treatments either pre or post-partum. Plasma non-esterified fatty acids, glucose and β-hydroxybutyrate were also unaffected by treatment but did indicate a state of negative energy balance in early lactation. The results of this experiment suggest that pre-partum adaptation to fresh PRG would not benefit milk production in dairy cows in early lactation in Ireland.

Introduction

The Irish dairy industry is a pasture-based production system and success is largely reliant on maximising the annual milk production from grazed pasture (Dillon *et al.* 1995; Horan *et al.* 2005). The use of pasture silage is also pivotal within the industry, however grazed pasture is cheaper (Finneran *et al.* 2010) and nutritionally superior (Dillon *et al.* 2002), providing the incentive for maximising its use. Due to the seasonality of pasture production, most dairy cows in Ireland are housed indoors and fed pasture silage during the non-lactating period. Immediately post-partum, these cows are turned out to graze pasture, resulting in an abrupt diet change. When a ruminant's diet is changed in either level of feeding or composition of diet, the rumen requires time to adapt in order to avoid digestive or metabolic disorders (Kaufmann *et al.* 1980). Poor adaptation impacts on dry matter intake (DMI) (Tremere *et al.* 1968) and consequently milk yield.

The transition period, typically referred to as three weeks immediately before and after calving, is recognised as the most dramatic and difficult time for a dairy cow due to the onset of both parturition and lactation. The difficulties at this time are highlighted by the fact that the risk of disease is greatly increased (Shanks *et al.* 1981; Curtis *et al.* 1985). During the transition period, a decline in DMI occurs (Grummer *et al.* 2004; Douglas *et al.* 2006) despite nutritional demands on the body rising. Cows in early lactation commonly enter a state of negative energy balance (NEB), where body reserves are mobilised to meet the deficit between energy intake and lactation needs (Bauman and Currie 1980). Entering a state of NEB can result in health concerns for the animal, as well as impacting on long term milk production. Effects of nutrition during the transition period carry over to the subsequent lactation, health and reproductive performance (Grummer 1995). Thus, it is recommended to use nutritional strategies during this period to minimise NEB, allowing for an improved metabolic state. An increase in DMI during early lactation can reduce the deficit between energy intake and expenditure, thereby improving energy balance (EB).

Irish dairy cows face an abrupt dietary change immediately after calving, an already stressful period for the cow, which may result in reduced DMI and subsequent milk yields. Any means of increasing DMI pre-partum can help improve metabolic parameters and decrease the incidences of metabolic disorders post-partum (Grummer 1995). Previous work has shown that introducing cows to pasture in early lactation results in greater DMI and yields of milk, fat, protein and lactose when compared to offering pasture silage as the sole forage source (Dillon *et al.* 2002).The objective of this experiment was to determine if offering harvested fresh pasture even earlier, in late pregnancy, and thus adapting cows to the new diet sooner, could improve health and productivity outcomes following the transition period for dairy cows, leading to increased DMI and milk production in early lactation. The hypotheses tested were (1) that cows fed fresh PRG pre-partum will have a greater pre-partum DMI than cows fed silage pre-partum and (2) that cows adapted to fresh PRG pre-partum will have a greater DMI and MY post-partum than cows fed silage pre-partum and PRG post-partum.

Materials and methods

The experiment was conducted in late winter through to early spring of 2016 at Teagasc Moorepark Research Centre, Fermoy, Co. Cork, Ireland (55°10'N, 8°16'W). All experimental procedures were carried out in accordance with European Union Directive 2010/63/EU and S.I. No.516 of 2016.

Experimental design and dietary treatments

Fourteen spring calving Holstein-Friesian primiparous cows were allocated to one of two groups. Allocation was random, subject to balancing for expected calving date, body weight and estimated economic breeding index (EBI). Each group was assigned one of two feeding strategies: pasture silage pre-partum followed by fresh cut perennial ryegrass (PRG) post-partum (control), or fresh cut PRG both pre-partum and post-partum (GG). Prior to the experiment all cows were on a diet of pasture silage only.

Three weeks prior to their expected calving date, cows were randomly allocated to individual pens with separate feed boxes and all feed was offered *ad libitum.* Cows were penned adjacent to each other with both visual and tactile contact. Due to differences between expected and actual calving dates, the number of days for which treatments were imposed varied between cows. On average, experimental rations were fed for 11 (±7) days pre-partum and for 14 (± 0) days post-partum. Pasture was harvested fresh daily and all feed was given in the morning at 1030 h. All refusals were collected, weighed and subsampled the next morning, prior to feeding. A mineral block (Welmin dry cow elite block; Agritech, Ballyanny, Ireland) was provided to each cow in their individual stalls, pre-partum and was available *ad libitum*. Immediately following calving, all cows in the control treatment had their silage removed and replaced with freshly cut PRG, available *ad libitum.*

Intake and nutritive characteristics

Dry matter (DM) of all offered and refused feed was recorded for individual cows and the difference represented individual feed intakes. Daily, two subsamples of feed offered and refused were collected. An initial sample was oven dried at 90°C for 16 h to determine DM content. The second subsample was frozen, freeze dried, milled through a 0.5 mm sieve and analysed at a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY, USA) for nutritive characteristics by wet chemistry (AOAC 2000). Averages for silage and pasture offered during the experimental period are reported. Concentrations of estimated ME were calculated using the following formula (National Research Council 2001):
ME (MJ/kg DM) = ((1.01 × (0.04409 × TDN)) – 0.45) × 4.184, where TDN is total digestible nutrient (%). The nutritive characteristics of feed offered are presented in Table 3.1. Diet Check (Heard *et al.* 2004) was used to evaluate the nutrient intake of the cows in terms of energy and protein requirements.

Milk yield and composition

Following calving, milking took place twice daily at 0700 h and 1600 h. Individual MY (kg) were recorded at each milking (Dairymaster, Causeway, Co. Kerry, Ireland). Milk samples were taken at

every milking and composition (fat, protein, and lactose) was determined by automated infra-red analysis using a Milkoscan 203 (Foss Electric, Denmark). Energy corrected milk (ECM) yield was calculated using the following formula (Tyrrell and Reid 1965): ECM (kg) = MY (kg) × [376 × fat (%) + 209 × protein (%) + 948] / 3,138

Table 3.1. Nutritive characteristics of feed offered during the experimental period
All values are % of DM unless otherwise indicated

Feed	CP	aNDF	ADF	NFC	TDN	ME[1]
Silage	13	55	33	20	67	11
PRG	25	43	25	19	70	12

[1]Estimated ME (MJ/kg DM).

Body condition score and live weight

Cows were weighed once per week before the morning feed, and post milking if lactating. Weights were recorded electronically using a portable weighing scale and the Winweigh software package (Tru-test Limited, Auckland, New Zealand). At the same time as weighing, all cows were condition scored using a scale of 0 to 5 (Lowman *et al.* 1976).

Blood samples

The target day and the actual day of blood sampling relative to parturition for the GG and the control treatment groups were -14 and -13.6 (SD = 2.12, n = 10), -7 and -7.2 (SD = 2.20, n = 13), 0 and 0 (SD = 0.00, n = 13), 7 and 6.8 (SD = 0.43, n = 13) and 14 and 14.1 (SD = 0.51, n = 13), respectively. Blood samples were collected via the coccygeal vein using evacuated tubes containing lithium heparin (Becton Dickinson, Plymouth, United Kingdom) and immediately put on ice. Within 15 min of collection, samples were centrifuged (Kontron Centrikon T-324, Kontron Instruments, Milan, Italy) at 530*g* for 15 min at 4°C. The plasma was collected and stored at -20°C until analyses. Non-esterified fatty acids (NEFA), glucose, β-hydroxybutyrate (BHBA) and creatinine concentrations were analysed by enzymatic colorimetry using an ABX Pentra 400 auto-analyser (ABX Mira, Montpelier, France; BHBA and creatinine kits were supplied by Randox Laboratories Ltd., Crumlin UK; NEFA kit supplied by Wako Chemicals GmBH, Neuss, Germany; glucose kit supplied by Horiba BAX, Montpellier, France). Plasma urea concentrations were measured using an enzymatic kinetic method (Randox Laboratories, Ltd., Crumlin UK) on a Randox RX Imola multichannel autoanalyser (Randox Laboratories, Ltd., Crumlin UK).

Eating behaviour

Two weeks prior to their expected calving date, cows were fitted with halters containing pressure and movement sensors (RumiWatch, Itin + Hoch GmbH, Liestal, Switzerland) to quantify daily ruminating and eating time. The halters remained on the cows for 14 days post-partum.

Statistical analyses

One cow was removed from the control treatment due to low intakes during the initial two days. All data were analysed using Genstat for Windows (Genstat 18th edition, VSN International Ltd., Hemel Hempstead, UK). An average DMI, estimated ME balance, CP balance, metabolic live weight (MLW) and DMI:MLW were calculated for each cow pre and post-partum. These values were then subjected to ANOVA with the factorial treatment structure treatment by stage (pre or post-partum) and treatment blocking structure cow split for stage. Due to the inconsistencies in milk composition in the first few days of lactation, milk production data were refined to only include days 5 through 14. Using daily DMI and ECM, an individual feed conversion efficiency (FCE) was calculated for each cow on days 5 through 14 (post-partum). Additionally, a corrected FCE value was calculated following the methodology of Beever and Doyle (2007). Milk production and FCE data were then analysed using ANOVA with treatment used as a single factor in the treatment structure and a blocking structure of cow split for day relative to calving. Behaviour data were summarised daily for each cow as minutes spent eating, ruminating and not chewing. The data were then analysed using a mixed-effects model, with the random effects cow split for day relative to calving. The factorial fixed effects for the model were treatment by stage (pre or post-partum). Blood samples were categorised by stage as either pre-partum, at calving or post-partum and within stage by day relative to calving. Plasma metabolite data were then analysed by ANOVA with a factorial treatment structure of treatment by stage (pre-partum, at calving, or post-partum) split for day relative to calving, and a blocking structure of cow split for day relative to calving.

Results

Intake and eating behaviour

Mean DMI, live weight, body condition score (BCS), DMI as a proportion of MLW, nutrient balance information and eating behaviour of cows fed according to each of the treatments are presented in Table 3.2. There was no difference between the DMI of the treatment groups, pre or post-partum. The post-partum DMI was, on average, 2.9 kg DM/cow per day greater than pre-partum DMI. There were no differences in BCS between treatments, which declined by 0.25 units following parturition. An interaction effect between treatment and stage (pre or post-partum) occurred for time spent ruminating. Control cows spent less time ruminating post-partum while GG cows spent more time ruminating post-partum.

There were no differences in ME required or consumed between the treatments. Requirements increased post-partum and were met by an increase in ME consumed. Despite the increased intake, the cows were in deficit both pre and post-partum with no difference between the two, averaging 23 MJ/cow per day. There was however a tendency towards an interaction effect, such that control cows were in greater deficit pre-partum compared to post while the deficiency in the GG treatment was similar at both stages. Crude protein balance was affected by a treatment and stage interaction. Pre-partum, the

GG treatment group was consuming 804 g/cow per day in excess of requirements. The control cows, while still in excess, were consuming much lower quantities resulting in 55 g/cow per day in excess. While requirements for both treatments increased post-partum so did the amount consumed. This resulted in both treatment groups remaining in a positive balance, but there was no longer a difference between the two, due to a greater increase in amount consumed by the control group.

Table 3.2. Mean dry matter intake (DMI), DMI as a proportion of metabolic live weight, body condition score (BCS), eating behaviour and nutrient balances of cows fed according to each of the treatments

	Control[1]		GG[1]			*P*-value		
	Pre	Post	Pre	Post	SED	Treatment	Stage	Interaction
DMI (kg/cow per day)	5.6	8.6	6.3	9.0	0.26	0.571	<0.001	0.339
DMI (g/kg $BW^{0.75}$ per day)	52.6	88.5	58.3	90.8	4.32	0.768	<0.001	0.403
BCS	3.3	3.0	3.3	3.1	0.05	0.516	<0.001	0.448
Eating (min/cow per day)	400	374	420	389	6.1	0.222	<0.001	0.737
Ruminating (min/cow per day)	503[a]	463[bc]	449[b]	470[ac]	29.7	0.904	0.985	0.030
Not chewing (min/cow per day)	540	599	567	576	33	0.626	0.110	0.146
Intake rate (kg DM/cow per h)	0.6	0.9	0.8	1.0	0.09	0.672	<0.001	0.228
ME (MJ/cow per day)								
Required	94	118	93	130	7.2	0.351	<0.001	0.252
Consumed	60	101	74	106	5	0.189	<0.001	0.248
Excess/deficit	-34	-17	-19	-24	7.6	0.565	0.387	0.109
CP (g/cow per day)								
Required	657	1550	742	1631	71.1	0.378	<0.001	0.964
Consumed	712[a]	2094[b]	1546[c]	2203[b]	87.1	0.007	<0.001	<0.001
Excess/deficit	55[a]	545[b]	804[c]	573[b]	32.7	<0.001	0.001	<0.001

[1]Control, cows fed *ad libitum* silage pre-partum and fed *ad libitum* perennial ryegrass post-partum; GG, cows fed *ad libitum* perennial ryegrass pre and post-partum.
[a-c]Means within a row with different superscripts differ ($P < 0.05$).

Milk yield and composition

Mean daily MY, ECM yield, milk composition, estimates of FCE and corrected FCE from cows fed according to each of the treatments are presented in Table 3.3. There were no differences between treatments for any of the parameters. Correcting the measured FCE for milk produced from tissue mobilisation resulted in lower FCE values for both treatments. The amount of ECM produced from tissue mobilisation was not different between the treatments, nor was the amount produced from feed.

Plasma metabolites

Plasma NEFA, glucose, BHBA, urea and creatinine concentrations from days −14, −7, 0, 7 and 14 relative to parturition for both treatments are presented in Figure 3.1. An interaction between treatment and stage for circulating NEFA concentrations ($P = 0.005$) resulted in both treatments showing similar

increases pre-partum and both continued to increase post-partum. However, the post-partum increase was more pronounced for the control cows. Both treatments reached a peak in NEFA concentrations at 7 days post-partum. An interaction between stage and day showed an increase between the first and the second sample pre-partum measurements but no difference between the two post-partum measurements. Plasma glucose concentrations were unaffected by treatment ($P = 0.196$) but were affected by stage ($P < 0.001$). While there was no difference in glucose concentrations between the pre and post-partum samples; a peak occurred at parturition. There was a trend towards an effect of treatment on plasma BHBA concentrations ($P = 0.099$). The BHBA concentration in the plasma of the control cows increased steadily from day -14 to day 14, with a significant difference between the first and the last measurement. There was no difference in the concentrations from the GG treatment group. Plasma BHBA was affected by stage ($P = 0.023$) with concentrations being lowest pre-partum and highest post-partum, but this was driven by the differences in the control group. A treatment by stage interaction for plasma urea ($P < 0.001$) resulted in significantly higher concentrations in GG cows pre-partum and at calving. The concentration in the control cows increased post-partum, resulting in similar concentrations for the two treatment groups. Creatinine concentrations did not differ between treatments. Levels were similar in the pre-partum and calving samples, then declined post-partum.

Table 3.3. Mean daily milk yield (MY), energy corrected milk (ECM) yield, milk composition and estimates of feed conversion efficiency (FCE) and corrected FCE from cows fed according to each of the treatments

	Control[2]	GG[2]	SED	*P*-value
MY (kg/cow per day)	14.8	16.7	1.51	0.232
ECM (kg/cow per day)	17.7	20.0	0.28	0.283
Protein (%)	3.8	3.7	0.11	0.316
Fat (%)	5.4	5.4	0.33	0.881
Lactose (%)	4.6	4.6	0.07	0.426
Calculated FCE	2.0	2.2	0.24	0.459
ECM from body tissue (kg/cow per day)	2.7	3.7	1.50	0.529
ECM from feed (kg/cow per day)	15.0	16.4	1.32	0.331
Corrected FCE[1]	1.8	1.9	0.06	0.316

[1]FCE corrected for milk produced from tissue mobilisation (Beever and Doyle 2007).
[2]Control, cows fed *ad libitum* silage pre-partum and fed *ad libitum* perennial ryegrass post-partum; GG, cows fed *ad libitum* perennial ryegrass pre and post-partum.

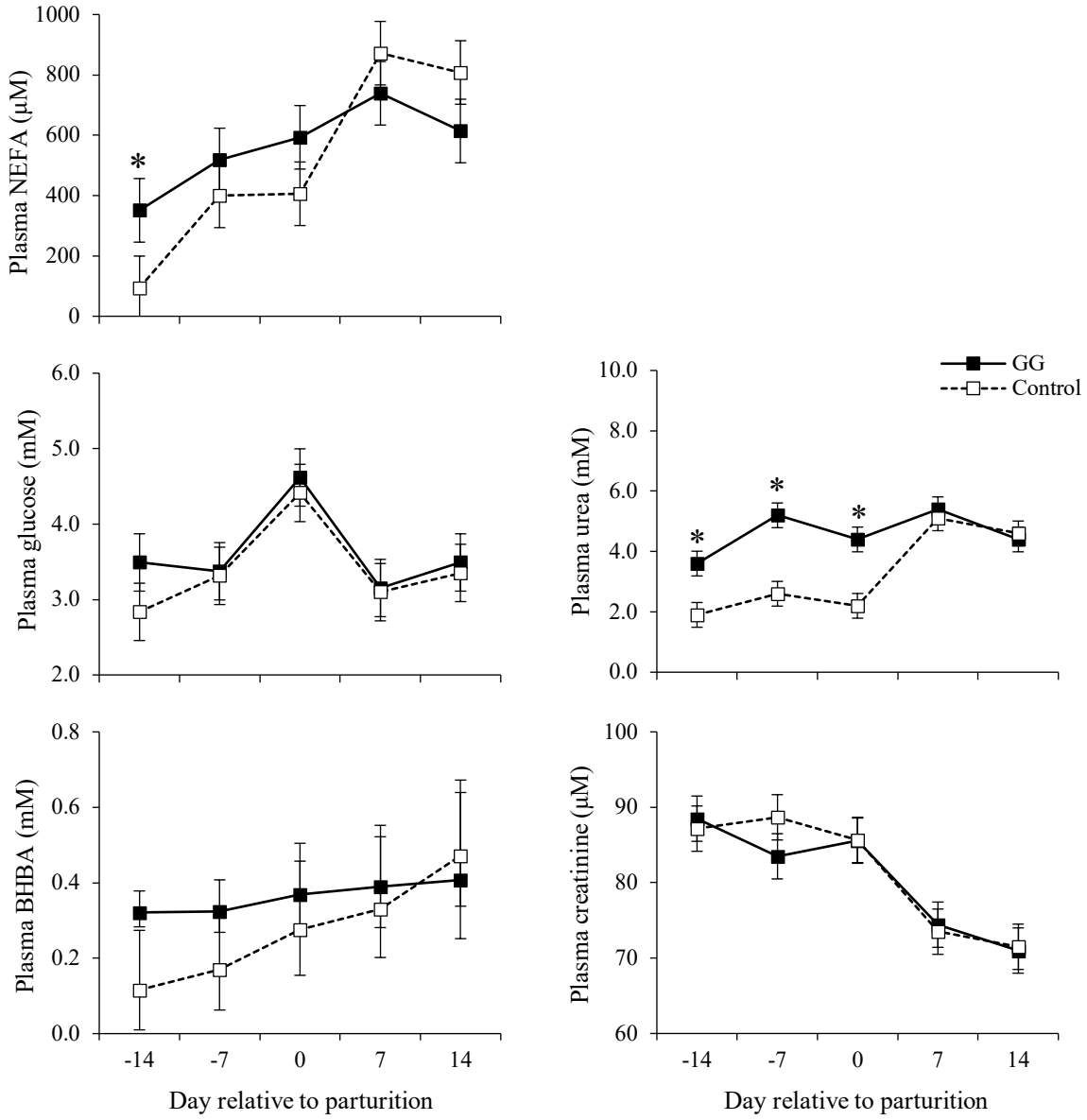

Figure 3.1. Mean circulating plasma metabolites during the transition period for cows fed either grass silage pre-partum and perennial ryegrass (PRG) post-partum (control; □) or PRG both pre and post-partum (GG; ■). Asterisks indicate a difference between treatments within day ($P < 0.05$).

Discussion

Increasing DMI pre-partum can help improve metabolic parameters and decrease the incidences of metabolic disorders (Grummer 1995). Under the conditions of the current experiment, early adaptation to fresh PRG did not increase DMI pre-partum, nor did it increase DMI post-partum or result in milk production benefits. The DMI of both the control and the GG group did increase with the onset of lactation by 35% and 30%, respectively. An increase of this level is consistent with other reports of dairy cows in transition experiments (McNamara *et al*. 2003b; Douglas *et al*. 2006). The increase in DMI serves to boost energy intake and moderate the need for excessive lipid metabolism to meet the energy demands of lactation. The DMI were reflected in the BCS of the cows with no differences

between the treatments, pre or post-partum, and all were considered within an optimum range (Roche *et al.* 2009). Despite the post-partum increase in DMI, the BCS of both treatment groups declined as was expected. During the transition from late gestation to early lactation, it is typical for dairy cows to undergo a period of NEB, resulting in a decline in BCS (Grummer 1995; Drackley 1999). The energy deficits reported for both treatments are similar to that reported in other transition experiments (Beam and Butler 1998; Vandehaar *et al.* 1999). McNamara *et al*. (2003a) fed cows only high-quality silage pre-partum and also found them to be in NEB in the final weeks of gestation. While the requirements for ME increased post-partum, so did DMI, negating some of the deficit. However, body reserves were still being mobilised as milk production needs were not being met by intake alone.

As the early lactation cows were in NEB, the measured FCE was exaggerated due to the contribution of tissue mobilisation to milk production (Beever and Doyle 2007). The corrected FCE, which adjusts for this, was much lower and there was no difference between treatments indicating an equal level of tissue mobilisation and that the GG cows were no better supported for beginning lactation and demonstrated no obvious benefits from early PRG adaptation. Counterproductively, consuming fresh PRG pre-partum may have actually been costing the GG cows energy. As the PRG was providing CP in excess of requirements, energy was being used to metabolise the extra protein and synthesise and excrete urea (Reed *et al*. 2017). The significantly higher plasma urea from the GG cows pre-partum and at parturition is indicative of high CP in the diet (Butler 1998). This is somewhat supported further by the plasma NEFA and BHBA levels being marginally higher pre-partum for GG cows indicating increased nutritional stress from a metabolic perspective, despite DMI and ME intakes being similar. The pattern of decreasing plasma creatinine levels post-partum and the concentrations reported for both treatment groups in the current experiment are similar to those reported in other transition experiments (Piccione *et al*. 2012) and are considered within the normal range for lactating dairy cows (Cozzi *et al*. 2011).

Increased levels of circulating hormones such as cortisol in the lead up to parturition (Goff and Horst 1997) signal an increase in gluconeogenesis in the liver (Drackley *et al.* 2001). This results in a peak in plasma glucose at parturition, as seen in both treatments in the current experiment. The reduction in these circulating glucocorticoids and the demand for glucose with the onset of milk synthesis then result in plasma glucose concentrations declining rapidly post-partum. Despite the abrupt changes in circulating glucose concentrations within the current experiment, they are considered within the normal range for dairy cows (Cozzi *et al.* 2011). As available glucose decreases at parturition, the body responds by increasing lipolysis, releasing NEFA into the bloodstream to meet the energy needs of the dairy cow (McNamara 1991). Circulating NEFA concentrations are regularly used as a proxy for energy balance, higher NEFA levels indicate a greater degree of NEB. It is typical for the levels of circulating NEFA to increase as parturition approaches (Bell 1995) and continue into early lactation as the animal enters a state of NEB. Within the current experiment, DMI decreased in the lead up to parturition (data

not shown); a time when energy demands are heightened, and so lipolysis increased to meet the energy shortfall (McNamara 1991; Bertics *et al.* 1992). Hence a peak in NEFA concentrations occurred at 7 days post-partum. Levels of serum NEFA >500 μM pre-are linked to post-partum health issues, as are post-partum levels >1000 μM (McArt *et al.* 2013). The NEFA concentrations within the current experiment were all below these thresholds but were still high, perhaps exacerbated by the withholding of concentrates (Petterson *et al.* 1994); an important source of energy for high producing dairy cows in early lactation. When the liver can no longer increase the amount of NEFA being oxidised and levels of stored triacylglycerol are exceeded, the concentration of circulating ketones increases. A BHBA threshold of 1.2 mmol/L is typically used to identify cows with excessive amounts of circulating ketones post-partum (McArt *et al.* 2013). The concentrations measured within the current experiment were well below this threshold, indicating the cows were not in excessive NEB and adequately adapting to lactation.

Despite both treatment groups eating more post-partum, time spent eating actually decreased with cows increasing their intake rates instead. This is likely a combination of lactation increasing the drive to eat and the temporary fasting imposed through milking increasing subsequent intake rates (Kennedy *et al.* 2009). The control cows spent more time ruminating pre-partum, likely driven by the greater NDF concentration in the silage (Beauchemin and Yang 2005). There were no differences in rumination time post-partum once all cows were consuming the same feed.

While there were some numerical differences in the production outcomes and the EB pre-partum, they were not statistically significant, possibly due to low cow numbers per treatment. Additionally, the experiment was conducted with heifers which may have confounded the results as they have not yet matured and reached peak milk yields. However, in terms of practical application, the lack of difference between treatments is a positive result. Given the seasonal production of PRG, particularly in Ireland (Hanrahan *et al.* 2017), pasture growth is low over the winter months when the pre-partum feeding would need to occur. If the strategy to offer pasture pre-partum proved beneficial it would have required the development of novel pasture management practices to overcome the pasture supply deficit.

Conclusion

Under the conditions of this experiment adapting dairy cows to PRG prior to parturition did not improve DMI or MY, showing no benefit over current feeding strategies. It is possible that the similarities between the forages mean that a change from pasture silage to fresh PRG post-partum does not disrupt normal rumen function and hence no early adaptation to PRG is required. Indicating that current feeding strategies are adequate in terms of a successful dietary changeover.

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Chapter 4

Effect of wheat adaptation strategies on rumen parameters and dry matter intake of late lactation dairy cows

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Effect of wheat adaptation strategies on rumen parameters and dry matter intake of late lactation dairy cows

V. M. Russo[A,B,C,D], *B. J. Leury*[B], *E. Kennedy*[C], *M. C. Hannah*[A], *M. J. Auldist*[A] *and W. J. Wales*[A]

[A]Agriculture Victoria, Department of Economic Development, Jobs, Transport and Resources, 1301 Hazeldean Road, Ellinbank, Vic. 3821, Australia.
[B]Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Vic. 3010, Australia.
[C]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland.
[D]Corresponding author. Email: victoria.russo@ecodev.vic.gov.au

Abstract. The effects of a major dietary change on ruminal fluid pH, volatile fatty acid (VFA), lactate and ammonia concentrations, dry matter intake (DMI) and milk yield were measured in 32 dairy cows in late lactation. All cows were initially fed 100% lucerne hay cubes and were then gradually introduced to a diet with wheat comprising 40% of total dry matter (DM) and lucerne hay cubes, the remainder. Wheat was gradually substituted for lucerne via one of four strategies, (1) in six small increments (each 6.7% of total DM) over 6 days; (2) in six small increments (each 6.7% of total DM) over 11 days; (3) in three large increments (each 13.3% of total DM) over 6 days; or (4) in three large increments (each 13.3% of total DM) over 11 days. The introduction of wheat in six small increments resulted in a lower daily minimum ruminal fluid pH (pH 5.95) when compared with using three large increments (pH 6.05). Despite this difference none of the treatments exhibited a ruminal fluid pH that would have compromised ruminal function, nor were there differences in DMI (19.7 kg DM/cow.day) or milk yield (16.0 kg/cow.day). Additionally, there were no differences between ruminal fluid VFA, lactate or ammonia concentrations. It is speculated that the properties of the lucerne cubes, including a high buffering capacity, helped the ruminal contents resist the pronounced declines in pH often seen with the fermentation of large amounts of wheat. Under the conditions of this experiment the wheat adaptation strategies used did not lead to any critical differences in rumen parameters. These results suggest that changes to rumen function are driven not only by the characteristics of the concentrate being introduced but also by those of the forage.

Additional keywords: dietary change, ruminal pH, volatile fatty acids.

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Introduction

Grazed grass can be a cheap feed source for lactating dairy cows, and many dairy farms in countries such as Australia, Ireland and New Zealand are predominantly pasture based for this reason (Dairy Australia 2014). Perennial ryegrass, white clover and lucerne are the most common pasture varieties on dairy farms in the temperate regions of Australia (Wolfe 2009; Nichols *et al*. 2013). The availability and quality of this pasture changes throughout the year, as does the energy and protein requirements of a lactating cow (Moller *et al*. 1996; Doyle and Stockdale 2011). Hence, many farmers rely on supplementing pasture with cereal grain or pelleted concentrates in order to meet the nutrient demands of the herd (Bargo *et al*. 2003*a*). The introduction and withdrawal of supplements to and from the diet is done tactically throughout the year, but it can be challenging to change diets rapidly and efficiently. A common and problematic change is the introduction of a large amount of cereal grain-based concentrate into a predominantly forage-based diet. In countries with pasture-based dairy systems, such as Australia, pasture supply can become limited or of poor quality from summer onwards and large amounts of cereal grains are introduced to meet energy demands.

Changing from a high-forage diet to one containing large amounts of ruminal fermentable starch causes substantial alterations to the ruminal microbial environment as well as alterations to the proportions and amounts of volatile fatty acids (VFA) (Bargo *et al*. 2003*b*). The recommended time to allow the rumen to adapt to diets high in fermentable starch varies from 10 to 21 days, with concentrate increases occurring at 2–3-day intervals (Warner 1962; Kellaway and Harrington 2004); the exact timing depends on amount and type of starch supplementation. If appropriate adaptation steps are not taken, there can be significant declines and fluctuations in average daily ruminal fluid pH, leading to acidosis, compromised digestion and variable dry matter intake (DMI) (Owens *et al*. 1998; Krause and Oetzel 2006). In light of this, adaptation strategies should be designed to maintain ruminal fluid pH within an optimal range, although identifying this range is contentious, particularly the lower threshold (Beauchemin *et al*. 2003; Plaizier *et al*. 2008). The target pH range should also depend on the particular feeding

system employed. A pH range of 6.00–6.20 has been suggested as the optimum for digestion of forage-concentrate diets (Hutjens and Overton 1996; Pitt *et al*. 1996) and, accordingly, pH 6.00 is commonly referred to as the minimum for optimum rumen function (Mould *et al*. 1983; Shriver *et al*. 1986).

Information on the progressive effects of gradually increasing the amount of cereal grain to a high amount from an all forage diet in lactating dairy cows is very limited. Due to the widespread occurrence of acidosis when adapting feedlot cattle to high concentrate diets there has been extensive research in the area for beef cattle. Much of this research has investigated the effects of transitioning from a diet containing ~40% to one containing ~90% concentrate (Brown *et al*. 2006). This is a level much higher than typically fed in the dairy industry. It has been found that rapid adaptation strategies result in greater variance of pH values (mean, minimum, maximum and time below the curve) compared with gradual adaptation strategies, and has been linked to cases of acidosis (Bevans *et al*. 2005). Another common observation in cattle adapting to a high concentrate diet is a reduction in DMI (Tremere *et al*. 1968; Burrin *et al*. 1988). Tremere *et al*. (1968) reported that strategies using a larger daily increment to increase the level of concentrate in the diet of yearling dairy heifers resulted in them beginning to refuse feed sooner.

The objective of this experiment was to determine the effects of different adaptation strategies for introducing a large amount of crushed wheat grain to dairy cows previously fed forage only, on ruminal fluid pH, VFA and ammonia concentrations, DMI and milk yield (MY). The hypotheses tested were (1) that introducing a large amount of crushed wheat grain (40% of total DMI) to cows on a forage only diet in larger increments will result in greater fluctuations in the mean daily DMI and more time per day when ruminal fluid is below pH 6.00, compared with smaller increments; and (2) that if the wheat adaptation is conducted over a 6-day period, it will result in greater fluctuations in the mean daily DMI and more time per day when ruminal fluid is below pH 6.00 than when adaptation is conducted over 11 days.

Materials and methods

Experimental design and dietary treatments

The experiment was conducted at the Department of Economic Development, Jobs, Transport and Resources (DEDJTR), Ellinbank Centre, Vic., Australia (38°14′S, 145°56′E). All procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council 2004). Approval to proceed was obtained from the DEDJTR Agricultural Research and Extension Animal Ethics Committee. The experiment was conducted over 35 days. The initial 21 days were used as a covariate period during which the cows were fed *ad libitum* on only lucerne cubes, in order to minimise the effects of previous cereal grain consumption. Following the covariate period a 14-day measurement period began, during which the treatments were imposed.

The experiment used 32 rumen-fistulated Holstein-Friesian dairy cows, in their 2nd to 11th lactation. All cows were in late lactation (263 ± 15.8 DIM, mean ± s.d.) with a bodyweight of 662 ± 52.7 kg. They were milked twice daily at ~0700 hours and 1500 hours through a common parlour. Based on data collected during the covariate period, one of the four treatments were allocated to cows at random (8 cows per treatment), subject to the treatment groups being balanced for bodyweight, age, calving date, DMI and current MY. This was achieved using the COVDESIGN procedure in Genstat software (Genstat 18th edition, VSN International, Hemel Hempstead, UK).

During the measurement period each cow was fed at a rate of ~95% of her DMI during the covariate period. Each of the treatments began on a diet of 100% lucerne hay cubes, different strategies were then used to partially substitute crushed wheat grain for lucerne hay cubes, until wheat comprised 40% of total DM offered. The treatments were as follows:

(1) Small6: introduction of crushed wheat grain in six small even increments (6.7% of total DM) over 6 days.
(2) Small11: introduction of crushed wheat grain in six small even increments (6.7% of total DM) over 11 days.
(3) Large6: introduction of crushed wheat grain in three large even increments (13.3% of total DM) over 6 days.
(4) Large11: introduction of crushed wheat grain in three large even increments (13.3% of total DM) over 11 days.

A schedule of the dietary proportion of wheat each day for each of the treatments is presented in Table 1. Crushed wheat was provided by a commercial feed company (Evison Grain and Transport, Drouin, Vic., Australia) and particle size as a percentage of DM retained on sieve, was 62% coarse (2 mm), 27% medium (1 mm), 11% fine (<1 mm).

Following each milking, cows were moved to individual stalls where they were given half their daily ration (i.e. half in the morning and half in the afternoon). The wheat allocation was offered first and once that had been consumed, or had been on offer for 30 min, the lucerne cubes were given. The cows were allowed 4 h to consume their feed and were offered water twice

Table 1. Proportion of wheat (%, DM basis) in diets offered to each of the treatment groups

Small6, wheat introduced in six small increments over 6 days; Small11, wheat introduced in six small increments over 11 days; Large6, wheat introduced in three large increments over 6 days; Large11, wheat introduced in three large increments over 11 days

	Day of wheat introduction													
Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Small6	7	13	20	27	33	40	40	40	40	40	40	40	40	40
Small11	7	7	13	13	20	20	27	27	33	33	40	40	40	40
Large6	13	13	13	27	27	40	40	40	40	40	40	40	40	40
Large11	13	13	13	13	13	27	27	27	27	27	40	40	40	40

during this time. In between feeding periods, cows were returned to a bare paddock with no feed available but with free access to water.

Intake and nutritive characteristics

The amount of lucerne cubes and wheat offered and refused at each feeding was recorded for each cow, and samples were collected at each feeding. All samples were oven-dried at 100°C for 24 h to determine DM concentration. The amount of DM offered and refused was then determined, to enable the calculation of DMI of lucerne cubes and wheat grain. Additional samples of all feed offered and refused were collected, bulked by feed type or, in the case of refusals, by individual cow and stored at 4°C before being freeze-dried and ground through a 0.5-mm sieve. The samples were then dispatched for chemical analyses of nutritive characteristics by near-infrared spectroscopy (AOAC 2000) at a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY, USA). Concentrations of estimated metabolisable energy (ME) were calculated using the following formula (NRC 2001):

$$\text{ME (MJ/kg DM)} = (((1.01 \times (0.04409 \times \text{TDN})) - 0.45) \times 4.184),$$

where TDN is total digestible nutrient (%). The nutritive characteristics of feed offered are presented in Table 2.

Milk yield and composition

Individual MY were measured and recorded at every milking using a DeLaval Alpro milk metering system (DeLaval International, Tumba, Sweden). Three times during each measurement week a sub-sample was taken from all cows at consecutive evening and morning milkings, using in-line milk sampling meters. Milk samples were tested for concentrations of protein, fat and lactose using an infrared milk analyser (Model 2000, Bentley Instruments, Chaska, MN, USA). Energy-corrected milk (ECM) yields were calculated using the following formula (Tyrrell and Reid 1965):

$$\text{ECM(kg/cow.day)} = \text{MY(kg/cow.day)} \times [376 \times \text{fat(\%)} + 209 \times \text{protein(\%)} + 948]/3138$$

Ruminal fluid pH, VFA, ammonia and lactate

A bolus for measuring pH (KB5, Kahne, Auckland, New Zealand) was inserted *per fistula* into the rumen of each cow at the commencement of the final week of the covariate period, and remained in the cows until the end of the measurement period. On the day before insertion, each bolus was calibrated according to the manufacturer's instructions. Each bolus was attached to a 750-g weight to ensure it migrated to, and remained at the bottom of the rumen. Ruminal fluid pH was logged every 5 min. Boluses were removed weekly for 8 h, to validate the pH readings, and a linear interpolation was used to correct for any drift in individual bolus pH readings. Following the validation, all data were downloaded and boluses were re-calibrated before re-insertion.

Ruminal fluid samples were collected from all cows on the last day of the measurement period. On these days a sample was taken immediately before the morning feed and then 4 h after feeding had commenced. Samples were collected *per fistula* using a 100-mL plastic syringe connected to a copper pipe inserted into the rumen. Fluid was collected from four sites within the rumen and mixed thoroughly. A 50-mL subsample was immediately poured off and placed on ice while the pH of the remainder was analysed using a portable pH meter (Mettler-Toledo FG2 pH meter, Schwerzenbach, Switzerland). Following the collection of all samples the 50-mL aliquots were centrifuged (4°C, 4000*g*, 10 min) and a 0.5-mL aliquot of supernatant was transferred to a tube containing 4.5 mL of dilute acid (1% formic acid and 1% ortho-phosphoric acid) for later analysis of ammonia concentration. An additional 5-mL aliquot of supernatant was dispensed into a tube for later analyses of VFA and lactate concentrations. Both subsamples were stored at −20°C until subsequent analyses. Volatile fatty acid concentrations were determined by capillary gas chromatography using a flame ionisation detector, auto-sampler and auto-injector (Agilent 6890; Agilent Technologies, Santa Clara, CA, USA) and a wide bore capillary column (BP21 column; 12-m × 0.53-mm internal diameter (i.d.) and 0.5-μm film thickness; SGE International, Melbourne, Vic, Australia) with a retention gap kit (including a 2-m × 0.53-mm i.d. guard column). Analyses were conducted following the methodology described by Packer *et al*. (2011) using 4-methyl-valeric acid (184 ppm) as the internal standard. All results were calculated as ppm and converted to mmol/L for subsequent statistical analyses. Lactate concentrations were determined using a D/L lactate kit (K-DLATE; Megazyme, Bray, Ireland) and a microplate reader (AMR-100; Hangzhou Allsheng Instruments, Hangzhou, China). Ammonia concentrations were determined using flow-injection (Lachat Quik-Chem 8000; Lachat Instruments, Milwaukee, WI, USA) according to an alkaline phenol-based method (method 12–107–06–1-A; Lachat Instruments) and analysed against standard ammonia solutions.

Statistical analyses

All data were analysed using GENSTAT for Windows (GENSTAT 18th edition, VSN International). The DMI data were analysed using a general mixed model with a linear covariate term for the mean DMI during the covariate period, and factorial fixed effects for treatment by day, and the random effects model with an

Table 2. Mean nutritive characteristics of feeds offered during the measurement period

All values are % of DM unless otherwise indicated. CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre; NFC, non-fat carbohydrates; CF, crude fat (ether extract)

	CP	ADF	NDF	Lignin	NFC	Starch	CF	Ash	ME (MJ/kg DM)
Lucerne cubes	18.1	38.4	49.6	7.3	21.4	0.8	2.0	9.0	9.2
Wheat	14.2	4.2	11.0	0.4	71.2	57.3	1.8	1.8	14.8

autoregressive, order 1, process (AR1) for repeated-measures within cows over time. The AR1 model was selected as a suitable repeated-measures model for the databased on the Akaike information criterion. Distributional assumptions were checked using graphs of residuals.

Ruminal fluid pH data were summarised for each cow within the measurement and covariate periods before statistical analysis. Several summaries were used: average pH, average maximum daily pH, average minimum daily pH, the average change in pH relative to the previous day per additional kg DM wheat on days of incremental increases, total time for the period that pH was below 6.00, the total area (pH/h) of pH below 6.00, and the number of days on which pH fell below 6.00. The number of days on which pH fell below 6.00 in the measurement period was analysed using a generalised linear model with dispersed binomial error and logit link function, with factorial effects of increment size (large or small) by length of adjustment period (6 or 11 days), and covariate effects for mean pH in the covariate period, bolus position during the covariate period, and mean bolus position during the measurement period. Other pH variables were analysed by ANCOVA with covariates for the outcome variable in the covariate period. Treatment structure was factorial in increment size (small or large) by length of adjustment period (6 or 11 days). *P*-values for variables with non-normal distribution, namely total time for the period that pH was below 6.00, and the total area (pH × h) of pH below 6.00, were calculated by permutation tests within the ANCOVA.

The concentrations of VFA, ammonia and lactate in the sampled ruminal fluid for each treatment were analysed by ANCOVA, with covariate terms for the variable taken from the ruminal fluid sampled during the covariate period. The factorial treatment structure was increment size (small or large) by number of adjustment days (6 or 11 days) by sampling time (pre- or post-feed) and a blocking structure of cow split for time of sampling. The MY and composition data were analysed by ANCOVA, with covariate terms for the mean of the variable during the covariate period. The factorial treatment structure was increment size (small or large) by length of adjustment period (6 or 11 days) by day, and a blocking structure of cow split for day.

Results

Dry matter intake

Overall DMI averaged 19.7 kg/cow.day for the 14-day measurement period. Once the maximum proportion of wheat (40% DMI) had been reached, mean daily DMI (kg/cow) of wheat were 7.8 ± 0.87, 8.0 ± 0.47, 7.9 ± 0.71 and 7.8 ± 1.21, and of lucerne cubes were 11.6 ± 1.29, 11.8 ± 0.76, 11.8 ± 1.09 and 11.6 ± 1.74, for Small6, Small11, Large6 and Large11, respectively. During the 2-week measurement period there was one refusal of wheat; 1.09 kg from a cow on Small6 treatment on Day 9. There were two lucerne cube refusals; both from cows in the Small6 treatment group, on Days 1 and 9 (2.5 and 2.4 kg DM, respectively).

Ruminal fluid pH

Features of the ruminal fluid pH for each of the adaptation strategies, averaged over the treatment period, are presented in Table 3. Neither the number of adaptation days nor the increment size had an effect on either mean or maximum ruminal fluid pH. The increment size of the wheat affected the minimum pH, the introduction of wheat in small increments resulted in a lower daily minimum ruminal fluid pH compared with the use of large increments (pH 5.95 and 6.05, respectively). The increment size also affected the change in pH per kg DM wheat that occurred immediately after an incremental increase in wheat, but this was not affected by number of adaptation days. When wheat was fed out in six smaller increments the ruminal fluid pH dropped by a greater amount than if wheat was fed out in three larger increments (–0.05 and –0.04 pH/kg DM wheat, respectively). There were no differences between the treatments for time ruminal fluid spent under pH 6.00 or area under pH 6.00. The daily mean ruminal fluid pH values for each of the adaptation strategies are presented in Fig. 1. There was a significant effect of day ($P < 0.001$) on mean ruminal fluid pH. Mean daily ruminal pH declined as the proportion of wheat in the diet increased for all treatments; however, once the proportion of wheat stopped increasing and remained at 40% of DMI, the mean pH exhibited no further declines. None of the strategies showed a mean daily pH value below 6.00 on any of the measurement days.

Table 3. Covariate adjusted means of the ruminal fluid pH features during the 14-day measurement period for cows fed according to each of the four adaptation strategies

Small6, wheat introduced in six small increments over 6 days; Small11, wheat introduced in six small increments over 11 days; Large6, wheat introduced in three large increments over 6 days; Large11, wheat introduced in three large increments over 11 days. No interactions between increment size and number of adaptation days were significant and are therefore not presented

	Treatment					*P*-value	
	Large6	Large11	Small6	Small11	s.e.d.	Increment size	Number of adaptation days
Mean daily pH	6.36	6.48	6.33	6.35	0.074	0.122	0.234
Maximum pH	6.85	6.93	6.84	6.78	0.074	0.148	0.903
Minimum pH	6.00	6.09	5.90	6.00	0.067	0.047	0.052
Time under pH 6 (h/day)[A]	2.40	0.97	3.84	3.65	1.610	0.073	0.473
Area under pH 6 (pH × h)[B]	0.37	0.07	0.54	0.53	0.279	0.114	0.435
ΔpH/kg increase of wheat[C]	–0.04	–0.03	–0.05	–0.05	0.007	0.011	0.184

[A]Mean time per day during which ruminal pH was below 6.00.

[B]Area of the pH versus time of day curve below pH 6.00 (pH × h).

[C]The average change in pH relative to the previous day per additional kg DM wheat on days of incremental increases.

An interaction ($P < 0.001$) occurred between day and number of adaptation days. Wheat introduced in 6 days resulted in significantly lower mean ruminal fluid pH from Day 4 to Day 8, compared with wheat introduced in 11 days. However, from Day 9 until the end of the measurement period mean ruminal pH did not differ with number of adaptation days. An interaction effect ($P < 0.001$) between day and increment size also occurred. Introducing wheat in large increments resulted in a higher mean ruminal fluid pH from Day 10 until the end of the measurement period, compared with the use of small increments. No three-way interactions were significant.

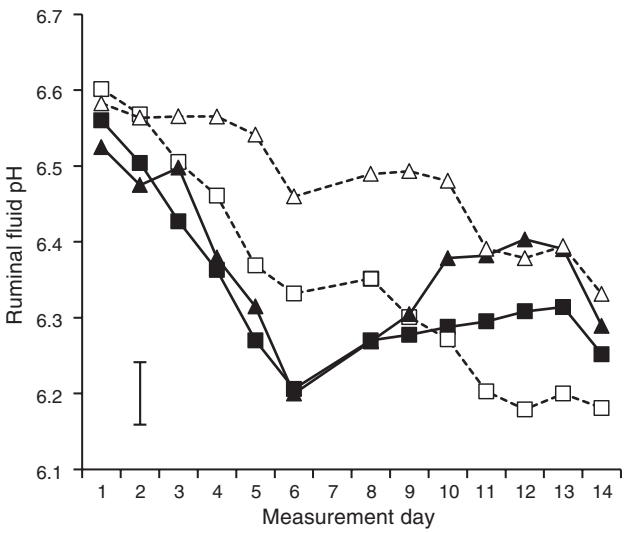

Fig. 1. Mean daily ruminal fluid pH of cows fed via each of the different adaptation strategies: Small6 (solid squares, solid line); Small11 (empty squares, dashed line); Large6 (solid triangles, solid line); Large11 (empty triangles, dashed line). Small6, wheat introduced in 6 small increments over 6 days; Small11, wheat introduced in six small increments over 11 days; Large6, wheat introduced in three large increments over 6 days; Large11, wheat introduced in three large increments over 11 days. The error bar is the s.e.d. (0.082) for comparing strategy treatments within each day.

Ruminal fluid VFA, ammonia and lactate

The concentrations of total VFA, ammonia and lactate, and proportions of individual VFA (% of total VFA) in ruminal fluid sampled on the final morning of the measurement period are presented in Table 4. None of the measured parameters were affected by increment size or number of adaptation days. Volatile fatty acids, ammonia and lactate concentrations as well as the proportions of individual VFA; propionic and valeric acid, all increased post feeding. The proportion of acetic and butyric acid decreased post feeding. The lipogenic to glucogenic VFA ratio [(acetic acid + butyric acid)/propionic acid] was not affected by treatments or feeding.

Milk yield and composition

Mean daily MY and ECM yield, as well as the proportions of protein, fat and lactose, (averaged over the treatment period), are presented in Table 5. Neither the number of adaptation days nor the increment size had an effect on MY, ECM yield or any of the milk composition variables (protein, fat, lactose). Measurement day affected overall ECM yield with an increase occurring between Day 1 and Day 4 (data not shown). However, ECM yield did not differ between Day 1 and Day 14 for any of the treatments. The three-way interaction between number of adaptation days, increment size and measurement day was significant for MY and ECM ($P < 0.05$) but not for any composition variables. No other interactions with measurement day were significant.

Discussion

The present experiment has provided information about the effects of different strategies for adapting lactating forage-fed dairy cows to a diet high in wheat grain, on ruminal fluid parameters, DMI and MY. The first hypothesis tested was that

Table 4. Covariate adjusted mean concentrations of total volatile fatty acids (VFA), ammonia and lactate, and proportions of individual VFA (% of total VFA) in ruminal fluid sampled on the final day of the experiment from cows fed according to each the treatment adaptation strategies

Small6, wheat introduced in six even increments over 6 days; Small11, wheat introduced in six even increments over 11 days; Large6, wheat introduced in three even increments over 6 days; Large11, wheat introduced in three even increments over 11 days

	Treatment									*P*-value[A]		
	Small6		Small11		Large6		Large11			Increment	Number of	Sampling
	Pre-feed[B]	Post-feed[B]	Pre-feed	Post-feed	Pre-feed	Post-feed	Pre-feed	Post-feed	s.e.d.	size	adaptation days	time
Total VFA (mmol/L)	78	169	88	173	70	165	79	173	14.0	0.161	0.062	<0.001
Acetic acid (%)	66.8	66.0	65.9	65.1	67.0	65.4	66.9	65.9	0.68	0.347	0.387	<0.001
Propionic acid (%)	12.5	14.7	14.4	17.2	12.8	15.9	13.0	15.7	2.44	0.531	0.103	0.004
Butyric acid (%)	17.1	14.7	16.3	13.6	16.7	14.1	16.5	14.0	1.01	0.817	0.327	<0.001
Valeric acid (%)	0.9	1.8	1.0	1.9	0.9	1.9	0.9	1.8	0.24	0.747	0.742	<0.001
(A +B)/P[C]	6.4	5.9	5.6	5.4	6.0	5.6	6.1	5.7	0.81	0.940	0.214	0.493
Ammonia (mg/L)	115	185	123	187	101	176	122	199	25.5	0.687	0.117	0.011
D-Lactate (mmol/L)	0.02	0.06	0.02	0.05	0.02	0.05	0.02	0.06	0.007	0.862	0.401	<0.001
L-Lactate (mmol/L)	0.04	0.06	0.04	0.07	0.04	0.06	0.05	0.06	0.005	0.707	0.062	<0.001

[A]No interactions were significant and are therefore not presented.
[B]Pre-feed, samples were taken immediately before the morning feed; post-feed, samples were taken 4 h after feed was offered to the cows in the morning.
[C]The lipogenic to glucogenic VFA ratio [(acetic acid + butyric acid)/propionic acid].

Table 5. Covariate adjusted mean daily milk yield (MY) and composition of milk from the cows fed according to each the adaptation strategies

Small6, wheat introduced in six small increments over 6 days; Small11, wheat introduced in six small increments over 11 days; Large6, wheat introduced in three large increments over 6 days; Large11, wheat introduced in three large increments over 11 days. No interactions between increment size and number of adaptation days were significant and are therefore not presented. ECM, energy-corrected milk yield

	Treatment					*P*-value	
	Small6	Small11	Large6	Large11	s.e.d.	Increment size	Number of adaptation days
MY (kg/cow.day)	13.7	13.9	15.2	13.7	0.77	0.228	0.257
ECM (kg/cow.day)	15.4	15.8	17.1	15.8	0.97	0.208	0.495
Protein (%)	3.71	3.63	3.65	3.66	0.046	0.689	0.367
Fat (%)	4.83	4.99	4.89	5.05	0.135	0.570	0.110
Lactose (%)	4.68	4.70	4.74	4.74	0.039	0.120	0.763

if wheat was introduced in larger but fewer increments it would result in greater fluctuations in the mean daily DMI and more time when ruminal fluid was below pH 6.00. This was based on previous research that showed introducing ground wheat in large increments twice daily to dairy heifers on an all hay diet, resulted in a decline in DMI that occurred sooner than when increments were smaller (Tremere *et al*. 1968). However, this was not observed in the present experiment; with few exceptions cows consumed all of the feed offered. Thus the size of the wheat increments did not affect DMI. Furthermore, the daily mean ruminal fluid pH did not drop below 6.00 on any of the days and MY did not change over the measurement period for any of the adaptation strategies. Contrary to what was expected, the introduction of wheat in smaller increments resulted in a lower minimum ruminal fluid pH at 5.95. This value still remained within a range (5.80–6.20) that has previously been associated with some of the highest producing pasture-based dairy cows (Kolver and De Veth 2002). Introducing wheat in smaller more frequent intervals caused greater declines in pH. It could be that the increased variability in the diet over a short time-frame resulted in a less stable rumen environment. The difference in pH change between the strategies was however, biologically insignificant, at only 0.01 pH/kg DM of wheat added, it had no subsequent effects on DMI or MY. It is possible that the lack of MY response was due to the cows being in late lactation, as response to supplementation decreases as lactation progresses (Kellaway and Harrington 2004). It has been demonstrated that neither the mean nor the minimum daily pH are the most important measurements, but rather the period of time ruminal fluid remains at a suboptimal pH (Stewart 1977; Mackie and Gilchrist 1979; de Veth and Kolver 2001). There were no differences between the treatments for the amount of time ruminal fluid was below pH 6.00, and the treatments did not remain at a suboptimal pH for any extended period of time. Furthermore, as daily mean ruminal pH was above 6.10 for all treatments, any diurnal variation would have likely had negligible effects on digestion parameters (Wales *et al*. 2004). The steady DMI throughout the experiment further supports this, as variable intake patterns have been suggested as another feature of disturbances from acidotic diets (Britton and Stock 1987). As DMI was steady and there were no differences in amount of time ruminal fluid was below pH 6.00 between the different incremental strategies, the first hypothesis is not supported.

When Leddin *et al*. (2009) fed up to 36% of DMI as wheat to previously adapted mid-lactation dairy cows on a pasture hay diet for 19 days, cows fed ≥25% wheat grain exhibited a mean ruminal fluid pH below 6.00 and a minimum pH of 5.40. The cows in the present experiment had no previous adaptation, were fed a greater proportion of wheat at 40% of DMI, and yet the lowest mean ruminal fluid pH was 6.33 for the measurement period, and 6.18 for an individual day. Furthermore, the lowest ruminal fluid pH exhibited by a treatment group was 5.90 from the Small6 treatment. These differences are likely driven by the different forages. Despite the pH levels in the present experiment being higher than expected, the pattern in ruminal fluid pH changes within each strategy is consistent with previous work showing a decrease in pH with increasing proportions of wheat in a dairy cows diet (Leddin *et al*. 2009). Although feeding wheat as 40% of total DM is at the upper end of what would typically be fed to cows in late lactation, it does occur in situations when pasture supply is limiting or if body condition needs to be improved before drying off. The ingestion of a large amount of readily fermentable carbohydrate, such as wheat grain, usually leads to a decline in ruminal fluid pH. This is due to a build-up of fermentation acids such as VFA and lactate, and is more common in cows that have not been previously conditioned to the feed (Bramley *et al*. 2008). The increase in total VFA, from 79 to 170 mmol/L, and more specifically propionic (13.2–15.9%) and valeric acid (0.94–1.86%) concentrations seen post-feeding in the present experiment reflects the increase in carbohydrate fermentation that was occurring. D-lactate is also known to build up in the rumen if a cow changes to a ration with a large amount of readily fermentable carbohydrate too rapidly (Counotte and Prins 1981). High concentrations of lactate in the rumen can be indicative of acidosis, with concentrations above 40 mmol/L exhibited in cows with severe acidosis (Owens *et al*. 1998). Although there was a significant increase in ruminal D-lactate post-feeding the total concentration measured was still well below any level symptomatic of lactic acidosis.

The second hypothesis tested was that the introduction of wheat in a shorter time frame (6 vs 11 days) would result in greater fluctuations in the mean daily DMI and more time when ruminal fluid was below pH 6.00. The number of adaptation days used did not affect any of the ruminal fluid pH parameters. The amount of time ruminal fluid spent below pH 6.00 did not differ between the 6- and 11-day adaptation strategies. Additionally, the

time spent below pH 6.00 was far less than previously reported in lactating dairy cows fed forage supplemented with wheat (Auldist *et al*. 2014; Greenwood *et al*. 2014). During the wheat adaptation period the ruminal pH for Large11 remained consistently higher than for all other strategies. This treatment successfully maintained a pH above 6.00, with a mean of 6.48 and minimum of 6.09; however, it does not necessarily indicate an ideal adaptation strategy. A ruminal fluid pH range of 6.00–6.20 has been identified as optimal for lactating dairy cows on a forage-concentrate diet (Mould *et al*. 1983; Pitt *et al*. 1996). Additionally, research has found that grazing dairy cows with the greatest MY had mean ruminal fluid pH values in the range of 5.80–6.20 (Kolver and De Veth 2002). It is possible that if the cows in the Large11 adaptation strategy maintained pH values within the range 5.80–6.20, it may have provided more desirable rumen conditions resulting in increased production. Although there was some daily variation between the treatments in regards to mean ruminal fluid pH, it was always above 6.00 and therefore of dubious biological significance (Wales *et al*. 2004). There were no differences between the 6- and 11-day adaptation strategies for DMI or amount of time when ruminal fluid was below pH 6.00 and so the second hypothesis is not supported.

It was expected that excessive acid production would occur in the more abrupt adaptation strategies used in the present experiment, and that this would result in increased fluctuations in ruminal fluid pH parameters and lower overall pH levels. It is possible that the reason this was not observed was due to the choice of forage. Lucerne hay cubes were used instead of fresh pasture; this was to ensure a consistent nutrient and DM concentration across the measurement period. Lucerne was selected specifically because it is of high nutrient concentration as a sole forage source for ruminants. However, lucerne is also known to have one of the highest buffering capacities among ruminant feedstuffs (Playne and McDonald 1966; Crawford *et al*. 1983; McBurney *et al*. 1983). Several studies have investigated the intrinsic buffering capacity of ruminant feedstuffs, and these studies have highlighted the high buffering capacity of legumes (McDonald and Henderson 1962; Greenhill 1964; Wohlt *et al*. 1987). It is well documented that high protein feeds generally have the highest buffering capacity (Jasaitis *et al*. 1987), possibly due to the fact that protein degradation leads to the production of ammonia, the main alkali in the rumen (Crawford *et al*. 1983). All the current adaptation strategies showed relatively high ammonia levels that further increased after feeding, suggesting that the high protein content of lucerne cubes helped prevent the anticipated depression in ruminal pH. The ruminal ammonia concentrations were comparable to other studies that incorporated high dietary CP (17–19%) (Gustafsson and Palmquist 1993; Hristov *et al*. 2004). The majority of previous research shows that grasses tend to have a low buffering capacity, whereas legumes have a high one. It is possible that if the present study was conducted with a grass as the base forage we may have seen more variable ruminal fluid pH, greater declines in pH and signs of acidosis. We speculate that in the present study the high buffering capacity of the lucerne cubes helped the ruminal contents resist the drop in pH that is normally seen when feeding large amounts of a readily fermentable carbohydrate (Owens *et al*. 1998). A similar result has been observed when steers were transitioned from a 100% lucerne hay diet to a 100% concentrate (65% corn, 25% wheat, 5% soybean meal, 5% molasses, 1% minerals) diet in 7 days, in an attempt to induce acidosis (Coe *et al*. 1999). Although, Coe *et al*. (1999) did not elucidate as to why there were no acidotic effects seen, it is likely due to a combination of the buffering effects of the lucerne and that the majority of the concentrate was made up of corn, a slowly fermentable starch source. The present results were surprising as introducing wheat to the equivalent of 40% of total DMI over a 6-day period with no previous adaptation is far more rapid than would typically be practiced on commercial dairy farms.

Conclusions

The different strategies used for adapting forage-fed cows to a high grain diet had very little effect on ruminal fluid pH variables and fermentation characteristics. There was no detectable effect on DMI, and the MY on the final measurement day was not different to initial MY for any of the treatment groups. The ruminal fluid pH did not decline to levels of biological concern, possibly due to the buffering effect of the lucerne. Under the conditions of this experiment, there appeared to be no advantage to lengthening the adaptation period, nor was there a benefit of introducing the wheat in smaller increments. This points to an opportunity to quickly introduce large amounts of grain to lactating dairy cows, while avoiding negative effects such as acidosis, and indicates a possibility for tailoring grain adaptation strategies to specific forages. However, these results may only apply when lucerne hay comprises the majority of the diet and so further research is required into the differing buffering effects of a variety of forages and concentrates *in vivo*.

Conflicts of interest

The authors declare no conflicts of interest.

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Chapter 5

Forage type influences milk yield and ruminal responses to wheat adaptation in late lactation dairy cows

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Forage type influences milk yield and ruminal responses to wheat adaptation in late-lactation dairy cows

V. M. Russo,*†‡[1] B. J. Leury,† E. Kennedy,‡ M. C. Hannah,* M. J. Auldist,* and W. J. Wales*
*Agriculture Victoria Research, 1301 Hazeldean Road, Ellinbank, Victoria 3821, Australia
†Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Victoria 3010, Australia
‡Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, P61 C996 Ireland

ABSTRACT

The effects of different wheat adaptation strategies on ruminal fluid pH, dry matter intake (DMI) and energy-corrected milk (ECM) were measured in 28 late-lactation dairy cows. Cows were fed either perennial ryegrass (PRG) hay or alfalfa hay and had no previous wheat adaptation. Wheat was gradually substituted for forage in 3 even increments, over 6 or 11 d, until wheat made up 40% of DMI (~8 kg of dry matter/cow per day). We found no differences in DMI between adaptation strategies (6 or 11 d) within forage type; however, cows fed alfalfa hay consumed more overall and produced more ECM. The rate of ruminal pH decline after feeding, as well as the decrease in mean, minimum, and maximum ruminal pH with every additional kilogram of wheat was greater for cows fed alfalfa hay. Cows fed alfalfa hay and on the 6-d adaptation strategy had the lowest mean and minimum ruminal fluid pH on 3 consecutive days and were the only treatment group to record pH values below 6.0. Despite ruminal pH declining to levels typically considered low, no other measured parameters indicated compromised fermentation or acidosis. Rather, cows fed alfalfa hay and adapted to wheat over 6 d had greater ECM yields than cows on the 11-d strategy. This was due to the 6-d adaptation strategy increasing the metabolizable energy intake in a shorter period than the 11-d strategy, as substituting wheat for alfalfa hay caused a substantial increase in the metabolizable energy concentration of the diet. We found no difference in ECM between adaptation strategies when PRG hay was fed, as there was no difference in metabolizable energy intake. The higher metabolizable energy concentration and lower intake of the PRG hay meant the increase in metabolizable energy intake with the substitution of wheat was less pronounced for cows consuming PRG hay compared with alfalfa hay. Neither forage type nor adaptation strategy affected time spent ruminating. The higher intakes likely contributed to the lower ruminal pH values from the alfalfa hay treatments. However, both forages allowed the rumen contents to resist the large declines in ruminal pH typically seen during rapid grain adaptation. Depending on the choice of base forage, rapid grain introduction may not result in poor adaptation. In situations where high-energy grains are substituted for a low-energy, high-fiber basal forage, rapid introduction could prove beneficial over gradual strategies.
Key words: ruminal pH, buffering capacity, alfalfa hay, perennial ryegrass hay

INTRODUCTION

In dairying industries where pasture makes up the majority of the cows' diet, such as those in Victoria, Australia, energy needs are often met through supplementation with cereal grains or pelleted concentrates (Bargo et al., 2003). Due to large seasonal variability in the nutrient supply from pasture (Roche et al., 2009) and changing energy demands throughout lactation (NRC, 2001), the amount of concentrates provided may be altered accordingly. This could mean increasing concentrate feeding rates several times throughout a lactation, which is known as stepped flat-rate feeding (Leaver, 1988). The introduction of increasing amounts of rapidly digestible concentrates and their subsequent fermentation results in the rapid production of VFA, and possibly lactate, causing declines in ruminal fluid pH (Wales and Doyle, 2003). Low ruminal fluid pH, in turn, can lead to compromised fiber digestion, variable feed intake, and metabolic diseases such as acidosis (Mould et al., 1983; Owens et al., 1998; Krause and Oetzel, 2006).

Despite the widespread use of concentrates in Australian dairy systems, forages still make up the majority of the diet and play a pivotal role in optimizing rumen function. The contribution of forages to maintaining

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[1] Corresponding author: victoria.russo@ecodev.vic.gov.au

a stable ruminal pH derive largely from their effects on fermentation and rumination (Allen et al., 2006). Due to a relatively low NDF fraction, legumes are more rapidly digested than grasses, allowing for greater DMI and a faster rate of acid production (Van Soest, 1965; Smith et al., 1972). However, legumes have a high intrinsic buffering capacity relative to other forages (Greenhill, 1964; Wohlt et al., 1987), thought to be a consequence of a higher cation exchange capacity and higher protein concentration (McBurney et al., 1983). An ability to buffer the ruminal contents when large amounts of rapidly fermentable starch are consumed is critical in high-concentrate systems. Grasses, which are fermented more slowly but have a lower intrinsic buffering capacity (McBurney et al., 1983), are likely to have less ability to resist ruminal pH changes. As well as influencing DMI and digestion, the fiber concentration of forages also has a role in buffering the rumen during fermentation of concentrates by positively influencing rumination time, which determines saliva production, the main source of buffers within the rumen (Allen, 1997; Allen et al., 2006; Krause and Oetzel, 2006).

Part of a successful adaptation strategy is maintaining ruminal pH within an optimum range, which for forage-concentrate diets is suggested to be pH 6.0 to 6.3 (Hutjens and Overton, 1996; Pitt et al., 1996). Strategies such as combining concentrates with forages as a mixed ration or offering a greater number of smaller meals positively influences ruminal pH (Kaufmann, 1976; Auldist et al., 2013). However, the majority of dairy farmers in Australia do not feed mixed rations and offer relatively large amounts of concentrates (>1.0 t/cow per year) during milking, with the most common being barley grain and wheat grain (Dairy Australia, 2015). This type of feeding system creates the challenge of a twice-daily rapid increase in fermentation acids within the rumen. The guidelines around introducing large amounts of cereal grains to forage-fed dairy cows are vague, with recommended introduction periods ranging from 10 to 21 d and the quantity being gradually increased every 2 to 3 d (Warner, 1962; Tremere et al., 1968; Kellaway and Harrington, 2004). It is desirable to shorten this adaptation period in order to simplify management strategies and increase the ME intake of the herd to maximize milk production. However, making such abrupt dietary changes heightens the risk of acidosis and animals refusing feed, particularly when the concentrate offered is ground wheat (Tremere et al., 1968) due to high rumen fermentability (Gonzalez-Rivas et al., 2016).

The objective of our experiment was to compare 2 strategies to introduce large amounts (~8 kg of DM/cow per day) of crushed wheat grain into the diet of late-lactation dairy cows previously fed only perennial ryegrass (**PRG**) hay or alfalfa hay and determine the effects on ruminal fluid pH, milk yield (**MY**), and DMI. The decision to use conserved forages was based on situations where fresh grazed forages are limiting in pasture-based systems. The hypotheses tested were (1) that feeding PRG hay in combination with wheat would result in a lower mean ruminal fluid pH than feeding alfalfa hay and wheat, and (2) that the mean ruminal fluid pH of cows introduced wheat in 6 d would not differ from that of cows introduced wheat in 11 d, irrespective of forage type.

MATERIALS AND METHODS

Experimental Design and Dietary Treatments

The experiment was conducted at the Department of Economic Development, Jobs, Transport and Resources, Ellinbank Centre, Victoria, Australia (38°14′S, 145°56′E). All procedures were conducted in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (National Health and Medical Research Council, 2013). Approval to proceed was obtained from the Department of Economic Development, Jobs, Transport and Resources Agricultural Research and Extension Animal Ethics Committee.

Twenty-eight rumen-fistulated Holstein-Friesian dairy cows in lactation 2 to 9 were used. All cows were seasonally calving and were in late lactation, having calved between July and October 2015 (235 ± 27.4 DIM; mean ± SD). Milking occurred twice daily at 0600 and 1500 h. The experiment was conducted over a 35-d period, composed of a 7-d covariate period, a 14-d forage adaptation period, and a 14-d measurement period that included a 6- or 11-d introduction of wheat. Cows were moved to individual pens for feeding and were kept in a bare paddock between feeding bouts with no available feed but water available ad libitum. During the covariate period, cows were individually offered a 50:50 mix of PRG hay and alfalfa hay ad libitum and DMI were measured over the final 3 d. Following this, 4 treatments were each allocated to 7 cows, balancing treatment groups for DMI, BW, age, DIM, and current MY using the method of Harville (1974) implemented in the software GenStat for Windows as the procedure COVDESIGN (GenStat 18th ed.; VSN International Ltd., Hemel Hempstead, UK). Each treatment substituted wheat for forage in 3 even increments (each 13.3% of total DM) until wheat comprised 40% of DM offered. The 4 treatments were (1) alfalfa hay with a 6-d wheat adaptation strategy (**ALF6**), (2) alfalfa hay

Table 1. Wheat (% of DM) offered over the experimental period for each of the treatment diets

	Treatment day													
Item[1]	1	2	3	4	5	6	7	8	9	10	11	12	13	14
ALF6	0	13	13	13	27	27	40	40	40	40	40	40	40	40
ALF11	0	13	13	13	13	13	27	27	27	27	27	40	40	40
PRG6	0	13	13	13	27	27	40	40	40	40	40	40	40	40
PRG11	0	13	13	13	13	13	27	27	27	27	27	40	40	40

[1]Treatments: ALF6 = cows fed alfalfa hay with wheat introduced over 6 d; ALF11 = cows fed alfalfa hay with wheat introduced over 11 d; PRG6 = cows fed PRG hay with wheat introduced over 6 d; PRG11 = cows fed PRG hay with wheat introduced over 11 d.

with an 11-d wheat adaptation strategy (**ALF11**), (3) PRG hay with a 6-d wheat adaptation (**PRG6**), and (4) PRG hay with an 11-d wheat adaptation strategy (**PRG11**).

On the first day of the forage adaptation period, the diet of individual cows was changed to only include their allocated forage. They remained on this forage-only diet for the entirety of the adaptation period, during which individual DMI were measured. During the measurement period, each cow was fed at a rate equivalent to 90% of her DMI during the adaptation period to minimize refusals and the opportunity for cows to select. Wheat introduction began on the second day of the measurement period. A schedule of the dietary proportion of wheat offered each day to individual cows within each treatment is presented in Table 1. Following each milking, cows were moved to individual stalls and given half their ration in the morning and half in the afternoon. Wheat was offered first, and after 30 min any remaining wheat was removed before forage was offered. All cows were given 3.5 h to consume their forage, and water was offered twice during this time.

Intake and Nutritive Characteristics

All feed offered and refused was weighed and a representative sample was collected per cow at each feeding. Part of each sample was then dried at 100°C for 24 h to determine DM concentration, which facilitated the calculation of individual DMI. The remainder of the samples were then bulked by feed type or, in the case of refusals, by individual cow and stored at 4°C. At the completion of the experiment, bulked samples were thoroughly mixed and representative subsamples were freeze-dried and ground to pass through a 1-mm screen. The samples were then analyzed for CP, amylase-treated NDF (**aNDF**), ADF, lignin, NFC, starch, crude fat (**CF**), ash, TDN, and minerals by wet chemistry in a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Concentrations of estimated ME were calculated using the formula (NRC, 2001)

$$\text{Estimated ME (MJ/kg of DM)} = \{[1.01 \times (0.04409 \times \text{TDN \%})] - 0.45\} \times 4.184.$$

Particle size distribution of the wheat grain was determined following the method described by Moate et al. (2017).

Eating Behavior

On d 1 and 14 of the measurement period, the eating behavior of all cows was monitored over a 24-h period. On d 1, all cows were on a forage-only diet, whereas on d 14 all cows were offered the maximum amount of wheat at 40% of total diet DM. During the 24-h periods, cows were observed every 10 min. Their activity was recorded as either eating, ruminating, or not chewing. It was assumed that each observation represented the activity for the previous 10 min (Gary et al., 1970).

Milk Yield and Composition

Milk yields were recorded using a milk metering system (MM25; DeLaval International, Tumba, Sweden), which is calibrated every 2 wk and has an accuracy of ±3%. A proportionate subsample was collected at each milking from each individual cow using in-line milk samplers. Individual samples were analyzed for protein, fat, and lactose concentrations using an infrared milk analyzer (Model 2000; Bentley Instruments, Chaska, MN). Energy-corrected milk yield was calculated using the formula (Tyrrell and Reid, 1965)

$$\text{ECM (kg/cow per day)} = \text{MY (kg/cow per day)} \times [376 \times \text{fat (\%)} + 209 \times \text{protein (\%)} + 948]/3{,}138.$$

Ruminal Fluid pH, VFA, Ammonia, and Lactate

At the commencement of the covariate period, loggers for measuring ruminal pH (KB5; Kahne Ltd., Auckland, New Zealand) were calibrated and inserted

per fistula into the rumen of each cow. The capsules remained in the cows until the end of the measurement period. A 750-g weight was attached to each capsule to ensure it remained on the floor of the rumen. An average ruminal pH was logged for every 5-min interval, and data were automatically stored in the devices. Capsules were removed once a week for 8 h to validate the pH readings, and a linear interpolation was used on each individual bolus data to correct for any drift in readings between calibration and validation, assuming a uniform rate of drift. Following the validation, all data were downloaded and boluses were recalibrated before reinsertion.

Samples of ruminal fluid were collected on measurement d 1, 4, 9, and 14. On these days, immediately before the morning feeding, cows were individually moved to a squeeze chute and the first sample was collected (prefeed). A second sample was collected 4 h after feeding had commenced (postfeed) while cows remained in their individual stalls and were restrained using a locking head yoke. Samples were collected per fistula using a 100-mL plastic syringe connected to a copper pipe directly inserted into the rumen. Fluid was collected from 4 sites within the rumen (in the fiber mat, below the fiber mat, anterior to the fiber mat, and midway down the posterior end of the fiber mat) and mixed thoroughly. A 50-mL subsample was immediately poured off and centrifuged (4°C, 4,000 × *g*, 10-min) and the pH of the remainder was determined using a benchtop pH meter (Orion star A211; Thermo Fisher Scientific, Scoresby, Victoria, Australia). A 0.5-mL aliquot of supernatant was then transferred to a tube containing 4.5 mL of dilute acid (2% formic acid) for later analysis of ammonia. An additional 5-mL aliquot was stored for analyses of VFA and lactate. Both subsamples were kept at −20°C until analyzed. Volatile fatty acid concentrations were determined by capillary GC (Agilent 6890 GC; Agilent Technologies, Santa Clara, CA) with a flame ionization detector, autosampler and auto-injector, and a wide-bore capillary column [BP21 column, 12 m × 0.53 mm internal diameter (i.d.) and 0.5 μm film thickness; SGE International, Ringwood, Victoria, Australia] with retention gap kit (including a 2 m × 0.53 mm i.d. guard column). Analyses were conducted following the methodology described by Packer et al. (2011) with 4-methyl-valeric acid (184 μL/L) used as the internal standard. Lactate analyses were conducted with a microplate reader (AMR-100; Hangzhou Allsheng Instruments, Hangzhou, China) using a D-/L-lactate kit (K-DLATE; Megazyme, Bray, Ireland). Ammonia-N concentrations were determined by flow-injection (Lachat Quik-Chem 8000; Lachat Instruments, Milwaukee, WI) according to an alkaline phenol-based method (method 12–107–06–1-A; Lachat Instruments) and analyzed against standard ammonia solutions (QuikChem Systems, 2008).

Titratable Acidity and Buffering Capacity

The methodology of Playne and McDonald (1966) was used to perform an acid titration on each of the forages. A subsample of each of the forage samples collected for nutritive analyses was stored at −18°C. After the experiment, each forage sample was divided into 3 replicates, each weighing approximately 10 g (exact weights were recorded). Each replicate was mixed with 250 mL of deionized water and blended for 20 s (NutriBullet 1000; NutriBullet, Brisbane, Queensland, Australia). The solution was then stirred continuously and, after 5 min, a pH value was obtained (Orion star A211; Thermo Fisher Scientific) and recorded as the initial pH. An automatic potentiometric titrator (809 Titrando; Metrohm AG, Herisau, Switzerland) was used to perform an acid titration on each sample. Hydrochloric acid (0.1 *N*) was added gradually to reduce the pH to 5.50. The method of Jasaitis et al. (1987) was applied to describe the results. The volume of acid added was multiplied by the normality to calculate the titratable acidity. The known DM percentage was used to calculate grams of DM of sample, and the titratable acidity was described as the milliequivalents of HCl required to decrease the pH of 100 g of DM of sample to 5.50. All sample titrations were corrected for a 250-mL water blank. Acid buffering capacity was calculated by dividing the titratable acidity by the total change in pH units (i.e., initial pH minus 5.50); therefore, acid buffering capacity describes the amount of acid required to generate a unit change in pH of 100 g of DM of the sample.

Statistical Analyses

All data were analyzed using Genstat for Windows (Genstat 18th ed.; VSN International Ltd.). Milk production data, including the covariate period, forage adaptation period, and the measurement period, were analyzed using a mixed effects model comprising 1 fixed effect and crossed random effects. The fixed effect consisting of a single factor with 1 level for forage (PRG and alfalfa mix) in the covariate period, 2 levels for forage in the adaptation period (PRG or alfalfa), and a level for each combination of forage by adaptation strategy by day in the measurement period. The random effects of the mixed model were cow crossed with day. Other models for repeated measures, such as autocorrelation, in the random effects were compared

by Akaike information criterion, but not required. Statistical tests for the hypothesized effects within the measurement period for forage (alfalfa hay vs. PRG hay), adaptation strategy (11 vs. 6 d), and for their interaction, were obtained as *t*-tests by defining contrasts between relevant means. The main effect treatment means, for example, were obtained by linear combinations averaging over the relevant diet by adaptation strategy by day means predicted by the fitted mixed model. Standard errors, used in the *t*-test denominators of the contrasts, were computed correspondingly from the variance-covariance matrix of the predicted means. Behavior data from d 1 and 14 were analyzed using an ANOVA with a blocking structure of cow split for day and factorial treatment structure of forage type by adaptation strategy by wheat percentage. Nonsignificant interaction terms were dropped from the model to simplify presentation. The composition of ruminal fluid sampled on d 1, 4, 9, and 14 were subjected to mixed model analysis. Random effects for cow split for day split for time were used along with factorial treatment structure of adaptation strategy by forage-type by time by percentage of wheat in the diet. Ruminal fluid pH pre- and postfeed for each cow on d 1, 4, 9, and 14 were analyzed using a similar mixed model with random effects for cow split for day split for sampling time (pre- or postfeed) and factorial fixed effects for forage by adaptation strategy by wheat proportion by sampling time. Adaptation strategy was not significant and was dropped from the model to simplify presentation. Rates of decline in pH over the 4 h following the morning feeding were calculated for each cow on d 1, 4, 9, and 14. These were subjected to ANOVA with blocking structure of cow split for day and factorial treatment structure of adaptation strategy by forage by day. The analysis was also conducted using a factor for the percentage of wheat in the diet to replace the factor for day in the treatment structure. This was required analysis by ReML software to allow for imbalance on d 9 when the 6- and 11-d adaptation strategies had differing percentages of wheat in the diet (40 and 27%, respectively). Nonsignificant interaction terms were dropped from the model to simplify presentation. Ruminal fluid pH data from several boluses were unavailable. The number of cows having bolus data available were 6, 5, 5, and 7 for ALF6, ALF11, PRG6, and PRG11, respectively. Bolus data were summarized daily for each cow as daily mean, minimum, and maximum, and data were subjected to mixed model analysis with a fixed effect for the data in the covariate period and factorial fixed effects for forage-diet by adaptation strategy by day. The random effects were specified as an autoregressive order 1 process for day within cow. An ANOVA was performed on change in pH per kilogram of wheat added to the diet between the day before applying a wheat increment and the day on which the increment was applied (or the following day in the case of d 7 when no bolus data were recorded). These pH change rate data were calculated and averaged for each cow before analysis. The ANOVA had factorial treatment structure, forage diet by strategy, and cow as the unit. Three variables were analyzed: change in daily mean, maximum, and minimum pH per change in kilograms of DMI wheat. Days (i.e., measurement days) were defined from 0800 to 0759 h. The amount of milliequivalents of HCl per 100 g of DM added to reach a pH of 5.50 was recorded from the acid titrations for each of the 3 replicates of the 2 forage samples; these data were analyzed by ANOVA.

RESULTS

Nutritive and Dry Matter Intake

Concentrations of CP, aNDF, ADF, lignin, NFC, starch, CF, ash, TDN, estimated ME, DCAD, and cation fraction of the feeds offered are presented in Table 2. The particle size distribution of the crushed wheat grain as a percentage of DM retained on sieve was 61% large ($\geq$2 mm), 29% medium (between 1 and 2 mm), and 10% fine ($<$1 mm). The mean ($\pm$SD) particle size distribution of the hays as a percentage of DM retained on sieve was 77% ($\pm$2.2) large ($\geq$2 mm), 11% ($\pm$3.1) medium (between 1 and 2 mm), and 12% ($\pm$4.3) fine ($<$1 mm). Mean DMI of forage and wheat for each of the treatments are shown in Figure 1. The mean DMI for cows fed alfalfa hay and PRG hay during the measurement period was 18.6 and 15.7 kg DM/cow per day, respectively. Once the maximum proportion of wheat (40% DM) had been reached, daily DMI (kg/cow) of wheat were 7.4 $\pm$ 0.07, 7.5 $\pm$ 0.13, 6.2 $\pm$ 0.07, and 6.3 $\pm$ 0.12 and of forage were 11.3 $\pm$ 0.13, 11.3 $\pm$ 0.22, 9.3 $\pm$ 0.13, and 9.4 $\pm$ 0.21 (mean $\pm$ SEM) for ALF6, ALF11, PRG6, and PRG11, respectively. The mean daily estimated ME intake for each of the treatment groups are presented in Figure 2. The estimated ME intake increased over the duration of the experiment ($P < 0.001$) and cows fed alfalfa hay consumed more estimated ME overall than cows fed PRG hay ($P < 0.001$). Whereas the total amount of estimated ME consumed during the experiment was not different between adaptation strategies, on d 5 through 10 ALF6 cows were consuming an average of 14.2 MJ/d more than cows in the ALF11 treatment group ($P < 0.05$).

Table 2. Nutritive characteristics of feed offered during the experimental period[1]

Item	CP	aNDF[2]	ADF	Lignin	NFC	Starch	CF[3]	Ash	TDN	ME[4]	DCAD[5]	Cation
Alfalfa hay	18.5	46.0	35.8	7.3	20.3	0.7	2.9	12.4	56	8.6	62	3.9
PRG hay	14.9	57.3	35.9	4.3	17.5	0.3	2.1	8.4	61	9.4	42	2.4
Wheat grain	14.7	10.8	4.1	0.9	70.5	63.9	2.2	1.9	86	14	−1	0.5

[1]All values are % of DM unless otherwise indicated.

[2]Amylase-treated neutral detergent fiber (aNDF), analyzed by using heat-stable amylase and sodium sulfite.

[3]Crude fat (ether extract).

[4]Estimated (MJ/kg of DM).

[5]Milliequivalents/100 g of DM, DCAD = [(% Na/0.023) + (% K/0.039)] − [(% S/0.016) + (% Cl/0.0355)].

Ruminal Fluid pH, VFA, Ammonia, and Lactate

Pre and postfeed ruminal fluid pH, concentrations of total VFA, proportions of individual VFA, ammonia concentrations, and lactate concentrations are presented in Table 3. The average prefeed ruminal fluid pH was not different for cows being offered either of the forages (pH 6.68, $P = 0.508$). The difference occurred postfeed ($P < 0.001$), as the ruminal fluid pH of cows fed PRG hay declined to 6.39, whereas the pH of those fed alfalfa hay declined to 6.10. Ruminal fluid pH consistently declined between the prefeed and the postfeed sample ($P < 0.001$). An average of the change per hour for the 2 forage types is presented in Figure 3. The adaptation strategy had no effect ($P = 0.563$), thus the results have been averaged across both the

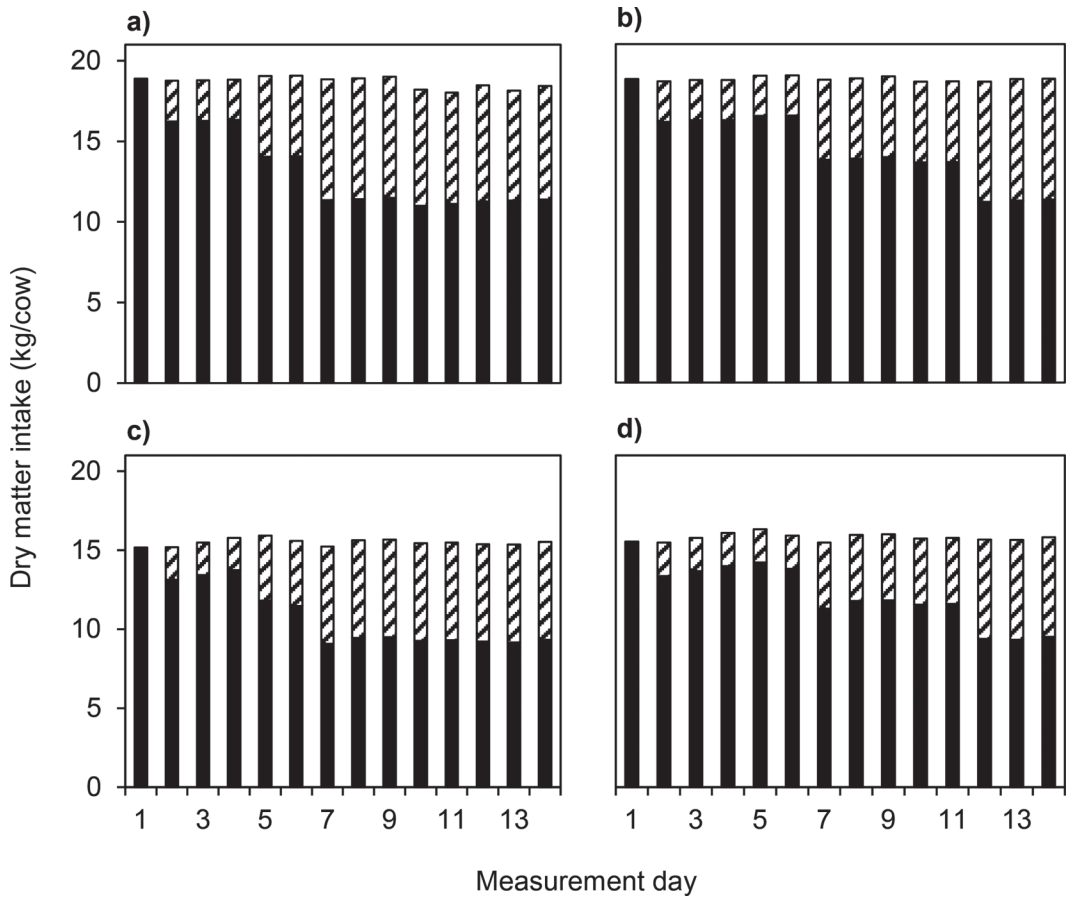

Figure 1. Mean DMI for each of the treatment groups. (a) ALF6 = cows fed alfalfa hay with wheat introduced over 6 d; (b) ALF11 = cows fed alfalfa hay with wheat introduced over 11 d; (c) PRG6 = cows fed perennial ryegrass hay with wheat introduced over 6 d; (d) PRG11 = cows fed perennial ryegrass hay with wheat introduced over 11 d. Intake of individual feed components is represented as solid black bars for forage and lined bars for wheat.

6- and 11-d strategies. The rate at which ruminal fluid pH declined following feeding was affected by the type of forage being consumed. On average, the ruminal fluid pH of cows consuming alfalfa hay declined by 0.14 pH units/h, whereas that of cows consuming PRG hay declined by 0.07 pH units/h. The difference between the forages was significant at all levels of wheat inclusion. We noted a greater rate of decline when wheat comprised 40% of the diet compared with all other wheat proportions. We found no differences in rates of pH decline between 0, 13, and 27% wheat. The average change in mean, minimum, and maximum ruminal fluid pH, relative to the previous day, for every additional kilogram of wheat consumed is presented in Table 4. All pH parameters declined when wheat was added to the diet, irrespective of treatment. However, we observed a difference between forages. Feeding alfalfa hay resulted in a greater decrease in mean (−0.05 vs. −0.02), minimum (−0.07 vs. −0.03), and maximum pH (−0.04 vs. −0.01) with every additional kilogram of DM of wheat. We noted a forage by adaptation strategy interaction for minimum pH. The decline in minimum pH with every additional kilogram DM of wheat was not different for the 2 alfalfa hay treatments, but PRG11 showed a greater decline than PRG6. The daily maximum, mean, and minimum ruminal fluid pH averages for each treatment are shown in Figure 4. Of note are the significantly lower values in mean and minimum pH for ALF6 cows compared with all other treatments on d 8, 9, and 10 ($P < 0.001$). On these days, ALF6 cows had daily minimum pH values below 6.00, including the lowest pH reached for any of the treatments (5.72 on d 9). None of the other treatments resulted in any ruminal fluid pH values below 6.00.

We observed a strong negative correlation between ruminal fluid pH and VFA concentrations ($r = -0.95$). Total VFA concentrations were higher in the ruminal fluid of cows consuming alfalfa hay than in those consuming PRG hay (Table 3). For all treatments, the total concentration of VFA increased after feeding but the increase was much greater for cows fed alfalfa hay. The lipogenic-to-glucogenic VFA ratio [(acetate + butyrate)/propionate] decreased as more wheat was included in the diet and also with feeding. Sampling time affected the proportion of propionate, with an increase occurring postfeed, a difference that was more pronounced in cows fed alfalfa hay. Sampling time also affected the proportion of butyrate for cows fed alfalfa hay, with a lower proportion in the postfeed sample. For both forage treatments, acetate proportion declined as the amount of wheat in the diet increased. For cows fed PRG hay, the acetate proportion also decreased after feeding, a difference that was not observed in alfalfa hay-fed cows. The proportion of valerate increased postfeed and the difference was much greater in PRG hay-fed cows. Ammonia concentrations were greater in cows fed alfalfa hay than those fed PRG hay. For both forages, the concentration declined as the proportion of wheat in the diet increased. The concentration of D-lactate increased postfeed in the ruminal fluid of cows consuming alfalfa hay. We observed no difference between the pre- and postfeed concentrations of D-lactate in the rumen fluid of cows consuming PRG hay, but we noted a trend in the same direction. L-Lactate concentrations increased postfeed irrespective of forage type. Both D- and L-lactate concentrations increased for all treatments with increasing proportions of wheat.

Milk Yield and Composition

Mean daily MY and ECM yield, as well as the proportions of protein, fat, and lactose (averaged over the measurement period) are presented in Table 5. Cows fed alfalfa hay had higher yields than cows fed PRG hay. We observed an interaction between forage type and adaptation strategy for both MY and ECM yield.

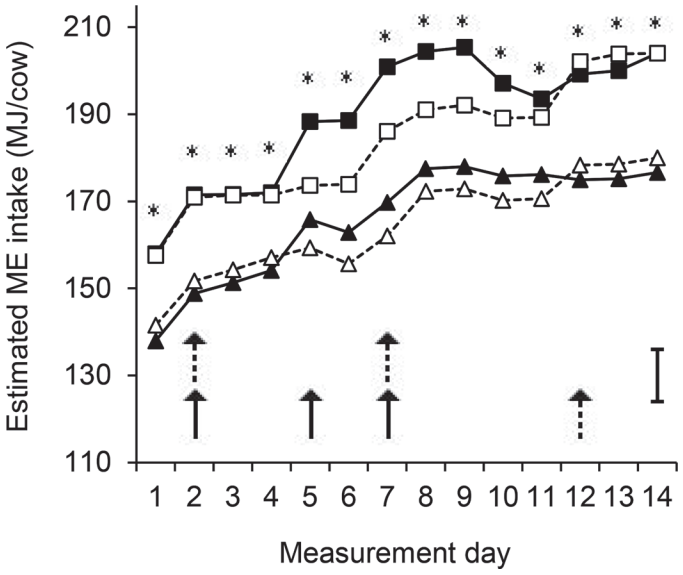

Figure 2. Daily estimated ME intakes of cows fed via each of the treatments. ALF6 (solid squares) = cows fed alfalfa hay and introduced wheat over 6 d; ALF11 (empty squares) = cows fed alfalfa hay with wheat introduced over 11 d; PRG6 (solid triangles) = cows fed perennial ryegrass hay with wheat introduced over 6 d; PRG11 (empty triangles) = cows fed perennial ryegrass hay and introduced wheat over 11 d. Values presented are means. The solid arrows indicate days an increase in wheat occurred for ALF6 and PRG6. Dotted arrows indicate days an increase in wheat occurred for ALF11 and PRG11. An asterisk indicates days when means differ ($P < 0.05$) and the error bar is the least significant difference for comparing treatments within each day.

Table 3. Effect of forage and proportion of wheat in the diet on ruminal fluid pH and fermentation characteristics

Forage	Wheat (% of DM)	Time[1]	Ruminal fluid pH	Total VFA (m*M*)	% of total VFA							
					Acetate (A)	Propionate (P)	Butyrate (B)	Valerate	(A +B)/P[2]	Ammonia-N (mg/L)	D-Lactate (m*M*)	L-Lactate (m*M*)
Alfalfa hay	0	Pre	6.73	92	71.0^{ef}	13.9	10.0	1.0	5.9	94^{g}	0.02	0.02
		Post	6.19	139	71.2^{ef}	17.1	8.7	1.0	4.7	81^{f}	0.03	0.04
	13	Pre	6.88	87	70.7^{e}	13.4	10.6	0.9	6.1	89^{g}	0.03	0.10
		Post	6.26	137	70.6^{e}	17.1	8.8	1.2	4.6	80^{f}	0.04	0.08
	27	Pre	6.56	101	68.5^{cd}	15.5	11.4	1.1	5.2	45^{bcde}	0.02	0.03
		Post	6.06	147	68.4^{cd}	19.0	8.9	1.3	4.1	58de	0.06	0.04
	40	Pre	6.56	107	67.0^{ab}	16.0	12.4	1.1	5.0	49^{bcde}	0.04	0.02
		Post	5.89	157	66.6^{a}	18.0	10.1	1.5	4.1	56^{e}	0.05	0.05
Perennial ryegrass hay	0	Pre	6.62	94	71.8^{f}	16.0	9.0	0.8	5.1	44^{bcd}	0.02	0.02
		Post	6.37	115	70.6^{e}	17.4	8.6	1.6	4.5	54^{cde}	0.03	0.06
	13	Pre	6.75	87	71.9^{f}	15.2	9.6	0.7	5.4	42^{b}	0.03	0.09
		Post	6.50	98	69.0^{d}	17.3	9.6	1.8	4.6	43^{bc}	0.04	0.12
	27	Pre	6.67	87	71.2^{ef}	16.1	9.4	0.8	5.1	19^{a}	0.03	0.03
		Post	6.38	108	67.8^{bc}	18.7	9.4	1.8	4.2	25^{a}	0.05	0.03
	40	Pre	6.66	93	69.5^{d}	16.2	10.3	0.9	5.0	29^{a}	0.04	0.03
		Post	6.29	111	67.1^{ab}	18.0	9.8	2.0	4.3	30^{a}	0.04	0.04
SED[3]			0.058	5.1	0.51	0.79	0.68	0.05	0.20	5.9	0.008	0.021
P-value[4]	Forage		0.001	<0.001	0.061	0.074	0.207	<0.001	0.085	<0.001	0.358	0.518
	Wheat		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Time		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.487	<0.001	0.016
	Forage.Wheat		<0.001	<0.001	0.004	0.309	0.533	0.212	0.027	0.001	0.557	0.877
	Forage.Time		<0.001	<0.001	<0.001	0.04	<0.001	<0.001	<0.001	0.131	0.023	0.545
	Wheat.Time		0.020	0.808	0.003	0.289	0.122	<0.001	0.010	0.149	0.178	0.514
	Forage.Wheat.Time		0.540	0.368	0.039	0.576	0.167	0.785	0.236	0.010	0.415	0.160

[a–f]Means within a column with different superscripts differ ($P < 0.05$).

[1]Pre = samples were taken immediately before the morning feed; post = samples were taken 4 h after feed was first offered to the cows in the morning.

[2]The lipogenic-to-glucogenic VFA ratio [(acetate + butyrate)/propionate].

[3]Standard error of difference between means.

[4]The adaptation strategy was not significant, nor were any interactions with the adaptation strategy and are therefore not presented.

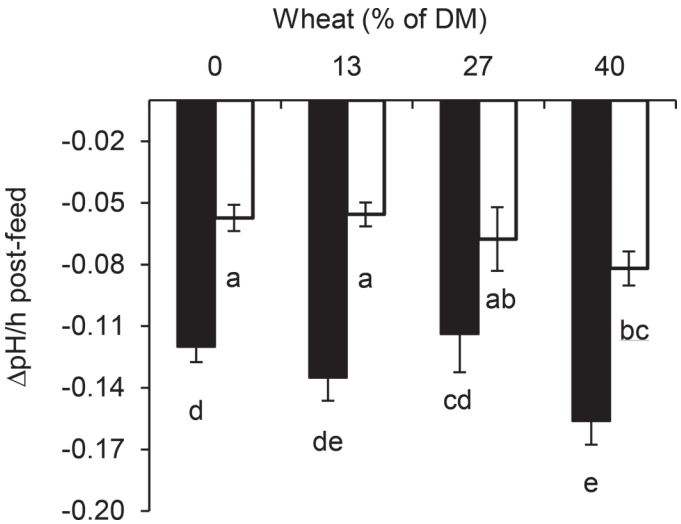

Figure 3. Change in ruminal fluid pH/h during the 4 h after the morning feed commenced, when cows from each treatment group were fed on each of the 4 wheat proportions. Data were averaged across the 2 adaptation strategies. The 2 types of forages are represented as black bars for alfalfa hay and white bars for perennial ryegrass hay. Values presented are means. Error bars indicate SEM. Means with different letters (a–e) differ ($P < 0.05$).

When alfalfa hay was fed the 6-d adaptation strategy resulted in greater yields, but we found no differences between the adaptation strategies when PRG hay was fed. The cows fed PRG hay had a greater concentration of protein in their milk. We noted an interaction between the effects of adaptation strategy and forage type resulting in a greater concentration of lactose in the milk from PRG6 cows compared with PRG11 cows, but we observed no difference between the 2 alfalfa hay treatments. The ECM yields for both of the alfalfa hay treatments increased ($P < 0.001$) throughout the measurement period (daily data not shown). Despite the overall difference in mean ECM yield, both alfalfa hay treatments had equal yields on d 1 (17.6 kg/cow per day) and equal yields again by d 14 (18.7 kg/cow per day). On d 5 through 9, cows in the ALF6 treatment were producing greater quantities of ECM than cows in the ALF11 treatment group, with the difference ranging from 1.6 to 2.9 kg/cow per day.

Eating Behavior

Eating behavior data are presented in Table 6. Forage type had a small effect on the amount of time per day cows spent eating. Cows consuming alfalfa hay spent more time eating than those fed PRG hay (208 and 189 min/cow per day, respectively). The amount of time spent ruminating and the amount of time spent not chewing were not different between the forage types. All behaviors were affected by the proportion of wheat in the diet. Cows spent less time eating and ruminating when wheat made up 40% of DM compared with an all-forage diet, which translated to more time spent not chewing.

Forage Buffering Capacity

The results of the acid titrations for both of the forages are presented in Table 7. The titratable acidity of the 2 forages differed. Due to a higher initial pH, almost twice as much acid was required to reduce the pH of PRG hay from initial pH to 5.50 compared with alfalfa hay. The buffering capacity, which describes the amount of acid required to produce a unit change in pH regardless of the initial pH, was twice as much for alfalfa hay.

DISCUSSION

We found marked differences in ruminal pH parameters between the 2 forage types. Both forages provided good buffering within the rumen, but, contrary to our

Table 4. Effect of additional wheat on ruminal fluid mean, minimum, and maximum pH

	Treatment[1]					*P*-value		
Item	ALF6	ALF11	PRG6	PRG11	SED[3]	Forage	Adaptation strategy	Interaction
Δ mean pH/kg of wheat[2]	−0.05	−0.04	−0.01	−0.03	0.013	0.004	0.928	0.197
Δ minimum pH/kg of wheat[2]	−0.08[a]	−0.06[a]	−0.01[b]	−0.06[a]	0.022	0.030	0.527	0.013
Δ maximum pH/kg of wheat[2]	−0.04	−0.04	−0.01	−0.01	0.018	0.020	0.855	0.950

[a,b]Means within a row with different superscripts differ ($P < 0.05$).

[1]Treatments: ALF6 = cows fed alfalfa hay with wheat introduced over 6 d; ALF11 = cows fed alfalfa hay with wheat introduced over 11 d; PRG6 = cows fed perennial ryegrass hay with wheat introduced over 6 d; PRG11 = cows fed perennial ryegrass hay with wheat introduced over 11 d.

[2]The average change in mean, minimum and maximum pH (relative to the previous day) per additional kg of DM of wheat consumed on days of incremental increases.

[3]Standard error of difference between treatments.

first hypothesis, cows consuming alfalfa hay had a greater decline in ruminal pH after eating and a lower daily mean pH. A main driver of this was likely the higher DMI of cows consuming alfalfa hay compared with cows offered PRG hay. The voluntary intake of grasses is typically less than that of legumes due to greater NDF fractions contributing to a slower rate of passage and increased ruminal fill (Thornton and Minson, 1973; Dado and Allen, 1995). The higher intake of cows fed alfalfa hay would have meant more microbial fermentation of feed within the rumen, resulting in a greater production of VFA, and lower ruminal pH (Krause and Oetzel, 2006). In support of this, total VFA concentrations were consistently higher in cows fed alfalfa hay, with a greater quantity produced after feeding.

The higher DMI would also explain the more rapid decline in pH after eating, as rate of ruminal pH decline increases with meal size (Allen, 1997). Additionally, as saliva is the main contributor to buffering within the rumen (Bailey and Balch, 1961), the eating behavior of the treatment groups needs to be considered. We observed no differences in time spent ruminating between cows on the 2 forages, but cows fed alfalfa hay spent an extra 19 min eating per day. As saliva flow is greater during eating compared with resting (Cassida and Stokes, 1986), this would lead to the assumption that cows fed alfalfa hay had a greater influx of saliva into the rumen each day. However, as their ruminal pH was lower overall and declined further after eating, it would appear that the buffering benefits from the additional saliva were not enough to overcome the greater reduction in pH due to feed fermentation. Cows offered alfalfa hay had a maximum wheat intake of 7.4 kg of DM/cow per day, whereas cows fed PRG hay had a maximum wheat intake of 6.3 kg of DM/cow per day. The differences in wheat intake could have influenced ruminal pH, and so pH was expressed as change in pH per kilogram of extra wheat consumed to account for the variability. The mean, maximum, and minimum ruminal pH (relative to the day before) declined with every extra kilogram of wheat consumed, irrespective of forage type. However, the drop in each of the parameters was at least twice as much for the alfalfa hay treatments. Despite the fact that grasses are typically of higher digestibility, legumes have a larger rapidly digested fraction (Thornton and Minson, 1973; Schofield and Pell, 1995) breaking down quicker within the rumen (Van Soest, 1994), perhaps explaining the greater decline in pH seen immediately after eating within the current experiment.

The greater concentration of lactic acid postfeed for the alfalfa treatments would have also played a role in reducing ruminal pH, as lactic acid is particularly

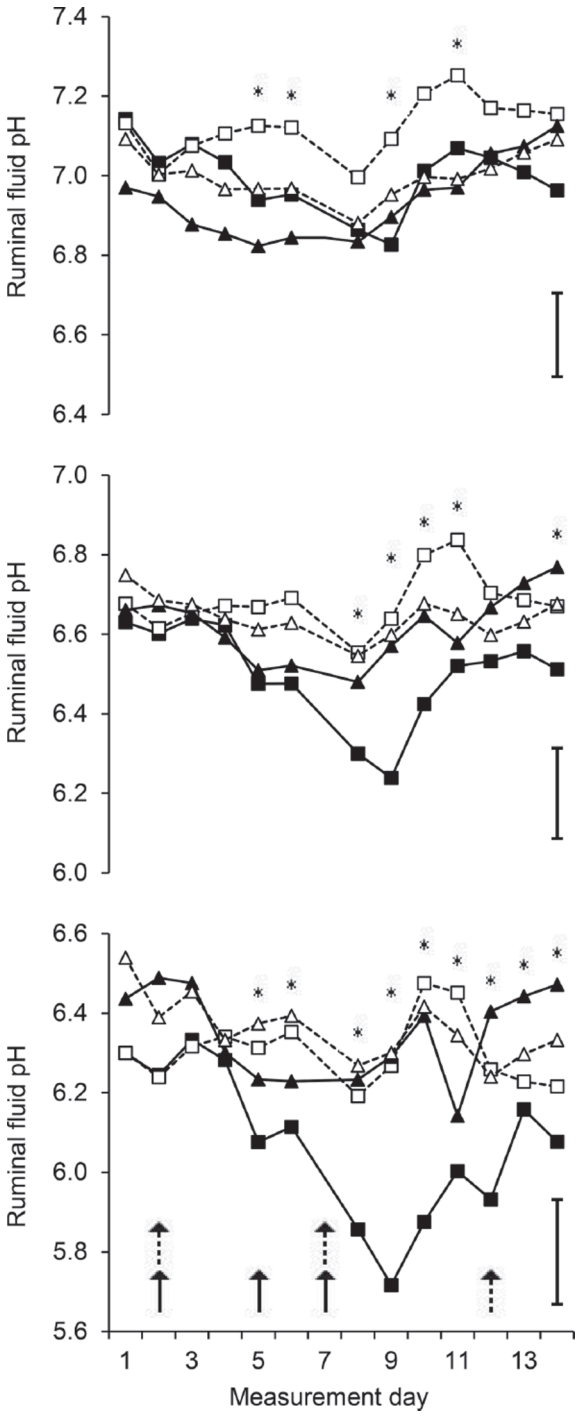

Figure 4. The daily (a) maximum, (b) mean, and (c) minimum ruminal fluid pH of cows fed via each of the different treatments. ALF6 (solid squares) = cows fed alfalfa hay with wheat introduced over 6 d; ALF11 (empty squares) = cows fed alfalfa hay with wheat introduced over 11 d; PRG6 (solid triangles) = cows fed perennial ryegrass hay with wheat introduced over 6 d; PRG11 (empty triangles) = cows fed perennial ryegrass hay with wheat introduced over 11 d. Values presented are means. The solid arrows indicate days an increase in wheat occurred for ALF6 and PRG6. Dotted arrows indicate days an increase in wheat occurred for ALF11 and PRG11. An asterisk indicates days when means differ ($P < 0.05$) and the error bar is the least significant difference for comparing treatments within each day.

Table 5. Mean daily milk yield (MY), ECM yield, and composition of milk from cows fed according to each of the treatments

	Treatment[1]					*P*-value		
Item	ALF6	ALF11	PRG6	PRG11	SED[2]	Forage	Adaptation strategy	Interaction
MY (kg/cow per day)	17.8[a]	16.5[b]	13.4[c]	13.6[c]	0.30	<0.001	0.020	0.002
ECM (kg/cow per day)	19.5[a]	18.0[b]	14.8[c]	15.0[c]	0.37	<0.001	0.019	0.002
Protein (%)	3.66	3.65	3.74	3.67	0.03	0.020	0.051	0.123
Fat (%)	4.66	4.63	4.73	4.67	0.09	0.406	0.510	0.853
Lactose (%)	4.76[a]	4.74[a]	4.83[a]	4.69[b]	0.03	0.696	0.005	0.023

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]Treatments: ALF6 = cows fed alfalfa hay with wheat introduced over 6 d; ALF11 = cows fed alfalfa hay with wheat introduced over 11 d; PRG6 = cows fed perennial ryegrass hay with wheat introduced over 6 d; PRG11 = cows fed perennial ryegrass hay with wheat introduced over 11 d.

[2]Standard error of difference between treatments.

influential in depressing ruminal pH (Dijkstra et al., 2012). Lactic acid accumulation is typical during dietary adaptation, particularly with the introduction of readily fermentable carbohydrates (France, 1975; Counotte and Prins, 1981), and so the increase in ruminal concentrations of D- and L-lactate with the introduction of wheat is not surprising. Despite the increase of lactic acid, it did not accumulate to a level symptomatic of lactic acidosis, reported to be above 40 m*M* in severe cases (Owens et al., 1998).

The acid buffering capacity of alfalfa hay was almost double that of PRG hay, yet this did not result in a greater pH stability within the rumen. Previous work has described the high intrinsic buffering capacity of alfalfa hay compared with the majority of other ruminant feeds (Crawford et al., 1983). Despite the varied intrinsic buffering capacity of feeds, it has been reported to have little influence within the rumen, particularly compared with saliva and VFA (Counotte et al., 1979; Wohlt et al., 1987; Allen, 1997); however, few have tested this in vivo. The results of the titrations within the current experiment appear to further support this. It would appear the initial pH and the titratable acidity, rather than the buffering capacity, is a better predictor of effects within ruminal fluid. The higher initial pH of PRG hay meant more acid was required to reduce the pH to 5.50, resulting in a greater titratable acidity at pH levels relative to normal rumen function, indicating PRG hay as better forage for rapid grain introduction.

The DCAD of the diets within the current experiment ranged from 25 to 62 mEq/100 g of DM and were mostly within an optimum range for lactating dairy cows, 25 to 50 mEq/100 g of DM (Sanchez et al., 1994). In vitro acid buffering capacity correlates strongly with the total cation fraction of feeds (Jasaitis et al., 1987). Diets with a greater DCAD have been associated with higher ruminal fluid pH and increased DMI (Tucker et al., 1988; Wildman et al., 2007). The alfalfa hay diets had a higher DCAD, yet this did not translate to a greater ability to buffer the ruminal contents. Another

Table 6. Eating behavior data as observed for 24 h on d 1 and d 14 when wheat made up 0 and 40% of the diet, respectively

Treatment[1]	Wheat (% of DM)	Eating (min/cow per day)	Ruminating (min/cow per day)	Not chewing (min/cow per day)
ALF6	0	274	460	706
	40	136	385	919
ALF11	0	294	472	674
	40	126	325	989
PRG6	0	283	506	651
	40	104	383	953
PRG11	0	266	461	713
	40	103	356	981
SED[2]		18.2	28.4	36.3
P-value[3]	Forage	0.039	0.325	0.896
	Adaptation strategy	0.809	0.069	0.250
	Wheat	<0.001	<0.001	<0.001

[1]Treatments: ALF6 = cows fed alfalfa hay with wheat introduced over 6 d; ALF11 = cows fed alfalfa hay with wheat introduced over 11 d; PRG6 = cows fed perennial ryegrass hay with wheat introduced over 6 d; PRG11 = cows fed perennial ryegrass hay with wheat introduced over 11 d.

[2]Standard error of difference between treatments.

[3]No interactions were significant so are not presented.

Table 7. Results of the acid titrations conducted on alfalfa hay and perennial ryegrass (PRG) hay from initial pH to pH 5.50

Item	Alfalfa hay	PRG hay	SED[1]	*P*-value
Initial pH	5.77	6.50	0.07	<0.001
Titratable acidity[2] (mEq/100 g of DM)	5.9	11.0	0.93	0.005
Acid buffering capacity[3]	22.0	11.0	0.12	<0.001

[1]Standard error of difference between treatments.

[2]Milliequivalents of HCl required to lower the pH of 100 g of DM of forage to the specified target pH.

[3]Titratable acidity (mEq) divided by pH change (initial pH minus pH 5.50).

driver of buffering capacity might be CP concentration, as ammonia is the main alkali within the rumen (Crawford et al., 1983) and ruminal fluid ammonia concentrations are typically a reflection of the CP concentration of a diet (Elizalde et al., 1996). The higher CP concentration of alfalfa hay explains the higher ammonia concentrations in the ruminal fluid of cows consuming it within the current experiment. However, contrary to what we expected, the greater CP concentration and subsequent rumen ammonia concentrations from alfalfa hay did not result in increased ruminal pH relative to PRG hay. This suggests the increase in VFA production, driven by the higher DMI, outweighed the possible pH benefits of the increased ammonia concentrations.

Our second hypothesis, that the mean ruminal fluid pH of cows would not differ between the 2 wheat adaptation strategies, irrespective of forage type, was not supported. Large differences were observed between the daily mean and minimum ruminal pH values of the 2 alfalfa hay treatments (see Figure 4). The differences in the daily pH values appeared to be driven by the rapid increase of wheat over 6 d versus the more gradual increase over 11 d. As the 6-d adaptation strategy was rapidly increasing the ME content of the diet, it did not allow for the ruminal pH of cows to recover, resulting in a declining ruminal pH. Despite the mean daily pH falling to levels considered low (<6.0; Pitt et al., 1996), cows on the ALF6 treatment produced 1.5 kg/cow per day more ECM over 14 d than cows on the ALF11 treatment. This difference was driven by higher yields on d 5 through 9, when ALF6 cows were consuming ~14 MJ/cow per day more than ALF11 cows. This also corresponds with some of the days ALF6 cows were generating their lowest ruminal pH values.

No difference in ECM yields was observed between the adaptation strategies, when cows were fed PRG hay. This is likely due to the higher estimated ME concentration and lower intake of the PRG hay, which resulted in similar estimated ME intakes from the 2 PRG hay treatment groups. Substituting wheat for alfalfa hay caused larger variation to estimated ME intake compared with the same proportional substitution for PRG hay (see Figure 2). Despite the lower estimated ME proportion of the forage, cows consuming alfalfa hay had a greater estimated ME intake overall, as they were eating 3.1 kg of DM/cow per day more. This difference in DMI, and subsequently estimated ME intake, resulted in a greater MY from the alfalfa hay-fed cows. The combination of the 6-d adaptation strategy paired with a reduced buffering ability and the lower estimated ME concentration of the alfalfa hay resulted in only the cows in the ALF6 treatment exhibiting daily mean ruminal pH values below 6.00. Despite the pH reaching levels considered to compromise digestion, no other measured parameter suggested poor adaptation or acidosis. In fact, it was these cows, with the lowest ruminal pH values that had the highest MY. This relationship is consistent with that observed by Kolver and De Veth (2002), who reported that the performance of pasture-fed dairy cows was not adversely affected by a mean ruminal pH of 5.80 to 6.20.

CONCLUSIONS

Milk yield and rumen responses to the wheat adaptation strategies varied depending on the base forage. Both forages demonstrated good buffering within the rumen and sufficiently stimulated rumination and associated saliva secretion, allowing cows to cope with the rapid starch load of the 6-d adaptation strategy. However, the greater intake of the alfalfa hay and its lower estimated ME concentration meant cows on the ALF6 treatment benefited substantially from the rapid input of wheat and increase in dietary ME. This resulted in cows on the 6-d strategy producing more milk than those on the 11-d adaptation strategy, whose increase in dietary estimated ME was more gradual. We found no differences between the 6- and 11-d adaptation strategies when PRG hay was fed. These results indicate that some subtle changes to grain introduction methods can lead to increased MY, depending on intake and forage choice. Feeding alfalfa hay produced lower ruminal fluid pH, possibly driven by a greater intake and increased fermentation within the rumen. Despite

the rapid introduction of large amounts of wheat and some differences seen between the forages, none of the treatment groups indicated compromised production, with neither adaptation strategy posing any significant threats to biological function. Both forages buffered the rumen against potentially detrimental pH changes often seen with the introduction of large amounts of rapidly fermentable starch, highlighting the important role forages play when adapting dairy cows to large amounts of concentrates.

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Chapter 6

Forage type influences ruminal responses to a wheat grain challenge in early lactation dairy cows

V. M. Russo[A,B,C,D], B. J. Leury[B], E. Kennedy[C], M. C. Hannah[A], M. J. Auldist[A], G. Morris[A] and W. J. Wales[A]

[A]Agriculture Victoria Research, Ellinbank, Vic. 3821, Australia.

[B]Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Vic. 3010, Australia.

[C]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland.

[D]Corresponding author. Email: victoria.russo@ecodev.vic.gov.au

Abstract

The role that forage plays in ruminal, behavioural and production responses to a wheat grain challenge was investigated in 16 early lactation, ruminally fistulated dairy cows. Cows were fed a forage only diet of either lucerne hay, perennial ryegrass (PRG) hay or one of two cultivars of fresh PRG pasture (Bealey or Base), for three weeks prior to the grain challenge. The forage diet was then supplemented with crushed wheat grain at a rate of 8 kg dry matter/cow per day, with no adaptation period. Wheat comprised between 32 and 43% of total dry matter intake and was fed over two meals, followed by forage, for one day only. During the wheat challenge and for two days prior, ruminal fluid pH was continually measured using intraruminal sensors. Ruminal fluid samples, analysed for volatile fatty acids, ammonia and DL-lactate, were taken prior to each meal and 6 h after. In general, on both the forage and forage-wheat diet, cows fed fresh pasture had a lower ruminal fluid pH than cows fed hay, and pH remained below 6.0 for longer each day. Following supplementation of wheat, cows fed pasture exhibited ruminal fluid pH levels associated with sub-acute ruminal acidosis. Hay created a ruminal environment that was better able to cope with the influx of acid produced as wheat was digested. A combination of increased ruminating time and a decreased rate of fermentation are likely responsible for the higher ruminal fluid pH values. The ruminal environment of cows fed lucerne hay remained most stable throughout the grain challenge, with ruminal fluid spending the least amount of time below pH 6.0. In practice, rapid grain introduction means a rapid increase in the energy concentration of the diet as well as improving convenience and efficiency. However, forage plays a critical role and must be considered when determining wheat introduction strategies. Traditional, gradual adaptation strategies must still be employed with highly digestible fresh forages, while more aggressive adaptation strategies can be implemented when hays are used as the base forage.

Introduction

Although most dairy farms in Victoria, Australia rely on pasture as their main feed source, it alone cannot fully meet the nutritional requirements of a high producing dairy cow. Both dry matter intake (DMI) and energy limit milk production on a pasture only diet (Kolver and Muller 1998). Because of this, even in spring, dairy farmers typically incorporate additional nutrients into cows' diet, commonly as cereal grains fed during milking and conserved fodder offered in the paddock. According to a recent survey, supplements are fed at an average rate of 1.6 t/cow per year in Australia (Dairy Australia 2018). Wheat and barley grain are the most commonly used concentrates and are typically fed twice daily. The amount of concentrates fed at different stages of lactation can vary depending on the nutrients supplied from pasture and the energy requirements of the cows. This is known as stepped flat-rate feeding (Leaver 1988). The sudden introduction or increase in the amount of starch offered can cause dramatic changes to the ruminal environment, including a rapid production of acids as a result of fermentation, to which rumen microbes require time to adapt. If large quantities of concentrates are introduced abruptly to unadapted cows, the ruminal environment may not be able to cope with the increased acid load, leading to metabolic issues such as acute or sub-acute ruminal acidosis (SARA) (Krause and Oetzel 2006). Therefore, adaptation processes are typically implemented over several weeks with the amount of grain being offered gradually increasing.

Rumen microbes have adapted to efficiently digest forages, however the responses within the rumen to different forages are not equal. A ruminal fluid pH below 6.0 for extended periods of time can severely inhibit fibre digestion (Mould and Ørskov 1983), hence a lower threshold of pH 6.0 is typically used to identify optimal rumen function. Williams *et al.* (2001) reported a ruminal fluid pH consistently below 6.0 when dairy cows were consuming 19 kg DM/cow per day of highly digestible Persian clover (*Trifolium resupinatum*). The same study reported a ruminal fluid pH below 6.0 for at least 15 h/day when cows were grazing perennial ryegrass (PRG) (*Lolium perenne*). In contrast, Leddin *et al.* (2009) reported a ruminal fluid pH that remained consistently above 6.0 when lactating dairy cows were consuming a diet of solely pasture hay. Ruminal responses to increasing amounts of crushed wheat grain also varies depending on forage type (Leddin *et al.* 2009; Leddin *et al.* 2010).

Eating behaviour and intake rate varies with forage type and both impact ruminal fluid pH, mainly through saliva production (Allen 1997; Williams *et al.* 2000). Introducing or increasing concentrate supplements in a forage-based diet also alters eating behaviour, with both the amount of time spent eating and ruminating decreasing as the proportion of wheat in the diet increases (Russo *et al.* 2018).

The process of gradually adapting cows to large amounts of concentrates can come at a cost of convenience and efficiency. It is therefore desirable to accelerate the process while still optimising rumen function and milk production. This experiment investigated the effects of different forages during an abrupt grain introduction, with an aim of providing some insight into the possibility of using forages

for improving grain adaptation processes in the dairy industry. The hypotheses tested were that 1) the daily amount of time ruminal fluid pH is below 6.0 will be greatest for fresh forages, then lucerne (Medicago sativa) hay, then PRG hay; 2) there will be no difference in duration below pH 6.0 for the two fresh forages; 3) the minimum ruminal fluid pH will be lowest for cows fed fresh forages, then lucerne hay, then PRG hay; and 4) the minimum ruminal fluid pH will not differ between the two fresh forage treatments.

Materials and methods

Experimental design and dietary treatments

The experiment was conducted at the Agriculture Victoria Research Centre, Ellinbank, Victoria, Australia (38°14'S, 145°56'E) in September 2017. All procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council 2004). Approval to proceed was obtained from the DEDJTR Agricultural Research and Extension Animal Ethics Committee and was contingent on having thresholds for minimum ruminal fluid pH for removal of animals.

Sixteen rumen-fistulated Holstein-Friesian dairy cows in their 3rd to 9th lactation were used. While all cows were seasonally-calving, a combination of both fresh and carryover cows were used, either having calved between July and October 2016 or 2017 (230 ± 163.1 DIM; mean ± SD). Milking occurred twice daily at ~0600 and 1500 h. Leading up to the experiment, concentrates being fed to the cows were gradually reduced, and for one week prior to the experiment they were fed a forage only diet. The experiment then ran for 28 days divided into a 3-day covariate period, a 17-day adaptation period and a 4-day measurement period. During the covariate period, all cows grazed PRG as a single cohort and received no concentrates. Following the covariate period 4 treatments were randomly allocated to cows, such that the treatment groups were balanced for mean ruminal fluid pH, MY, body weight, DIM and age as reported in the covariate period.

Each treatment group received one of the following forages: lucerne hay, PRG hay, fresh PRG cultivar Bealey or fresh PRG cultivar Base. During the adaptation period, all cows were moved to individual pens indoors for feeding and offered their allocated forage *ad libitum*. Cows were not given any concentrates during the adaptation period. In between feeding bouts cows were returned to an empty paddock, with free access to water. During the measurement period, forage was offered at a rate of 17 kg DM/cow per day. For the first two days of the measurement period all cows were on a forage only diet. On the third and fourth day crushed wheat grain was offered at a rate of 8 kg DM/cow per day and forage continued to be offered at a rate of 17 kg DM/cow per day. Following each milking, cows were moved to individual stalls and given half their ration in the morning and half in the afternoon. Wheat was offered first and after 20 min (or sooner if all cows had consumed their grain) any grain refusals

were removed, and forage was offered. All cows were given 4.5 hours to consume their forage and had free access to water during this time.

The experiment was designed with four measurement days. However, due to several cows reaching minimum ruminal fluid pH thresholds outlined in the animal ethics documentation, the experiment was concluded 6 h after the morning feed on day 4. No data collected on the fourth day is included in the analyses.

Intake and nutritive characteristics

All feed offered and refused was weighed and a representative sample was collected at each feeding. Part of each sample was then dried at 100ºC for 24 h to determine DM concentration, which facilitated the calculation of individual DMI. The remainder of the samples were then bulked by feed type or, in the case of refusals, by individual cow and stored at 4ºC. At the completion of the experiment bulked samples were thoroughly mixed and representative sub-samples were freeze-dried and ground to pass through a 0.5 mm sieve. The samples were then analysed for CP, NDF, ADF, lignin, NFC, starch, crude fat (CF), ash, total digestible nutrient (TDN) and minerals by wet chemistry in a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY, USA). Concentrations of estimated metabolisable energy (ME) were calculated using the following formula (National Research Council 2001):
ME (MJ/kg DM) = (((1.01 × (0.04409 × TDN%)) – 0.45) × 4.184. The nutritive characteristics of feed offered are presented in Table 6.1.

Table 6.1. Nutritive characteristics of feed offered during the experimental period[1]

	CP	ADF	aNDF	Lignin	NFC	Starch	CF[2]	Ash	TDN	ME[3]
Alfalfa hay	14	47	55	10	21	0.9	2.6	8.1	54	8.9
PRG hay	10	40	60	8	23	1.4	1.9	5.5	57	8.9
Bealey	28	40	46	10	10	1.3	5.8	10.4	59	10.2
Base	29	42	48	12	8	0.9	6.1	9.6	58	10
Wheat	14	5	11	2	71	58.7	2.2	1.8	84	14.4

[1]All values are % of DM unless otherwise indicated.
[2]Crude fat (ether extract).
[3]Estimated ME (MJ/kg DM).

Eating behaviour

Three days prior to the measurement period cows were fitted with halters containing pressure and movement sensors (RumiWatch, Itin + Hoch GmbH, Liestal, Switzerland), to quantify eating behaviour.

Milk yield and composition

Milk yield was recorded at each milking using a DeLaval Alpro milk metering system (DeLaval International; Tumba, Sweden) and a sub-sample was collected for each cow using in-line milk meters

(DeLaval International). Samples were analysed for protein, lactose and fat concentrations using an infrared milk analyser (Model 2000, Bentley Instruments, Chaska, MN, USA). Energy corrected milk (ECM) yield was calculated using the following formula (Tyrrell and Reid 1965):
ECM (kg/cow per day) = MY (kg/cow per day) × [376 × fat (%) + 209 x protein (%) + 948] / 3,138

Ruminal fluid pH and composition

At the commencement of the measurement period, capsules for measuring ruminal fluid pH (KB5; Kahne limited, Auckland, New Zealand) were calibrated and inserted per fistula into the rumen of each cow. The capsules remained in the cows until the end of the measurement period. A 750 g weight was attached to each capsule to ensure it remained on the bottom of the rumen. Ruminal fluid pH was logged every five minutes and the data were automatically stored in the devices. Capsules were removed once a week for 8 h to validate the pH readings, and a linear interpolation was used to correct for any drift in readings from individual boluses. Following the validation, all data were downloaded, and boluses were re-calibrated before re-insertion.

Beginning on day 3 of the measurement period, seven ruminal fluid samples were collected per cow per feed with the first sample collected immediately prior to feeding and a sample collected every hour thereafter. Samples were collected per fistula using a 100 mL plastic syringe connected to a copper pipe directly inserted into the rumen. Fluid was collected from four different sites within the rumen and mixed thoroughly. A 50 mL sub-sample was immediately poured off and centrifuged (4°C, 4,000*g*, 10 min) while the pH of the remainder was measured using a benchtop pH meter (Orion star A211; Thermo Fisher Scientific, Schwerzenbach, Switzerland). A 0.5 mL aliquot of supernatant was then transferred to a tube containing 4.5 mL of dilute acid (0.1 M HCl) for later analysis of ammonia concentration. An additional 5 mL aliquot was dispensed into a tube for analysis of VFA and lactate concentrations. Both sub-samples were stored at -20°C until analyses. Volatile fatty acid concentrations were determined by capillary gas chromatography (Agilent 6890 GC; Agilent Technologies, Santa Clara, CA) using a flame ionisation detector, auto-sampler and auto-injector, and a wide bore capillary column (BP21 column, 12 m x 0.53 mm internal diameter (ID) and 0.5 µM film thickness; SGE International, Ringwood, Victoria, Australia) with retention gap kit (including a 2 m x 0.53 mm ID guard column). Analyses were conducted following the methodology described by Packer *et al.* (2011) with 4-methyl-valeric acid (184 ppm) used as the internal standard. Lactate analyses were conducted with a microplate reader (AMR-100, Allsheng Instruments, China) using a D/L lactate kit (K-DLATE; Megazyme, Bray, Ireland). Ammonia concentrations were determined by flow-injection (Lachat Quik-Chem 8000; Lachat Instruments, Milwaukee, WI) according to an alkaline phenol-based method (method 12-107-06-1-A; Lachat Instruments, Milwaukee, WI) and analysed against standard ammonia solutions.

Statistical analyses

All data were analysed using Genstat for Windows (Genstat 18th edition, VSN International Ltd.). For all data sets, days were grouped according to diet, with day 1 and 2 categorised as forage only and day 3 categorised as forage and wheat. As day 4 only consisted of an AM period it was not included in the overall analyses. Comparisons between forage groups, fresh PRG (Bealey and Base) and hay (PRG and lucerne), as well as between forages within these groups, for all variables, were achieved by specifying contrasts on the factor for forage within the treatment structure employed in the ANCOVA. Daily yields (milk, ECM, and composition yields) were calculated as the sum of PM and AM values. Daily milk composition (%) was calculated as the ratio of daily composition yield to milk yield. Milk production and intake data were subject to an analysis of variance (ANOVA) adjusted for data collected during the covariate period. The factorial treatment structure was forage by wheat, with a blocking structure of cow split for period (forage, wheat and forage) split for day.

Ruminal fluid pH data collected via the intra ruminal boluses were summarised daily for each cow as daily mean, minimum, maximum, time under pH 6, area under pH 6 and rate of decline post-feeding. A day was considered 0700 to 0700 h. To calculate the rate of pH decline following each feeding, each daily set of pH data was also categorised into two 'peak' pH intervals and two 'trough' pH intervals. These intervals were derived visually from an average ruminal fluid pH (averaged over all cows, at each time) vs time graph. The daily intervals were peak: 0300 to 0900 h and 1400 to 1800 h, and trough: 0900 to 1400 h and 1800 to 0300 h. The maximum pH within each peak-interval and the minimum pH within each trough-interval was then identified and the slope as change in pH divided by change in time was calculated. The data were then summarised as an average daily rate of decline in pH for each cow, the amount of pH decline and the duration of the decline. All summary data for ruminal fluid pH variables were subjected to an ANCOVA with a blocking structure of cow by period (forage, wheat and forage) split for day, with covariate as the corresponding variable measured in the covariate period. The factorial treatment structure was period by forage. Ruminal fluid composition data consisted of pre- and 6 h post-feed measurements for AM and PM on each of days 2 and 3. These were subjected to analysis of variance with the factorial treatment structure of forage by period by sample (pre or post-feeding) plus time of day (AM or PM), and blocking structure of cow by period (i.e. day) split for time of day split for sample. Lactate data were log transformed prior to analysis. Eating behaviour data were analysed with an ANOVA using the treatment structure forage by wheat and the blocking structure cow by period split for day.

Table 6.2. Feed intake, eating behaviour, milk yield (MY)[1], energy corrected milk (ECM) yield and milk composition[1] from cows receiving each treatment both before and after wheat inclusion[2]

		Feed intake (kg DM/cow per day)			Eating behaviour (min/cow per day)			MY (kg/cow per day)	ECM (kg/cow per day)	Milk composition (%)		
Forage	**Diet**	**Forage**	**Wheat**	**Total**	**Eating**	**Ruminating**	**Not chewing**			**Fat**	**Protein**	**Lactose**
Alfalfa	Forage only	16.5	0.0	16.5	393	484	548	15.6	16.9	5.1	3.2	4.6
	Forage and wheat	13.7	8.0	21.3	451	478	498	15.8	15.9	4.5	3.3	4.6
PRG	Forage only	11.1	0.0	11.1	359	584	488	7.7	8.9	5.2	3.6	4.2
	Forage and wheat	9.7	8.0	17.3	284	454	690	8.1	8.7	4.8	3.5	4.3
Bealey	Forage only	13.9	0.0	13.9	355	295	782	17.9	20.2	4.9	3.7	4.9
	Forage and wheat	14.3	8.0	21.8	418	246	764	19.2	21.5	4.9	3.5	4.9
Base	Forage only	14.8	0.0	14.8	368	236	827	16.9	18.8	4.9	3.4	4.7
	Forage and wheat	15.3	8.0	22.8	446	237	745	21.2	22.4	4.5	3.4	4.7
	SED	0.86		0.86	36.5	21.9	19.4	0.92	1.27	0.38	0.14	0.14
P-value	Forage	<0.001		<0.001	0.185	<0.001	<0.001	0.002	0.001	0.950	0.277	0.072
	Fresh vs hay	0.031		0.031	0.643	<0.001	<0.001	0.001	<0.001	0.742	0.431	0.028
	PRG vs lucerne	<0.001		<0.001	0.042	0.169	0.651	0.004	0.006	0.746	0.167	0.153
	Bealey vs base	0.239		0.239	0.602	0.312	0.649	0.996	0.788	0.740	0.241	0.519
	Wheat	0.083		<0.001	0.002	0.034	0.532	0.005	0.164	0.093	0.326	0.426
	Forage.Wheat	0.060		0.060	<0.001	0.115	0.001	0.029	0.105	0.735	0.810	0.911
	Fresh vs hay	0.013		0.013	<0.001	0.275	0.008	0.019	0.034	0.466	0.745	0.622
	PRG vs lucerne	0.291		0.291	<0.001	0.042	<0.001	0.850	0.636	0.656	0.468	0.687
	Bealey vs base	0.930		0.930	0.503	0.382	0.279	0.040	0.218	0.487	0.598	0.760

[1]Values are covariate adjusted.
[2]Values are treatment means from days 1 and 2 (forage only), or day 3 (forage and wheat).

Results

Dry matter intake

Forage DMI varied with the type of forage (Table 6.2). Cows offered PRG hay consumed the least amount of forage (10.4 kg DM/cow per day), while there was no difference between the other three treatment groups (14.8 kg DM/cow per day). Cows in all treatments consumed all wheat that was offered and total DMI increased for all treatment groups on the day wheat was offered. Only lucerne fed cows exhibited substitution effects, with the amount of forage consumed reducing due to the consumption of wheat. This substitution effect resulted in an interaction between forage and wheat when comparing the pasture treatments to the hay treatments, such that the increase in total DMI when wheat was included was much greater for the pasture fed cows.

Eating behaviour

Introducing wheat into the diet had varied effects on eating behaviour depending on forage type. There were large differences between the hay treatments, with PRG hay fed cows spending less time eating and more time ruminating after wheat was introduced (Table 6.2). For lucerne hay fed cows, the effect was the opposite; they spent more time eating and less time ruminating, as did both the pasture treatment groups. Time spent ruminating varied with forage type. The PRG hay, lucerne hay, PRG cultivar Bealey and Base treatment groups spent an average of 519, 481, 270 and 237 min/cow per day ruminating, respectively. Cows spent an average of 46 min/day less ruminating once wheat was included in their diet.

Milk yield and composition

Mean yields of milk and ECM, and mean concentrations of milk fat, protein and lactose, for cows on the four dietary treatments are presented in Table 6.2. An interaction between forage type and wheat occurred, resulting in an increase in MY and ECM yield of pasture fed cows when wheat was offered, while there was no change for hay fed cows. With the addition of wheat to the diet the milk yield of the cows fed PRG cultivar Base increased, but this was not reflected in a difference in ECM yield. For the other three treatments, the inclusion of wheat in the diet did not affect MY or ECM. Neither forage type nor wheat inclusion had any effect on milk composition.

Ruminal fluid pH and composition

Changes in ruminal fluid pH over the entire measurement period is presented in Figure 6.1. Ruminal fluid pH data for the morning of day 4 is presented in the figure but is not included in any of the analyses. Ruminal fluid pH characteristics on days 1 to 3 are presented in Table 6.3. Both mean and minimum ruminal fluid pH varied with forage type; being greatest for PRG hay and lucerne hay, intermediate for Bealey and lowest for Base. Overall, for mean ruminal fluid pH, there was no interaction effect between forage type and wheat introduction, as the mean pH of all treatment groups declined with the introduction of wheat. However, there was an interaction when pasture was compared to hay. The

decline in mean ruminal fluid pH that occurred for the pasture treatment groups was much greater than that of the hay treatment groups (0.4 vs. 0.6 pH units). Minimum ruminal fluid pH also declined for all forages with the introduction of wheat, but no interaction effect occurred between forage and wheat. On average, the addition of wheat into the diet did not change the maximum pH of cows consuming hay but caused a reduction of 0.38 pH units for cows consuming pasture. The reduction was greatest for the Base treatment group (0.55 pH units).

The ruminal fluid of pasture fed cows had a pH below 6.0 for a greater proportion of the day than the ruminal fluid of hay fed cows, both on a forage only diet and when wheat was included. On a forage only diet the ruminal fluid pH of cows consuming hay only briefly fell below 6.0 (0.8 h/cow per day). Pasture fed cows had a ruminal fluid pH below 6.0 for significantly longer each day, particularly cows fed Base (11.2 h/cow per day). Following supplementation with wheat the time ruminal fluid pH was below 6.0 increased for all treatments. For cows fed pasture, ruminal fluid pH was below 6.0 for almost the entire day (21.5 h/cow per day). For cows consuming PRG hay, the duration increased from 0 to 12.9 h/cow per day, and while time below pH 6.0 increased for cows fed lucerne hay the increase was not as extreme, increasing from 1.5 to 9.0 h/cow per day.

Forage type affected the concentration of VFA in the ruminal fluid, with the greatest concentration in the pasture treatment groups, followed by the lucerne hay treatment group and the least in the PRG hay treatment group. The ruminal fluid mean concentration of acetate (expressed as a molar percentage of total VFA) was greater in cows fed hay compared to those fed pasture (68.2 and 60.7%), whereas the concentration of propionate was greater in the pasture fed cows (18.8 and 21.2%). The concentration of butyrate was greatest in the pasture fed cows, followed by PRG hay and lowest in the lucerne hay fed cows (13.1, 10.5 and 9.1%, respectively). There was a main effect of wheat introduction, which led to increased concentrations of total VFA, propionate and butyrate but decreased concentration of acetate and the acetate to propionate ratio. Adding wheat to the diet increased valerate concentrations for all treatments. However, the increase was twice as much for the PRG hay and pasture treatments compared to the lucerne hay treatment (0.4 vs. 0.2%). Both before and after the inclusion of wheat, the concentration of valerate was much greater in the pasture treatments compared to the hay treatments. DL-lactate concentrations were also affected by an interaction between forage and wheat. For cows fed pasture DL-lactate concentrations increased when wheat was added to the diet. For cows fed hay, however, DL-lactate concentrations did not change with the inclusion of wheat. Ammonia concentrations in pasture fed cows were more than double the concentrations measured in hay fed cows (125 and 260 mg/L) but were not impacted by wheat.

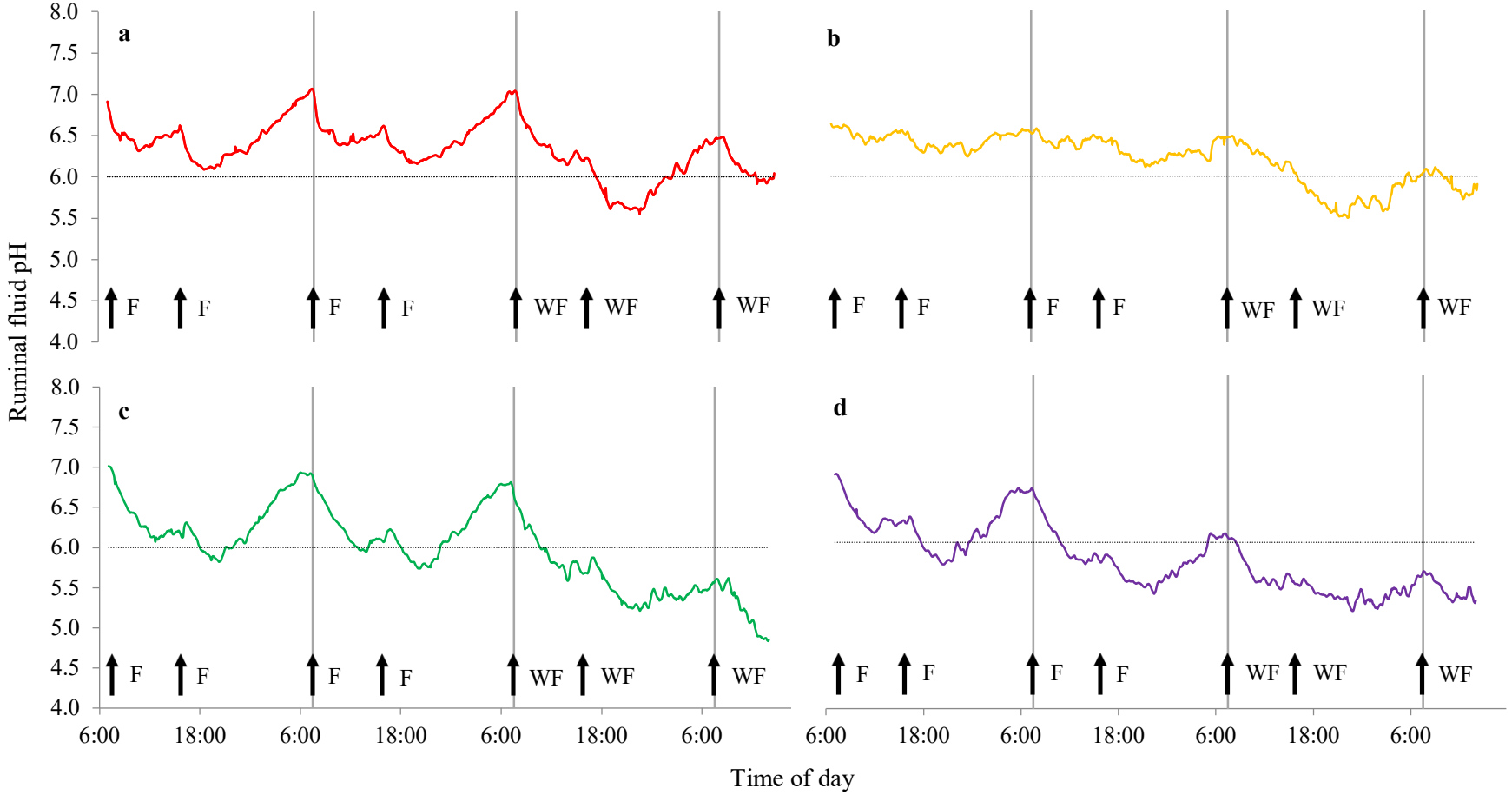

Figure 6.1. Changes in ruminal fluid pH over the 80 h measurement period for cows fed either a) lucerne hay, b) perennial ryegrass (PRG) hay, c) PRG pasture cultivar Bealey or d) PRG pasture cultivar Base. Values are the raw means for treatments. Arrows indicate when feed was offered, F is a meal of forage only and WF is when wheat was fed followed by forage. The dashed line at pH 6.0 defines the ruminal fluid pH below which fibre digestion theoretically declines. The vertical lines indicate the beginning and end of each defined day.

Table 6.3. Influence of forage type and the addition of wheat to the diet on mean ruminal fluid pH characteristics[1] and composition[2].

Forage	Diet	Ruminal fluid pH[3]					Total VFA[6] (mmol/L)	Individual VFA[6] (molar %)				Ac:Pr[7]	Ammonia (mg/L)	DL-Lactate (mM)[8]
		Mean	Minimum	Maximum	Time under pH 6[4]	Area under pH 6[5]		Acetate	Propionate	Butyrate	Valerate			
Alfalfa	Forage only	6.43	6.05	7.10	1.5	0.3	122	71.3	17.0	8.2	1.2	4.2	146	0.04
	Forage and wheat	6.08	5.47	7.05	8.7	2.7	123	67.2	19.0	10.0	1.4	3.6	159	0.02
PRG	Forage only	6.43	6.11	6.66	0.0	0.1	93	69.9	18.2	9.6	0.9	3.9	51	0.06
	Forage and wheat	5.97	5.37	6.57	12.9	4.2	111	64.4	21.0	11.4	1.3	3.1	145	0.02
Bealey	Forage only	6.26	5.76	7.04	5.8	1.0	141	62.3	20.8	12.2	1.4	3.0	208	0.52
	Forage and wheat	5.63	5.15	6.84	20.6	9.7	155	58.5	23.4	12.9	1.8	2.5	292	0.63
Base	Forage only	6.07	5.55	6.79	11.2	2.9	144	63.1	18.9	13.1	1.4	3.4	244	0.04
	Forage and wheat	5.53	5.06	6.24	22.3	12.0	162	58.9	21.5	14.1	1.8	2.8	293	0.37
	SED	0.084	0.130	0.090	1.79	1.03	7.9	1.19	1.12	0.72	0.09	0.22	45.2	0.670
P-value	Forage	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.013	<0.001	<0.001	<0.001	0.008	0.069
	Hay vs pasture	<0.001	<0.001	0.279	<0.001	<0.001	<0.001	<0.001	0.007	<0.001	<0.001	<0.001	0.001	0.043
	PRG vs lucerne	0.610	0.865	<0.001	0.839	0.610	0.002	0.067	0.146	0.016	0.021	0.055	0.248	0.068
	Bealey vs Base	0.039	0.029	0.001	0.017	0.011	0.311	0.556	0.088	0.063	0.752	0.129	0.689	0.918
	Wheat	<0.001	<0.001	<0.001	<0.001	<0.001	0.011	<0.001	<0.001	0.004	<0.001	<0.001	0.068	0.596
	Forage.Wheat	0.062	0.651	<0.001	0.025	0.002	0.475	0.472	0.824	0.686	0.040	0.578	0.769	0.014
	Hay vs pasture	0.018	0.424	<0.001	0.078	<0.001	0.451	0.339	0.763	0.252	0.086	0.297	0.834	0.005
	PRG vs lucerne	0.346	0.466	0.698	0.028	0.270	0.189	0.233	0.387	0.944	0.016	0.500	0.356	0.072
	Bealey vs Base	0.294	0.530	<0.001	0.070	0.761	0.733	0.738	0.976	0.812	0.872	0.548	0.686	0.558

[1]Summary of ruminal fluid pH characteristics days 1 and 2 (forage only), and day 3 (forage and wheat).
[2]Composition data are mean values from samples taken 6 h after feed was offered at both AM and PM on days 1 and 2 (forage only), and day 3 (forage and wheat).
[3]Values are covariate adjusted.
[4]Mean time per day during which ruminal fluid pH was below 6.0.
[5]Area of the pH vs. time of day curve below pH 6.0 (pH × h).
[6]Volatile fatty acids.
[7]Acetate to propionate ratio.
[8]Means were log transformed for analysis. Values presented are raw means, while the SED refers to log transformed values.

Discussion

The type of forage being consumed had significant effects on the ruminal fluid pH response to a wheat grain challenge. Compared with pasture, hay provided a rumen environment that was better able to cope with the influx of acid produced as a result of the sudden introduction and digestion of highly fermentable starch. Both with and without wheat in the diet, the daily mean and minimum ruminal fluid pH values were much greater for the cows consuming hays compared to the fresh forages. Furthermore, the ruminal fluid pH of cows fed fresh forages remained below 6.0 for a greater proportion of the day. The lower ruminal fluid pH from the pasture fed cows was most likely due to greater VFA production rates (Allen 1997). Although VFA production rates were not measured, the lower NDF and higher ME of the pasture would suggest faster degradation rates (Wales *et al.* 1999), and this was further supported by higher concentrations of VFA measured in the pasture fed cows (Sutton 1980). Saliva would have also played a major role in maintaining ruminal fluid pH of the hay fed cows. While intake is a driver of fermentation and hence acid production, saliva is the strongest buffer within the rumen (Van Soest 1994). Saliva production is greatest during rumination (Bailey and Balch 1961a, 1961b), and cows consuming hay were spending twice as long ruminating, driven by the greater NDF fraction (Allen 1997).

The introduction of wheat into the diet dramatically increased the amount of time ruminal fluid pH was below 6.0. The duration of time that pH remains below optimal is more critical than the daily mean pH (Hoover 1986; De Veth and Kolver 2001). If pH falls below the 6.0 threshold only temporarily, the negative implications on fibre digestion are only small and transient. When low pH (< 6.0) is sustained, however, the cellulolytic bacterial populations can be compromised (Hoover, 1986). Low ruminal fluid pH not only reduces fibre digestion (Stewart 1977) but can also limit energy intake and protein absorption due to the negative impacts on ruminal motility, microbial yield and appetite (Shinozaki 1959; Hoover 1986; Carter and Grovum 1990). If ruminal fluid pH is reduced to levels below 6.0 and remain there for extended periods, severe health problems can arise such as liver abscesses, laminitis, digestive tract tissue damage and in extreme cases, death (Slyter 1976; Nocek 1997; Nagaraja and Titgemeyer 2007).

Following wheat supplementation, the pasture fed cows had ruminal fluid pH values below 6.0 for almost the entire day. This is clear evidence that gradual adaptation strategies must be used to introduce large amounts of wheat when cows are consuming highly digestible spring pasture. The ruminal fluid of lucerne hay fed cows proved most resistant to the supplementation of wheat, exhibiting the smallest increase in time below pH 6.0. Despite having no prior wheat adaptation, the time below pH 6.0 was almost half that described in previous work when cows were grazing fresh Persian clover, at an average amount of 19 kg DMI/cow per day and adapted over 12 days to wheat fed at 3 kg DM/cow per day (Leddin *et al.* 2010). Comparatively, these results demonstrate how varied the adaptation process can

be with different forages. However, it is possible that time below pH 6.0 would have increased for the lucerne hay fed cows with continued wheat supplementation. It is also possible the results may have differed if the fresh pasture was grazed instead of harvested for feeding. Grazed pasture would have allowed for greater selection through more opportunity, possibly resulting in higher intakes and different nutritive profiles.

Although the maximum pH values reported for Bealey and Base on a forage and wheat diet are both above 6.0 (6.24 and 6.84, respectively) these values were recorded immediately after the morning feed was offered. From that time point onwards, ruminal fluid pH declined, and over the final 31 h remained at levels known to compromise NDF digestion (Mould *et al.* 1983). This downward trend continued further during the observations on day 4 (Table 6.4) when the maximum pH reached was 5.65 for Bealey and 5.81 for Base, again observed at the start of the day followed by a downward trend. This was likely driven by lower NDF concentrations and higher ME concentration of the pastures, resulting in a faster rumen passage rate and very little feed in the rumen prior to wheat consumption. This combined with reduced rumination times meant there were relatively less buffers available to resist further declines in pH with the fermentation of wheat. The ruminal fluid pH of cows in the pasture treatment groups showed very little ability to recover. It is possible that the sustained low pH levels reduced cellulolytic microflora to very low levels (Mould and Ørskov 1983), including protozoa that help maintain higher ruminal pH by engulfing starch granules (Mould *et al.* 2005). Hence, the low pH was further exacerbated. The ruminal fluid pH of cows in both the PRG hay and lucerne hay treatment groups recovered to levels above 6.0 at the beginning of day 4, values similar to those reported on a forage only diet.

On day 2, during the forage only period, the ruminal fluid pH of the Base treatment group was below pH 6.0 for almost the entire day, indicating that even without wheat in the diet, fibre digestion may have been impaired. Ruminal fluid pH levels this low on a diet of solely PRG pasture have previously been reported by Williams *et al*. 2001; 2005. The difference in ruminal fluid pH between day 1 and day 2 for the Base treatment group is due to a difference in DMI. The cows consumed ~4 kg DM/cow more on the second day compared to the first (12.6 vs. 16.8), which resulted in a lower ruminal fluid pH, a result previously reported in both stall fed and grazing dairy cows (Stockdale 1993; Williams *et al.* 2005). The already low ruminal fluid pH on the pasture only diet meant SARA was already prevalent in these cows prior to wheat supplementation. Despite the lower ruminal fluid pH values from the Base treatment group, the DL-lactate levels were significantly higher in the ruminal fluid of cows in the Bealey treatment group, when on a forage only diet. Following the grain challenge the DL-lactate concentration in the ruminal fluid increased dramatically for the Base treatment group.

Unlike the other three treatment groups, the average 24 h ruminal fluid pH pattern exhibited by cows fed PRG hay only was not a W-shaped pattern, as is typical when cows are fed twice daily (Greenwood

et al. 2014; Moate *et al.* 2017). Rather the ruminal fluid pH showed very little variation, varying by 0.55 pH units compared to 1.05 pH units for lucerne hay. This was likely due to lower and slower intakes by the cows fed PRG hay. While reduced variability benefits fibre digestion at low pH levels (Wales *et al.* 2004), the mean pH of lucerne fed cows was relatively high, remaining above pH 6.0 both before and after wheat supplementation. This indicates that the reduced variability would have provided no benefit for PRG hay fed cows over those fed lucerne hay. For the pasture treatments however, the large variability paired with a low mean pH on the forage-wheat diet, likely posed significant threats to fibre digestion.

There were greater proportions of propionate and butyrate in the ruminal fluid of pasture fed cows, which is consistent with the lower NDF concentration of the feed. While the greater proportion of valerate was likely driven by the higher CP concentration of the pasture (Bauman *et al.* 1971; Dijkstra 1994). The change in VFA proportion with the addition of wheat was consistent across treatments. The proportion of acetate declined while the proportions of propionic, butyrate and valerate all increased, reflecting the reduced proportion of VFA produced from NDF digestion and the greater contribution of starch digestion (Dijkstra 1994). The higher concentration of valerate in cows with SARA is supported by the results of Bramley *et al.* (2008).

Observations made on day 4 (Table 6.4) highlighted the degree to which the pasture fed cows were struggling to cope with the grain challenge and symptoms indicated acute acidosis (Owens *et al.* 1998). Rumination during the 7 h observation period had all but completely stopped for both Bealey and Base treatment groups. Cows in the Bealey treatment group appeared most compromised, exhibiting a minimum ruminal fluid pH of 4.78 and DL-lactate concentrations were 8 times greater than the previous day, contributing significantly to the total acid load, which is responsible for acidosis (Britton and Stock 1987). The order of the feeding, wheat before forage, may have played an important role in dictating pH patterns. Hay fed cows would have returned for the following feed with forage remaining in the rumen, allowing for buffering against the acids produced immediately by wheat fermentation. Cows consuming fresh pasture, however, consumed wheat with a near empty rumen, resulting in dramatic declines in ruminal pH.

The benefits of mitigating the impacts of dietary adaptation are extensive. Successful adaptation to a high concentrate diet improves the welfare of dairy cows by avoiding SARA and acute ruminal acidosis, both of which are concerns for the Victorian dairy industry (Garcia and Fulkerson 2005; Bramley *et al.* 2008). Furthermore, if the time required for successful adaptation to a high concentrate diet can be reduced, as indicated by the hay treatments within this study, total ME intake can be increased more rapidly, creating potential for increased milk production (Russo *et al.* 2018). The results of the current experiment indicate that there should be a focus on forage type when deciding on appropriate grain introduction strategies.

Table 6.4. Raw means of feed intake, eating behaviour, ruminal fluid pH and ruminal fluid composition of cows receiving each treatment as observed on day 4[1]

Item	Alfalfa hay	Perennial ryegrass hay	Perennial ryegrass cultivar Bealey	Perennial ryegrass cultivar Base
Feed intake (kg DM/cow)				
Forage	4.7	1.8	3.7	3.2
Wheat	4.0	3.0	2.9	2.9
Total	8.7	4.8	6.6	6.0
Eating behaviour (min/cow)				
Eating	164	102	141	130
Ruminating	102	86	3	6
Not chewing	149	227	274	282
Ruminal fluid pH				
Mean	6.14	5.93	5.26	5.44
Minimum	5.91	5.71	4.78	5.18
Maximum	6.55	6.26	5.65	5.81
Ruminal fluid composition[2]				
Total VFA[3] (mmol/L)	130	124	184	170
Acetate (molar %)	65.7	61.7	59.6	58.5
Propionate (molar %)	20.2	19.4	20.0	18.8
Butyrate (molar %)	10.1	15.1	16.0	17.1
Valerate (molar %)	1.4	1.2	1.4	1.8
Acetate: Propionate	3.3	3.2	3.0	3.1
Ammonia (mg/L)	96	12	377	340
DL-Lactate (mM)	0.03	0.01	5.38	1.03

[1]The observation period was from 0700 to 1400 h. Cows had received wheat and forage that morning.
[2]As sampled 6 h post feed.
[3]Volatile fatty acids.

Conclusion

The rumen environment of cows fed hay had an ability to resist the dramatic declines in ruminal fluid pH that are typically associated with rapid grain adaptation. This contrasted with cows fed pasture, who exhibited symptoms associated with SARA, including more than 20 h of the day with a ruminal fluid pH below 6.0. Overall, these findings highlight an ability to more rapidly introduce large amounts of wheat grain to forage fed cows when high quality hay is the basal forage.

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Chapter 7

General discussion and conclusion

7.1. Introduction

Most dairy systems in temperate parts of the world, such as Victoria, Australia and Ireland, utilise grazed pasture as their main feed source (Doyle and Stockdale 2011). However, due to high energy needs of lactating dairy cows and the challenges posed by both weather and the variability in pasture supply throughout the year, cows are often supplemented with conserved forage and either cereal grains or pelleted concentrates. This makes dietary changes involving grazed pasture, conserved forage and concentrates common practice in most pasture based dairy systems. Such changes often disrupt the ruminal environment leading to compromised intake and milk production. Four experiments were conducted using lactating dairy cows to help understand and mitigate the impacts of major dietary changes in pasture based dairy systems.

7.2. Type of dietary change

The data presented in Chapter 3 investigated a complete dietary change from one forage source to another. This type of change routinely occurs at calving in most pasture based dairy systems in Ireland as the forage source is changed from pasture silage to grazed pasture. Early adaptation to the new forage source showed no dry matter intake (DMI) nor milk yield (MY) benefits over traditional practices, suggesting that this specific change does not significantly disrupt normal rumen function. This may be due to the similarity of the two forages; grazed pasture and pasture silage. The rumen has evolved to successfully and efficiently digest forages. It is possible that the newly introduced diet of fresh pasture doesn't present a challenge to the ruminal microbiome. The microbial digestion of forages typically occurs slowly and results in a gradual release of energy, in the form of organic acids (Dijkstra 1994). So even when a complete change in diet occurs from one forage to another, there is not going to be a rapid influx of acid nor a need for a significant shift in the bacterial populations. The introduction of a diet containing a large amount of wheat grain, however, as described in Chapters 4, 5 and 6, can present unique challenges to the rumen environment, particularly when no previous adaptation exists.

A change from a predominantly forage diet to one containing large amounts of concentrate, not only promotes rapid fermentation and organic acid production, but counterproductively reduces rumination time due to decreased fibre intake (Allen *et al.* 2006). Saliva is a powerful buffer within the rumen, and rates of saliva production are greatest during rumination (Bailey and Balch 1961). The behavioural data presented in Chapter 5 shows rumination time significantly decreased from 7.9 h/cow per day to 6.0 h/cow per day on average, when 40% of the hay was removed and replaced with wheat grain (on a dry matter basis). This effect occurred regardless of the hay variety (PRG or lucerne) or the duration of the wheat adaptation strategy (6 or 11-day).

7.3. Management strategies

The experiments detailed in Chapter 3 and 4, indicate that management strategies do not always impact on the successful adaptation to a diet, but can influence milk production. Management techniques investigated as part of this thesis include both the timing of a dietary change regarding stage of lactation, as well as the method used to introduce the new diet. As described in Chapter 3, early introduction of a new diet, to avoid a dietary change at calving, did not yield any production benefits. Cows that avoided a major dietary change at calving did not produce more milk in early lactation compared with their control counterparts. Perhaps, as described earlier in this chapter, this was due to the dietary change posing little threat to the existing ruminal microbial population. Following these results, the focus of the research shifted to a dietary change that incorporated large amounts of wheat grain.

The experiment detailed in Chapter 4 described different strategies used for adapting forage-fed cows to a high grain diet. There was little variation between the strategies regarding ruminal fluid pH and fermentation characteristics. Under the conditions of this experiment, there appeared to be no benefit of introducing wheat in smaller increments nor was there an advantage of lengthening the introduction period. This premise was further developed in a subsequent experiment (Chapter 5), the results of which showed a milk production benefit of shortening the wheat adaptation period. Cows that were introduced to high levels of wheat in 6 days, compared to 11 days, benefited from the rapid input of wheat and subsequent increase in estimated dietary metabolisable energy (ME). This resulted in cows on the 6-day strategy producing an average of 1.5 kg/cow per day extra energy corrected milk than those on the 11-day adaptation strategy whose increase in dietary estimated ME was more gradual. However, these results were dependent on the base forage.

7.4. Forage choice

Both the research presented in this thesis and previous works have demonstrated that forage choice determines how the rumen functions, as forage makes up the majority of a dairy cow's diet in pasture-based systems. The rate of fermentation, the concentration of end products, eating behaviour and successful adaptation to a new diet are all influenced by the type of forage being consumed (Williams *et al.* 2000; Williams *et al.* 2001). The fermentation of fresh spring pasture occurs rapidly in the rumen (Wales *et al.* 1999) potentially leading to acidotic cows on a forage only diet (Williams *et al.* 2001). In Chapter 6 it is reported that cows fed pasture can exhibit symptoms of SARA, including more than 20 h of the day with a ruminal fluid pH below 6.0. These data show that acidosis isn't only a problem when introducing rapidly fermentable starch into a diet but can also occur when cows are fed a diet of high quality spring pasture with no supplement at all. Overall, these findings indicate that a cow's ability to adapt to large amounts of wheat grain is greatest when high quality hay is the basal forage, but also draw attention to the risk of acidosis when cow are grazing spring pasture only.

When introducing high amounts of concentrate into the rumen, the ruminal microbial population needs to withstand the increased rate and quantity of acid production. Appropriate use of forages can help achieve this. As described in Chapter 5, MY and rumen responses to wheat adaptation strategies varied depending on the base forage. Hays provided good buffering within the rumen and sufficiently stimulated rumination. This allowed the rumen environment to cope with the acid load imposed by a rapid grain introduction strategy. However, due to variation in intake and fibre and ME concentrations of the hays, the milk production and ruminal pH responses varied significantly. These results indicate that some subtle changes to grain introduction methods can lead to greater milk production depending on intake and forage choice. The rumen environment of cows fed hay had an ability to resist the dramatic declines in ruminal fluid pH that are typically associated with rapid grain adaptation.

7.5. Implications of this research and future research directions

Inadequate adaptation to high concentrate diets can result in both sub-acute and acute ruminal acidosis, both of which are major financial and welfare concerns for the dairy industry (Garcia and Fulkerson 2005; Bramley *et al.* 2008). The results of this thesis provide valuable insights for dairy farmers when introducing new diets, particularly high amounts of concentrate to forage fed cows, a common feeding strategy on Victorian dairy farms. When implemented appropriately, a rapid wheat adaptation strategy can increase total energy intake, potentially increasing MY while avoiding the threat of acidosis. This thesis highlights the importance of focusing on forage type when deciding on appropriate grain introduction strategies. There is a real opportunity to tailor grain adaptation strategies to specific forages. With the right forage choice, acidosis can be avoided during rapid changes to the amount of wheat offered. The traditional gradual introduction strategies for starch-based concentrates are still required when feeding with highly fermentable pasture but more aggressive wheat adaptation strategies can be used on a hay-based diet. The data presented throughout this thesis show clearly that there is no specific strategy to optimise the introduction of grain. It needs to be tailored to the forage type and intake. The nutritive and fermentation characteristics of the forage are just as important as that of the concentrates. Hays more adequately buffer the rumen which helps resist major pH drops, due to stimulating greater saliva production and slower rates of fermentation.

The results described in Chapter 6 highlight the risk of acidosis on high quality spring pasture, a concern that exists for dairy cows in both Ireland and Victoria, as large amounts of spring pasture are consumed in peak lactation. This stresses the importance of not only supplementing with a forage source or providing buffers as part of a mineral mix, but also being vigilant in monitoring for cases of acidosis where it may not have previously been a concern. For dairy farming in Ireland, the results described in Chapter 3, indicate little effect and no benefit of early introduction of pasture. This, if nothing else, is a reassurance that current methods are adequate for a successful dietary changeover.

While it has been made clear that wheat can be introduced rapidly when hay is the forage source, this is unlikely to occur regularly in pasture based dairy systems. Future research should investigate feeding a combination of both fresh and conserved forages to understand what proportion of the diet needs to be hay for a rapid adaptation to be successful. Additionally, the duration for which hay should be fed before grain introduction is also unknown. Throughout this thesis the buffering effect of lucerne hay was evident, due to effects on fermentation rates and rumination times. However, it is unclear what would result from feeding fresh lucerne or other legumes. If the benefits of feeding lucerne hay during rapid grain adaptation carried over to fresh lucerne, it could further support arguments for diversifying pasture species on farm (Pembleton *et al.* 2015). Questions were also raised about the rate of intake impacting on ruminal pH and how the form in which both the forage and the concentrate are presented to the cow. Most importantly there are several questions that address the basics of introducing high levels of grain to forage fed cows that remain unanswered. While most dairy farmers are aware of general guidelines around introducing large amounts of wheat to grazing dairy cows, there is no published research clearly defining the best adaptation strategy for grazing dairy cows. This poses the question of how gradual does the introduction of wheat to grazing dairy cows really need to be? What is the optimum adaptation strategy in a pasture-based system? The financial benefits of avoiding metabolic diseases are obvious. However, an investigation into the economics of rapid and gradual grain introduction is important to gain a full understanding of the financial benefits. In early lactation rapid adaptation resulted in milk production benefits but investigations into the effects at different stages of lactation is warranted.

7.6. References

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Appendix I

Table I.1. Mean and range of ruminal fluid pH for lactating dairy cows fed a forage-based diet reported in the published literature. Only studies which offered the forage and supplement separately, offered supplements twice daily and used lactating dairy cows have been included

Reference	Forage	Supplement	Forage DMI[1] (kg/cow per day)	Supplement DMI (kg/cow per day)	Ruminal pH		
					Min	Mean	Max
(Alvarez *et al.* 2001)	Mixed pasture (annual ryegrass and winter oats)	High moisture corn	14.6	6.4	NA	6.01	NA
	Mixed pasture (annual ryegrass and winter oats)	Dry cracked corn	14.8	5.6	NA	5.97	NA
(Auldist *et al.* 2013)	Perennial ryegrass (PRG) and pasture silage	Milled barley grain	9.4	7.9	5.64	6.18	6.87
(Auldist *et al.* 2016)	PRG	Mixed ration (milled wheat grain, crushed corn grain, canola meal and lucerne hay)	13.8	8.3	5.55	5.93	6.37
	PRG	Mixed ration (milled wheat grain, crushed corn grain, canola meal and lucerne hay)	12.6	16.4	5.56	6.00	6.20
(Bargo *et al.* 2001)	Winter oats	Ground corn, wheat bran, low protein sunflower meal and mineral mix	12.1	6.2	5.38	5.52	5.64
	Winter oats	Ground corn, wheat bran, high protein sunflower meal and mineral mix	14.4	6.4	5.35	5.53	5.63
	Winter oats	Ground corn, wheat bran, high protein feather meal and mineral mix	13.2	6.4	5.29	5.51	5.63

(Bargo *et al.* 2002)	Mixed pasture (smooth bromegrass, orchardgrass, Kentucky bluegrass)	Concentrate mix (corn, wheat, barley, soybeans, corn gluten meal, minerals and vitamins)	20.5	0.7	NA	6.40	NA
	Mixed pasture (smooth bromegrass, orchardgrass, Kentucky bluegrass)	Concentrate mix (corn, wheat, barley, soybeans, corn gluten meal, minerals and vitamins)	16.1	8.7	NA	6.29	NA
(Berzaghi *et al.* 1996)	Mixed pasture (tall fescue, orchardgrass and white clover)	Cracked corn and a vitamin and mineral mix	13.0	0.0	NA	6.40	NA
	Mixed pasture (tall fescue, orchardgrass and white clover)	Cracked corn and a vitamin and mineral mix	9.8	5.4	NA	6.20	NA
(Carruthers and Neil 1997)	High nitrogen PRG	Non-structural carbohydrate mix (cornflour and dextrose monohydrate)	14.3	1.3	NA	6.05	NA
	High nitrogen PRG	Control	14.5	0.0	NA	6.19	NA
	Low nitrogen PRG	Non-structural carbohydrate mix (cornflour and dextrose monohydrate)	14.0	1.3	NA	6.11	NA
	Low nitrogen PRG	Control	14.3	0.0	NA	6.17	NA
(Carruthers *et al.* 1997)	PRG	Control	14.1	0.0	5.70	6.08	6.60
	PRG	Non-structural carbohydrate mix (cornflour and dextrose monohydrate)	13.9	1.1	5.65	6.00	6.50
(Delagarde *et al.* 1997)	PRG	Soybean meal and formaldehyde-treated soybean meal	14.8	2.0	NA	6.01	NA
(Jones-Endsley *et al.* 1997)	Mixed pasture (lucerne and orchardgrass)	Rolled corn, soybean hulls and soybean meal	12.5	5.6	NA	5.90	NA

	Mixed pasture (lucerne and orchardgrass)	Rolled corn, soybean hulls and soybean meal	11.7	8.4	NA	5.82	NA
(King *et al.* 1990)	Mixed pasture (PRG and white clover)	High energy pellets (barley, millmix, citrus pulp, sunflower meal, vitamins and minerals)	16.3	3.3	NA	6.80	NA
(Kolver *et al.* 1998)	Orchard grass pasture	Ground shelled corn, soybeans and molasses	9.7	9.2	NA	6.06	NA
(Maekawa *et al.* 2002)	Barley silage	Barley grain	7.9	10.5	5.14	5.77	6.52
(McCormick *et al.* 2001)	Annual ryegrass	Ground corn, soyhulls, solvent soybean meal,	12.1	10.8	NA	6.19	NA
(Moate *et al.* 2014)	Lucerne hay	Crushed wheat with dried molasses and a mineral mix	13.2	4.1	NA	6.87	NA
(Moate *et al.* 2017)	Lucerne hay	Corn with canola meal and minerals	10.3	11.9	5.90	6.30	6.70
	Lucerne hay	Wheat with canola meal and minerals	9.7	11.4	5.25	6.10	6.60
	Lucerne hay	Barley with canola meal and minerals	10.8	11.9	5.85	6.75	6.80
(Reis and Combs 2000a)	Mixed pasture (lucerne, red clover, orchardgrass, smooth bromegrass)	Dry ground corn	10.8	9.2	6.40	6.57	6.82
	Mixed pasture (lucerne, red clover, orchardgrass, smooth bromegrass)	Dry ground corn plus lucerne hay	8.22	11.8	6.28	6.46	6.75
	Mixed pasture (lucerne, red clover, orchardgrass, smooth bromegrass)	Steam-rolled corn	10.7	9.1	6.32	6.48	6.75
	Mixed pasture (lucerne, red clover, orchardgrass, smooth bromegrass)	Steam-rolled corn plus lucerne hay	7.61	12.1	6.35	6.51	6.75

(Reis and Combs 2000b)	Mixed pasture (lucerne, red clover, orchardgrass, smooth bromegrass)	Control	13.9	0.0	NA	6.63	NA
	Mixed pasture (lucerne, red clover, orchardgrass, smooth bromegrass)	Ground dry corn with soybean meal, molasses and minerals	12.7	5.0	NA	6.72	NA
	Mixed pasture (lucerne, red clover, orchardgrass, smooth bromegrass)	Ground dry corn with soybean meal, molasses and minerals	9.8	10.0	NA	6.69	NA
(Russo *et al.* 2017)	PRG	Wheat, canola meal, maize grain, oaten hay	11.1	12.0	5.24	6.01	6.88
(Schor and Gagliostro 2001)	Mixed pasture (PRG, red clover, white clover and orchardgrass)	Corn grain, soybean meal and mineral vitamin mix	13.7	5.9	NA	5.70	NA
	Mixed pasture (PRG, red clover, white clover and orchardgrass)	Corn grain, blood meal and mineral vitamin mix	17.2	5.7	NA	5.80	NA
(Wales *et al.* 2000)	Mixed pasture (PRG and white clover)	Barley grain	9.7	5.8	5.85	6.26	6.5
(Williams *et al.* 2005)	PRG	Barley grain pellet	10.3	4.5	NA	5.87	NA
(Williams *et al.* 2016)	Lucerne cubes	Maize grain, canola meal and minerals	15.4	5.4	NA	6.61	NA
	Lucerne cubes and fresh forage brassica	Maize grain, canola meal and minerals	15.2	5.4	NA	6.60	NA
	Lucerne cubes and fresh perennial chicory	Maize grain, canola meal and minerals	12.3	5.4	NA	6.95	NA

[1]Dry matter intake.

Appendix II

Table II.2. Saliva production of lactating dairy cows during eating and resting as reported in published literature

Reference	Feed	Insalivation of feed (mL/g dry matter)	Rumination salivation rate (mL/min)	Resting salivation rate (mL/min)
(Bailey and Balch 1961)	Lucerne silage	2.7	105	
	Medium quality hay	4.7	146	
	Hay and dairy cubes	2.2	117	
	Hay, flaked maize and groundnut cake	1.9	150	
	Grass	6.9	190	
(Beauchemin *et al.* 2008)	Barley Silage	4.2		
	Lucerne silage	3.4		
	Lucerne hay	4.3		
	Barley straw	7.2		
(Bowman *et al.* 2003)	Total mixed ration[1] (45:55)[2]	3.3		138
(Cassida and Stokes 1986)	Hay crop silage	3.4		153
	Corn silage	3.1		144
(Maekawa *et al.* 2002)	Steam rolled barley grain	1.2		107
	Whole crop barley silage	14.4		
	Total mixed ration[3] (40:60)[2]	3.0		105
	Total mixed ration[3] (50:50)[2]	3.0		100
	Total mixed ration[3] (60:40)[2]	3.6		91
(Meyer *et al.* 1964)	Freshly cut lucerne	2.9		
	Lucerne hay	3.3		

[1]Barley silage, lucerne silage and steam rolled barley grain.
[2]Forage-to-concentrate ratio.
[3]Whole crop barley silage and steam rolled barley grain.

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