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Effects of level of concentrate supplementation on milk production and ruminal pH in lactating cows on pasture

Gatha R. Clevenger
West Virginia University

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EFFECTS OF LEVEL OF CONCENTRATE SUPPLEMENTATION ON
MILK PRODUCTION AND RUMINAL pH IN LACTATING COWS ON
PASTURE

Gatha R. Clevenger

Thesis submitted to the
Davis College of Agriculture, Natural Resources and Design at
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in partial fulfillment of the requirements for
the degree of

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in
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Division of Animal and Nutritional Sciences
Gene Felton, Ph. D
Thomas Griggs, Ph. D
K. Marie Krause, Ph. D, Chair

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2010

Keywords: dairy cows, grazing, pasture, supplementation, ruminal pH, milk production

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ABSTRACT

Effects of Level of Concentrate Supplementation on Milk Production and Ruminal pH in Lactating Cows on Pasture

Gatha R. Clevenger

The effects of concentrate supplementation on milk production and ruminal fermentation was evaluated in six ruminally cannulated lactating Holstein dairy cows in a Latin rectangle design. Cows were fed 4 (C4), 8 (C8), and 12 (C12) kg DM/d of a concentrate supplement. Increasing amounts of concentrate supplement decreased pasture intake, but increased overall dry matter intake (DMI) and increased milk production without changing milk composition. Daily mean and minimum ruminal pH was similar for all treatments. Increasing supplementation increased time spent below pH 5.8, and resulted in a reduction in the in situ degradability of DM and NDF at 24 hrs. Total tract digestibility, total volatile fatty acid, ammonia concentrations, and microbial protein synthesis were not affected by increasing levels of supplementation. The highest level of supplementation of 12 kg DM/cow/d had negative effects on rumen fermentation and may not be beneficial in a pasture based system.
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Finally, I dedicate this to my family. I want to thank my parents and sister for all their support and love. To my Pap, if it wasn’t for you my love of science may have never blossomed, so thank you. I am forever grateful for the love and support from my husband, Lucas Clevenger. If it wasn’t for his persistence and patience I would not be here today. Thank you all for your never-ending support and love, which I could not have done without during this process.
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Chapter 1

Introduction and Literature Review
INTRODUCTION, RESEARCH JUSTIFICATION, AND OBJECTIVES

In the United States today, pasture based grazing systems for dairy cows are becoming more popular. Farms using managed grazing typically produce less milk per cow than confinement farms (Kriegl, 2005). However, economic studies in Wisconsin show that grazing farms are economically competitive with confinement operations (Kriegl, 2005). A managed grazing dairy system is usually less expensive to set up and run than a confinement dairy. Key findings in an analysis by Kriegl (2005) show that in Wisconsin, managed grazing operations had more net farm income from operations per cow ($524 vs. $230) and per hundredweight equivalent than their confinement counterparts in the state.

The biggest challenge grazing dairy producers face is meeting the energy requirements of lactating dairy cows (Kolver and Muller, 1998). Inadequate nutrient intakes will result in cows entering a negative energy balance, resulting in loss of body condition which increases the likelihood of compromising the cow’s health and a decrease in milk production which is economically detrimental to the producer. Dairy cows which solely graze pasture have been shown to have a decreased dry matter intake (DMI) (Kolver and Muller, 1998; Bargo et al, 2002b), decreased milk production (Kolver and Muller 1998; Soriano et al, 2001; Bargo et al, 2002b), altered milk composition (Kolver and Muller, 1998; Bargo et al, 2002b), altered ruminal fermentation (Holden et al, 1994b; Bargo et al, 2002c) and an overall decrease in net energy for lactation (NE\textsubscript{L}) as well as metabolizable energy (ME) compared to cows receiving a total mixed ration (TMR) with no pasture (Kolver and Muller, 1998).

The decrease in energy intake can be overcome with the addition of an energy supplement in the form of grain, so that easily fermentable carbohydrates are available to the animal to compensate for the decrease in DMI and thereby energy intake, that occurs with
pasture intake. Appropriate strategies for supplementation of high producing dairy cows requires an understanding of the effect the supplement has on DMI, animal performance and digestion. Also, the nutrients provided must complement the nutrient content of the pasture and meet the nutrient requirements of the cow.

Cows grazing high quality pastures, supplemented with concentrates twice daily produce more milk, but with reduced fat content (Berzaghi, et al, 1996; Carruthers and Neil, 1997; Reis and Combs, 2000; Bargo et al 2002a), which may be associated with lower acetate to propionate ratios in supplemented cows due to increased propionate production. Supplementing with concentrates may also affect fiber digestibility. It is known that the inclusion of starch into the diet depresses fiber digestibility by reducing the cellulolytic activity of fiber digesters, which may be associated with the acidic conditions associated with rapid starch fermentation (Mertens et al, 1980). Bargo et al (2003) stated in a review that supplementation with concentrates did not affect digestibility of OM, but reduced digestibility of NDF. Compared with a pasture only diet, supplementation with 10 kg DM/d concentrate reduced the insoluble potentially degradable DM fraction of pasture, without affecting the soluble fraction, rate of degradation and the effective degradability of DM (Reis and Combs, 2000). A reduced ruminal pH may result in suboptimal digestion and nutrient supply and may also contribute to metabolic diseases such as ruminal acidosis and laminitis (Allen, 1997; Kolver and de Veth, 2002).

Knowledge of ruminal pH of grazing dairy cows is limited and no consistent relationship was found between ruminal pH and level of supplementation in a review by Bargo et al (2003). The number of samples collected and the timing of rumen sampling in relation to feeding could be affecting these results. Mean daily ruminal pH values of 5.6 to 7.0 have been reported for dairy cows grazing high quality pasture (Berzaghi et al, 1996; Carruthers and Neil, 1997; Reis
some authors reported reduced ruminal pH when feeding large amounts (> 8 kg DM/d) of concentrate supplements (Bargo et al, 2002a), but also when feeding small amounts (1.4 kg DM/d; Carruthers and Neil, 1997) of concentrates to grazing dairy cows. In contrast, other authors reported similar ruminal pH between pasture only diets and diets plus varying amounts supplementation (Berzaghi et al, 1996, Reis and Combs, 2000; Graf et al, 2005). The lack of consistency with the amount of concentrate supplementation on ruminal pH of grazing dairy cows suggests that there is not a simple relationship between amount of concentrate supplemented and ruminal pH.

The objectives of this experiment were to assess the impact of varying levels of concentrate supplementation on production performance, ruminal pH and fermentation products, nutrient utilization and fiber digestibility in grazing dairy cows.

**LITERATURE REVIEW**

**Dairy Production from Pasture Only**

**Dry Matter Intake**

Pastures used for lactating dairy cows are commonly described as high quality with 18 to 24% DM, 18 to 25% CP, 40 to 50% NDF and 1.53 to 1.67 Mcal/kg DM of NE\(_L\) (Bargo et al, 2003). Cows grazing pasture have a lower total DMI compared to cows consuming a TMR (Kolver and Muller, 1998; Bargo et al, 2003). Variables affecting DMI include the nutrient requirement of the cow, factors associated with satiety and distention, and limits resulting from pasture and animal factors that affect grazing behavior. Grazing time for lactating dairy cows is
one of the limiting factors to DMI because cows may only graze for 5-6 hrs per day (Ferris, 2007). Variables related to the cow, such as body weight, change in body weight and milk yield explain 71% of the variation in total DMI from pasture (Vazquez et al, 2000). A decrease in DMI is shown when cows are grazing pasture alone (Kolver and Muller, 1998; Reis and Combs, 2000). As shown in a study by Kolver and Muller (1998), pasture only cows consumed a total of 19.0 kg DM/d compared to 23.4 kg DM/d (P < 0.01) for cows consuming a TMR. One reason for a lower DMI may be because passage rate is lower in pasture diets which had a duodenal flow of 4.8 kg/d compared to grass silage with a duodenal flow of 7.3 kg/d (P <0.05) (Holden et al, 1994a). This lower rate of passage was also shown when comparing pasture only to a pasture plus corn supplementation: pasture duodenal flow rate was 6.6 kg/d compared to 8.6 kg/d (P < 0.02) in the cows consuming a supplemented diet (Berzaghi et al, 1996).

One way to increase intake in grazing animals is through increasing herbage allowance. An increase in DMI occurred when cows were given access to a higher pasture allowance of 40 kg DM/cow/day compared to a lower pasture allowance of 25 kg DM/cow/day: high pasture allowance DMI was 20.5 kg/d and low pasture allowance DMI was 17.5 (P <0.01) (Bargo et al, 2002a). Pasture DMI increases as pasture allowance increases, with a plateau in DMI at a pasture allowance of 60-70 kg DM/cow/day (Bargo et al, 2003). Another way to increase pasture intake is to increase sward structure. By doing this the bite size can be increased through increases in sward height and bulk density which will maximize intake per bite. As cows graze down through the sward the proportions of dead leaves and stems increase thus decreasing pasture intake, so intake tends to be higher on swards of greater density once height is removed by grazing (Ferris, 2007).
It has been shown that cows consuming a sole pasture diet consumed 19% less dry matter (DM), organic matter (OM) and NE\textsubscript{L} than cows fed a TMR, although on a live weight percentage grazing cows consumed more crude protein and NDF (Table 1-1; Kolver and Muller, 1998).

Kolver and Muller (1998) also showed that grazing cows lost more live weight and body condition than cows fed a TMR. An increase in beta-hydroxybutarate (BHBA) and non-esterified fatty acids (NEFA) concentrations were shown in cows grazing pasture, indicating an increased mobilization of body reserves. This led the researchers to conclude that the decrease in DMI rather than the energy content of pasture versus TMR (1.63 vs. 1.65 Mcal/kg) was responsible for a lower energy supply. Producers also need to realize that the grazing animal requires more NE\textsubscript{L} due to grazing activity and an increase in walking activity. This was demonstrated in a study by Holden et al (1994a) where grazing cows fed diets meeting NRC (1989) recommendations for DMI and with total DMI equal to or above NRC (1989) recommendations still experienced a decrease in body condition. Because the NE\textsubscript{L} requirement was underestimated for these cows their energy requirement was not met, making this the primary limiting factor in lactating cow’s production ability on pasture alone. Cows eat less on pasture than when fed a TMR due to factors that include sward height, pasture mass, pasture quality, and grazing time. Providing pasture that is of good quality and quantity is important in order to meet energy requirements for lactation.

**Milk Production and Composition**

Decreasing DMI on pasture will lead to a decrease in milk production. Kolver and Muller (1998) demonstrated that cows consuming an all pasture diet produced less milk with
lower protein content versus cows consuming a TMR, while Soriano et al (2001) found no differences in protein content milk from dairy cows consuming an all pasture diet compared to cows consuming a TMR (Table 1-2). Bargo et al (2002b) found an increase in milk production from 32.0 kg/d in cows consuming a pasture plus TMR to 38.1 kg/d in cows consuming a TMR. The percent milk fat did not change when comparing a pasture only diet to a TMR or when comparing a pasture plus TMR to a TMR (Reis and Combs 2000; Bargo et al, 2002b). Intake of nutrients, due to a decreased DMI, appears to be the primary factor that constrains the milk production of cows consuming pasture. High producing dairy cows will not be able to maintain production on pasture alone.

**Volatile Fatty Acids and Rumen Fermentation**

Few studies have compared VFA concentrations in cows grazing pasture alone compared to cows consuming a TMR. Bargo et al. (2002c) found no changes in total ruminal VFA concentrations which averaged 137.5 mmol/L or individual VFA molar proportions with cows grazing pasture plus TMR compared to cows consuming a TMR only. Total VFA concentrations showed no differences when comparing cows consuming a pasture only diet with consumption of a pasture plus corn silage diet (Graf et al, 2005). However, VFA concentrations were found to be higher in pasture only diets compared to hay or silage diets (131.7 vs. 118.4 mmol/L; Holden et al, 1994b). Despite the higher concentrations of total VFA, the mean concentrations of acetate and propionate were similar across forages. The increase in VFA is attributable to the extensive, rapid fermentation of grasses, thus substrates are more readily available in the rumen of cows on pasture than in the rumen of cows fed conserved forages.
Mean daily ruminal pH values of 5.6 to 6.4 have been reported for dairy cows fed diets of high quality pasture (Kolver and de Veth, 2002). In a more recent study rumen pH values ranging from 5.1 to 7.0 were reported for dairy cows grazing high quality pasture (Gibbs and Laporte, 2009). Bargo et al (2002c) found mean ruminal pH did not differ among cows consuming a pasture plus TMR diet versus a TMR only and averaged 5.87 ± 0.04. Graf et al (2005) also found no differences in mean ruminal pH of cows grazing pasture alone compared with cows consuming pasture plus corn silage or pasture plus grass hay and pH averaged 6.30 across the different treatments. Although the authors found no effect on mean pH, they did find that daytime mean rumen pH decreased from 6.46 to 6.23 ($P<0.03$), when comparing pasture only to cows consuming a pasture plus grass hay diet. In the study by Bargo et al (2002c) mentioned above a treatment by hour interaction was found for rumen pH ($P<0.05$), indicating that although the mean rumen pH did not differ, the variations in daily rumen pH patterns differed among treatments. Among the treatments, the pasture plus TMR resulted in the greatest variations in pH, with a minimum pH of 5.48 at 0100 h and maximum of 6.46 at 1700 h when cows remained indoors and consumed the TMR.

The diurnal variations in grazing dairy cows can be attributed to differences in grazing behavior during the day compared to the night. Grazing ruminants consume a larger meal before sunset (Bargo et al, 2002b). Cows consuming a pasture diet had an increased grazing time, a higher number of bites and higher bite rates during the evening compared to the daytime (Bargo et al, 2002b). In a study conducted by Taweel et al (2004) they concluded that cows grazed 71 minutes longer at dusk (1800 to 2400 h) than during the dawn (0600 to 1200 h) and 28 minutes longer than during the afternoon (1200 to 1800 h) bout as well as having an increased number of
bites in the dusk grazing bout compared to the other two. Because of this DMI increased from 3 kg during the dawn grazing bout to 7 kg during the dusk grazing bout.

Through meta-analysis Kolver and de Veth (2002) concluded that a low mean ruminal pH on diets of fresh pasture were associated with increasing VFA concentrations compared to conserved forages. A reduced ruminal pH may result in suboptimal digestion and nutrient supply and may also contribute to metabolic diseases such as ruminal acidosis and laminitis. Subclinical ruminal acidosis is a temporarily altered state of the rumen, whereby the pH is reduced below 5.8 (Maekawa et al, 2002). During acute ruminal acidosis the critical pH threshold of the rumen is < 5.0 resulting in very sick cows with impaired physiological functions and death may occur (Nocek, 1997). With a decrease in rumen pH the microbial profile of the rumen changes. Lactic acid accumulates in the rumen when the bacteria that synthesize lactic acid outnumber those that utilize lactic acid. Even with a mean pH less than 5.6 the occurrence of ruminal acidosis is low in cows consuming an all pasture diet (Kolver and de Veth, 2002). This may be due to the fermentable nature of fiber in fresh pasture along with the low ruminal concentration of lactate which is usually high in cases of acute ruminal acidosis.

Kolver and de Veth (2002) showed performance of dairy cows consuming fresh pasture was not affected by a mean ruminal pH of 5.8-6.2; in this range compared to a pH < 5.6 microbial N flow increased, VFA concentration increased and percent milk fat was unchanged. Only when the pH dropped below 5.4 did substantial reductions in microbial N flow occur along with a decrease in milk fat percentage (Kolver and de Veth, 2002). Three mechanisms were proposed by Kolver and de Veth (2002) as to why high levels of pasture digestion and microbial growth occur at a pH 5.6 - 6.2. First, the digestibility of high quality feed is less compromised by a low ruminal pH than a low quality feed. Second, low pH results from VFA production,
rather than lactic acid production which cause selection against fiber-digesting bacteria. Third, the preferential microbial degradation of starch instead of fiber does not occur on diets high in fresh pasture because there is no starch available. Rumen fermentation could be maintained despite a large variation in ruminal pH, if that pH was maintained at optimal levels (6.0-6.5) for a sufficient proportion of time (Kolver and de Veth, 2002).

Holden et al (1994b) and Bargo et al (2002c) found increased NH$_3$-N concentrations for cows on pasture compared to cows consuming conserved feeds. As with ruminal pH the cows on pasture also had a different fermentation pattern than cows consuming conserved feeds with respect to NH$_3$ -N. An increase was shown in both studies corresponding with gaining access to new a paddock; in the study by Bargo et al (2002c) two daily peaks were shown corresponding to ingestion of pasture after cows were moved to a new paddock.

**Concentrate Supplementation of Dairy Cows on Pasture**

**Purpose**

As stated above, the most important limiting factor in dairy cow production on pasture is intake of energy. This can be overcome with the addition of an energy supplement in the form of grain, so that easily fermentable carbohydrates are available to the animal to compensate for the decrease in DMI and thereby energy intake, that occurs with pasture intake. Objectives of supplementation include: increase milk production per cow, increase stocking rate and milk production per unit land, improve the use of pastures and in times of pasture shortages such as late summer, fall and winter in the Northeastern United States, maintain body condition score and increase length of lactation. Due to high milk production, the use of feeding systems
combining pasture plus the addition of a concentrate supplement is required (Bargo et al, 2003). Appropriate strategies for supplementation of high producing dairy cows requires an understanding of the effect the supplement has on DMI, animal performance and digestion. Also, the nutrients provided must complement the nutrient content of the pasture and meet the nutrient requirements of the cow.

**Effect of Supplementation on Substitution Rate and Total DMI**

Total DMI increases with increasing amounts of grain supplementation (Carruthers and Neil, 1997; Reis and Combs, 2000; Bargo et al, 2002a). However, when grazing cows are fed supplements, pasture DMI usually decreases which is known as substitution rate (SR) (Bargo, et al, 2003). A SR of < 1 kg pasture/kg concentrate means that total DMI is higher on the supplemented treatment than the unsupplemented treatment. The SR of concentrate to forage is extremely variable and depends on numerous factors including forage quality, the type of concentrate and the level of concentrate offered (Malossini et al, 1995). Grazing studies conducted with lactating dairy cows have shown an inconsistent relationship between the amount of supplement and the SR. Kellaway and Porta (1993) have suggested that SR increases with the amount of concentrate supplemented causing a decrease in pasture DMI of 0.5 to 0.9 kg for each kilogram of grain fed. However, it has been reported that in the range of 2 to 6 kg DM/day, amount of concentrate had no effect on substitution rate (Bargo et al, 2003). Reis and Combs (2000) saw a SR of 0.24 kg pasture/kg concentrate when comparing the unsupplemented cows to cows supplemented with 5 kg DM/d and a SR of 0.41 kg pasture/kg concentrate when comparing the unsupplemented cows to cows supplemented with 10 kg DM/d of a corn concentrate. In another study, the SR was lower at a low pasture allowance (25 kg DM/cow/d), 0.26 kg
pasture/kg concentrate, than at a high pasture allowance (40 kg DM/cow/d), 0.55 kg pasture/kg concentrate (Bargo et al, 2002a). It is reported that the higher the pasture quality the higher the SR which is attributed to increased pasture DMI (Penno et al, 2006).

There are two hypotheses that have been proposed to explain the substitution rate of forage by concentrate. The first is that SR may be caused by negative effects in the rumen of grazing cows: a reduction in rumen pH, a decreased rate of pasture digestibility and a decrease in apparent digestibility of NDF. The second hypothesis is that SR may be related to reductions in grazing time. In the study by Bargo et al (2002a), cows on the supplemented treatments at high and low pasture allowances had lower rumen pH, decreased degradation rates of pasture, lower digestibility of NDF and spent less time grazing compared to cows grazing pasture alone. It may not be a matter of which hypothesis is right, but that both hypotheses at differing extents may contribute to the substitution rates of grazing cows supplemented with concentrates. For example, Bargo et al (2002a) concluded that the reduction in grazing time explained 80% of the SR while the remaining 20% is explained by negative effects in the rumen.

**Effect of Supplementation on Milk Production and Composition**

Milk production increases with concentrate supplementation compared to pasture alone (Berzaghi et al, 1996; Reis and Combs, 2000; Bargo et al, 2002a) (Table 1-3). Most studies show a decrease in milk fat percentage with concentrate supplementation (Berzaghi et al, 1996; Carruthers and Neil, 1997; Reis and Combs, 2000; Bargo et al, 2002a). Reis and Combs (2000) saw a linear decrease in milk fat percentage with increasing levels of concentrate supplementation. Despite the greater milk output with supplementation, some studies reported similar fat yield (g/d) because of the lower milk fat percent (Berzaghi et al, 1996; Carruthers and
Neil, 1997). Bargo et al (2002a) found an increase in fat yield with supplementation, whereas Reis and Combs (2000) found a decrease in milk fat production with supplementation. The decrease in milk fat production found in the latter study could be related to the NDF level of this particular diet, which was calculated to be 26% NDF (DM basis) versus the 28% recommended by the NRC (1989). The lower fat percentage may be associated with the lower ratio of acetate to propionate in the rumen often reported in grazing cows supplemented with concentrate (Berzaghi et al, 1996; Reis and Combs, 2000; Bargo et al, 2002a).

In many studies, concentrate supplementation increases milk protein yield and percentage (Carruthers and Neil, 1997; Reis and Combs, 2000; Bargo et al, 2002a). This increase is attributed to increasing energy intakes in supplemented animals. Another explanation for increased milk protein percentage is that increased propionate concentrations lead to more substrate for gluconeogenesis, sparing amino acids used for gluconeogenesis and incorporating those amino acids into the milk. Berzaghi et al, (1996) found an increase in milk protein yield, but saw no difference in milk protein percentage with supplementation, directly reflecting the increase in milk production from supplemented cows (19.5 vs. 23.7 kg/d; \( P < 0.01 \)).

**Effect of Supplementation on Ruminal Fermentation**

Most studies report no effect of supplementation on total VFA concentration (van Vuuren et al, 1986; Berzaghi et al, 1996; Carruthers and Neil, 1997; Reis and Combs, 2000; Sayers et al, 2003) (Table 1-4). Molar concentrations of propionate and butyrate increased, while acetate concentrations remained unchanged with supplementation (Reis and Combs, 2000; Bargo et al, 2002a). Acetate to propionate ratios decreased as a result of the higher propionate concentrations due to grain feeding (Berzaghi et al, 1996; Reis and Combs, 2000; Bargo et al,
These changes were expected since propionate is the major end product of starch fermentation.

A lack of consistency with the effect of amount of concentrate supplementation on ruminal pH of dairy cows on pasture suggests there is not a simple relationship between amount of concentrate supplemented and ruminal pH. Reis and Combs (2000) reported no differences in rumen pH which averaged 6.68 or total VFA concentration which averaged 100.6 mmol/L in pasture only cows and cows supplemented with 5 or 10 kg DM/d of a corn concentrate. In this study rumen fluid was collected eleven times throughout a 24 h period with pH readings measured immediately. However, Sayers et al (2003) found differences in rumen pH in cows supplemented with 5 kg of concentrate compared to 10 kg concentrate (6.00 vs. 5.75, respectively). Berzaghi et al (1996) also reported no differences in ruminal pH which averaged 6.3 or total VFA production which averaged 149 mmol/L in cows grazing pasture only and cows supplemented with 5.4 kg DM/d of cracked corn. In this study rumen fluid was only collected once (0500 h on d 11) to determine pH and VFA concentrations. However, concentrate supplementation reduced rumen pH from 6.57 in unsupplemented cows to 6.25 (P < 0.01) in cows supplemented with 10 kg concentrate, and increased total VFA concentrations were also seen in supplemented cows (130.3 vs. 123.0 mmol/L) in a study by Bargo et al (2002a). In this study rumen fluid was sampled 6 times in a 12 h period and pH was measured immediately. The researchers attributed the high rumen pH of the unsupplemented cows to the medium quality of the pasture grazed. In a study by Carruthers and Neil (1997), which compared the effects of supplementation on pastures containing high nitrogen (2.8%) and low nitrogen (2.1%), no change in total VFA concentration was found with supplementation (average of 129.8 mmol/L),
but rumen pH decreased from 6.18 in unsupplemented cows to 6.08 (P < 0.001) in supplemented cows. Rumen fluid sampling occurred four times with pH measurements taken immediately.

No single dietary variable in diets based on fresh pasture could be used to predict ruminal pH with high reliability (Kolver and de Veth, 2002). For analysis in this study, a database was developed from 23 pasture based studies (121 treatment means), where pasture was the predominant feedstuff (40-100% of the diet). Relationships of measured variables and ruminal pH within study were investigated using mixed models. Regression relationships quantified the relationship between ruminal pH and animal and dietary variables within studies. Ruminal pH was positively related \( (P < 0.05; r^2 < 0.15) \) to forage NDF and NDF content within study, and negatively related \( (P = 0.001; r^2 = 0.14) \) to nonstructural carbohydrate across studies. One reason for a decrease in pH is that corn concentrates are high in fermentable starches which degrade rapidly in the rumen, which may increase VFA concentrations. Other reasons may be a decrease in chewing and ruminating, reduction in rumen motility and reduction in the buffering capacity of the feed all of which lead to a lowered ruminal pH (Kolver and De Veth, 2002).

Diurnal variations in rumen pH and VFA concentrations occur in concentrate supplemented and unsupplemented cows. The pattern of rumen pH variations differed between the supplemented and unsupplemented treatments (Bargo et al, 2002a). Supplemented cows had reductions in rumen pH after cows were fed concentrate and initiated grazing and unsupplemented cows had lower rumen pH after the evening milking. These variations in ruminal pH patterns were attributed to differences in grazing behavior. In a study by Reis and Combs (2000) rumen pH was lower (6.61 vs. 6.75) during the night which is in accordance with an increase in total VFA concentration (107.5 vs. 90.2 Mmol/L) during this time suggesting that more rumen fermentation occurred at this time. An increased proportion of starch in the diet
resulted in more pronounced diurnal patterns of VFA concentrations and reduced pH (Reis and Combs, 2000).

The reduction in ruminal NH\textsubscript{3}-N concentration of supplemented cows is the most consistent effect of supplementation on ruminal fermentation (Bargo et al, 2003). Ruminal NH\textsubscript{3}–N concentration was significantly reduced (Carruthers and Neil, 1997; Reis and Combs, 2000; Bargo et al, 2002a) and numerically reduced (Berzaghi et al, 1996) by supplementation. This reduction could be associated with a higher capture of NH\textsubscript{3}-N from the highly ruminally degradable CP of pasture (Reis and Combs, 2000; Bargo et al, 2002a), but also to a reduction in total CP intake because energy supplements are often lower in CP than pasture (Berzaghi et al, 1996). In the study by Bargo et al (2002a) the reduction in ruminal NH\textsubscript{3}-N concentration in supplemented cows compared to unsupplemented cows (15.3 vs. 8.9 mg/dl) was related to a higher utilization of NH\textsubscript{3}-N because microbial protein synthesis was increased and total CP intake was not reduced with supplementation. Reis and Combs (2000) attributed the linear reduction in ammonia concentrations to the ability of the ruminal bacteria to utilize larger amounts of ammonia because of increased supply of ruminally fermented organic matter in the supplemented cows, which suggests improved nitrogen utilization in these animals.

Effect of Supplementation on Microbial Protein Synthesis

The concentrations of purine derivatives (allantoin and uric acid) in urine have been proposed as a noninvasive method to estimate microbial protein flow (Bargo et al, 2000a). Allantoin is excreted at a constant proportion to other purine derivatives, which come from the breakdown of nucleic acids specifically adenine and guanine. Creatinine, the end product of phosphocreatinine degradation, can be used as in internal marker to predict metabolic processes
in intact animals, because urinary excretion of creatinine is not affected by energy or protein intake changes and is excreted in proportion to body weight. The allantoin/creatinine ratio in spot urine samples can be used as an index of total allantoin excretion in urine.

Few studies report microbial protein supply in supplemented grazing dairy cows. The increasing availability of fermentable energy from supplementation is important for microbial protein synthesis, resulting in the increased availability of amino acids in the small intestine for absorption by the animal. Allantoin and creatinine concentrations were increased in supplemented cows compared to unsupplemented cows (Bargo et al, 2000a). Similarly, the allantoin/creatinine ratio was increased in supplemented cows indicating an increased rumen microbial supply. Carruthers and Neil (1997) also found an increased microbial supply in supplemented cows vs. unsupplemented with a significant increase in allantoin concentration, but not uric acid concentration. However Sayers et al (2003) found no difference in the allantoin/creatinine ratio in cows supplemented with 5 kg compared to cows supplemented with 10 kg concentrate, indicating no change in microbial protein synthesis.

**Effect of Supplementation on In Situ pasture Digestion**

Compared with a pasture only diet, supplementation with 10 kg DM/d concentrate reduced the insoluble potentially degradable DM fraction of pasture, without affecting the soluble fraction, rate of degradation and the effective degradability of DM (Reis and Combs, 2000). Supplementation with 5 kg DM/d of concentrate did not, however, affect any degradation variables of pasture. Bargo et al (2002a) found the rate of degradation of DM pasture was reduced from 6.8 to 5.4 %/h with cows fed a concentrate supplement compared to a pasture only diet. They also found a reduced rate of pasture NDF degradation (4.1 vs. 5.1 %/h) with
concentrate supplementation compared with pasture only diets. Sayers et al (2003) reported that increasing concentrate from 5 kg DM/d to 10 kg DM/d had no effect on DM, ADF and NDF degradability. It is known that the inclusion of starch into the diet depresses fiber digestibility by reducing the cellulolytic activity of fiber digesters, which may be associated with the acidic conditions associated with rapid starch fermentation (Mertens et al, 1980).

**Effect of Supplementation on Post-ruminal Digestion**

Total tract digestibility of OM remained the same in cows consuming a pasture only diet and cows consuming 6.4 kg (as fed)/d of a concentrate supplement, however total tract disappearance of NDF was significantly reduced for cows fed pasture plus the concentrate supplement (70.4 vs. 64.5 %; Berzaghi, et al, 1996). The decrease in total tract disappearance of NDF indicates that the presence of readily degradable corn in the rumen might have altered the microbial population and limited the activity of the cellulolytic bacteria. Reis and Combs (2000) reported a linearly increase of the digestibility of DM and OM with increasing levels of supplementation. Supplementation with 5 kg of concentrate increased the apparent digestibility of DM and OM by 7.6 and 5.7% over the unsupplemented cows, and when the amount of supplement was increased from 5 to 10 kg, DM and OM digestibility values increased by 10.8 and 6.6%, respectively. This increase in digestibility may be due to the concentrate supplement having a high rate and extent of digestion, so an increase in supplementation would be expected to increase total tract digestibility. However, Sayers et al (2003) found that apparent digestibilities of DM and OM of pasture did not change in supplemented cows compared to cows consuming a pasture only diet. Bargo et al (2003) stated in a review that supplementation with concentrates did not affect digestibility of OM, but reduced digestibility of NDF. If NDF
digestibility is reduced one would expect to see a decrease in OM digestibility. However, that is not always the case because the higher digestibility of concentrates compared to pasture might compensate for the reduction in NDF digestibility.
LITERATURE CITED


Table 1-1. Nutrient intake of cows consuming pasture or TMR (adapted from Kolver and Muller, 1998).

<table>
<thead>
<tr>
<th></th>
<th>Pasture</th>
<th>TMR</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (kg/d)(^1)</td>
<td>19.0</td>
<td>23.4</td>
<td>0.6</td>
<td>0.01</td>
</tr>
<tr>
<td>OM (kg/d)</td>
<td>17.6</td>
<td>21.3</td>
<td>0.6</td>
<td>0.01</td>
</tr>
<tr>
<td>NE(_L) (Mcal/d)</td>
<td>32.4</td>
<td>40.2</td>
<td>1.8</td>
<td>0.02</td>
</tr>
<tr>
<td>CP (% of live weight)</td>
<td>0.85</td>
<td>0.75</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>NDF (% of live weight)</td>
<td>1.47</td>
<td>1.21</td>
<td>0.04</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(^1\)Pasture DMI was estimated using a Cr\(_2\)O\(_3\) marker; TMR DMI was calculated by weighing feed offered minus orts.
Table 1-2. Milk production and milk composition of cows consuming pasture or TMR.

<table>
<thead>
<tr>
<th></th>
<th>Kolver and Muller, 1998</th>
<th>Soriano et al, 2001</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pasture</td>
<td>TMR</td>
</tr>
<tr>
<td>Milk, kg/d</td>
<td>29.6</td>
<td>44.1</td>
</tr>
<tr>
<td>Fat %</td>
<td>3.72</td>
<td>3.48</td>
</tr>
<tr>
<td>Protein %</td>
<td>2.61</td>
<td>2.80</td>
</tr>
</tbody>
</table>
Table 1-3. Milk production and composition of grazing dairy cows with or without supplementation

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pasture only w/ 6.4 kg/d concentrate</td>
<td>Pasture only w/ 1.4 kg NSC&lt;sup&gt;1&lt;/sup&gt; supplement</td>
<td>Pasture only w/ 5 kg DM/d concentrate</td>
<td>Pasture only w/ 9 kg DM/d concentrate</td>
</tr>
<tr>
<td>Milk, kg/d</td>
<td>19.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.4</td>
<td>21.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat, kg/d</td>
<td>0.71</td>
<td>0.90</td>
<td>0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.79&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat %</td>
<td>3.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.81&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein, kg/d</td>
<td>0.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.62&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.60&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein %</td>
<td>2.84</td>
<td>3.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.85&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.96&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>NSC = nonstructural carbohydrates (50:50 cornflour and dextrose monohydrate).

<sup>abc</sup>Least square means in the same row within the same study with differing subscripts differ (P < 0.05).
Table 1. Ruminal fermentation variables of grazing dairy cows with or without supplementation.

<table>
<thead>
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<tbody>
<tr>
<td></td>
<td>Pasture only w/ 6.4 kg/d concentrate</td>
<td>Pasture only w/ 1.4 kg NSC supplement</td>
<td>Pasture only w/ 5 kg DM/d concentrate</td>
<td>Pasture only w/ 9 kg DM/d concentrate</td>
</tr>
<tr>
<td>Mean Ph</td>
<td>6.4</td>
<td>6.2</td>
<td>6.18&lt;sup&gt;a&lt;/sup&gt; 6.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.63 6.72 6.69</td>
</tr>
<tr>
<td>Total VFA, mmol/L</td>
<td>150.0</td>
<td>148.0</td>
<td>128.5 131.0</td>
<td>98.7 99.0 104.0</td>
</tr>
<tr>
<td>Acetate, mmol/L</td>
<td>63.2</td>
<td>62.4</td>
<td>51.2 50.9</td>
<td>65.7 63.1 64.2</td>
</tr>
<tr>
<td>Propionate, mmol/L</td>
<td>18.7</td>
<td>19.1</td>
<td>24.8 24.8</td>
<td>17.8&lt;sup&gt;b&lt;/sup&gt; 19.9&lt;sup&gt;b&lt;/sup&gt; 23.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Butyrate, mmol/L</td>
<td>12.9</td>
<td>13.5</td>
<td>16.6 16.7</td>
<td>10.0 10.9 11.3</td>
</tr>
<tr>
<td>A:P ratio</td>
<td>3.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND  ND</td>
<td>3.83&lt;sup&gt;a&lt;/sup&gt; 3.34&lt;sup&gt;b&lt;/sup&gt; 2.83&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> NSC = nonstructural carbohydrates (50:50 cornflour and dextrose monohydrate).
<sup>abc</sup> Least square means in the same row within the same study with differing subscripts differ ($P < 0.05$).
Chapter 2

EFFECTS OF LEVEL OF CONCENTRATE SUPPLEMENTATION ON MILK PRODUCTION AND RUMINAL pH IN LACTATING COWS ON PASTURE
Effects of level of concentrate supplementation on milk production and ruminal pH in lactating cows on pasture

G. R. Clevenger, K. M. Krause and L. R. Tager

Division of Animal and Nutritional Sciences, West Virginia University, Morgantown 26506-6125

Corresponding author: Gatha R. Clevenger, Division of Animal and Nutritional Sciences, West Virginia University, Morgantown 26506-6125, gpensis@mix.wvu.edu

ABSTRACT

The effects of concentrate supplementation on milk production and ruminal fermentation was evaluated in six ruminally cannulated lactating Holstein dairy cows in a Latin rectangle design. Animals were allowed daily access to 14 kg dry matter (DM) of new pasture in addition to the previous day’s paddock containing: 31.2 % acid detergent fiber (ADF), 44.5% neutral detergent fiber (NDF), 15.7% crude protein (CP) and 2.9% ether extract (EE). Cows were fed 4 (C4), 8 (C8), and 12 (C12) kg DM/d of a concentrate supplement consisting of (DM basis): 75.18% ground corn grain, 18.02% soybean meal, 3.0 % sugarcane molasses, 2.0% hydrolyzed feather meal, 0.6% limestone, 0.5% salt, 0.4% vitamin premix, and 0.3% calcium diphosphate. Increasing amounts of concentrate supplement decreased pasture intake from 14.9, 14.0, to 12.1 kg DM/d for C4, C8, and C12, respectively, but increased overall dry matter intake (DMI) to 18.3, 20.4, and 20.5 kg DM/d for C4, C8, and C12, respectively. This was associated with an increased milk production which was 19.9, 21.6 and 22.7 kg/d for C4, C8, and C12, respectively, with no changes in fat, protein or lactose content with increasing levels of concentrate supplementation. Continuous ruminal pH data was collected and daily mean ruminal pH was
similar for all treatments and averaged 6.32, as was daily minimum pH which averaged 5.78. However, an increased proportion of starch in the diet resulted in increased time spent below pH 5.8 (27.1, 71.2, and 162.4 min/d for C4, C8, and C12, respectively) with a trend for increasing numbers of bouts ≤ 5.8, and resulted in a reduction in the in situ degradability of DM (48.6 vs. 42.3% for C4 vs. C12) and NDF (15.5 vs. 9.0%) at 24 hrs. Total tract digestibility was not affected by supplementation. Total volatile fatty acid (VFA) and ammonia concentrations were not affected by increasing levels of supplementation, although diurnal effects were shown. Increasing level of supplementation did not increase microbial protein synthesis. The highest level of supplementation of 12 kg DM/cow/d had negative effects on rumen fermentation and may not be beneficial in a pasture based system.

Key Words: Dairy cow, grazing, pasture, supplementation, milk production, ruminal pH

INTRODUCTION

The economics of dairying in the United States have encouraged dairy farmers to search for new ways to reduce costs. Farms using managed grazing typically produce less milk per cow than confinement farms (Kriegl, 2005). However, economic studies show that grazing farms are economically competitive with confinement operations and a well-managed grazing dairy system is usually less expensive to set up and run with increased net returns compared to a confinement dairy (Kriegl, 2005). Pasture, as the sole diet, does not meet nutrient requirements for high producing dairy cows; therefore energy is the first limiting nutrient (Kolver and Muller, 1998). Dairy cows which solely graze pasture have been shown to have a decreased dry matter intake (DMI) (Kolver and Muller, 1998; Bargo et al, 2002b), decreased milk production (Kolver and Muller 1998; Soriano et al, 2001; Bargo et al, 2002b), altered milk composition (Kolver and
Muller, 1998; Bargo et al, 2002b), altered ruminal fermentation (Holden et al, 1994; Bargo et al, 2002c) and an overall decrease in net energy for lactation (NE\textsubscript{L}) compared to cows receiving a total mixed ration (TMR) with no pasture (Kolver and Muller, 1998).

The use of supplements is necessary in combination with pasture, to optimize DMI in order to maintain profitable milk production. Cows grazing high quality pastures and supplemented with concentrates twice daily produce more milk, but with reduced fat content (Berzaghi, et al, 1996; Carruthers and Neil, 1997; Reis and Combs, 2000; Bargo et al, 2002a), which may be associated with lower acetate to propionate ratios in supplemented cows due to increased propionate production.

Low ruminal pH has negative effects on rumen function. A reduced ruminal pH may result in suboptimal digestion and nutrient supply and may also contribute to metabolic diseases such as ruminal acidosis and laminitis (Allen, 1997; Kolver and de Veth, 2002). It is known that the inclusion of starch into the diet depresses fiber digestibility by reducing the cellulolytic activity of fiber digesters, which may be associated with the acidic conditions associated with rapid starch fermentation (Mertens et al, 1980). Bargo et al (2003) stated in a review that supplementation with concentrates did not affect digestibility of OM, but reduced digestibility of NDF. Compared with a pasture only diet, supplementation with 10 kg DM/d concentrate reduced the insoluble potentially degradable DM fraction of pasture, without affecting the soluble fraction, rate of degradation and the effective degradability of DM (Reis and Combs, 2000).

Knowledge of ruminal pH of grazing dairy cows is limited and no consistent relationship was found between ruminal pH and level of supplementation in a review by Bargo et al (2003). The number of samples collected and the timing of rumen sampling in relation to feeding could
be affecting these results. Mean daily ruminal pH values of 5.6 to 7.0 have been reported for
dairy cows grazing high quality pasture (Berzaghi et al, 1996; Carruthers and Neil, 1997; Reis
and Combs, 2000; Bargo et al, 2002a; Bargo et al, 2002c; Kolver and De Veth, 2002; Sayers et
al, 2003; Graf et al, 2005; Gibbs and Laporte, 2009). Some authors reported reduced ruminal pH
when feeding large amounts (> 8 kg DM/d) of concentrate supplements (Bargo et al, 2002a), but
also when feeding small amounts (1.4 kg DM/d; Carruthers and Neil, 1997) of concentrates to
grazing dairy cows. In contrast, other authors reported similar ruminal pH between pasture only
diets and diets plus varying amounts supplementation (Berzaghi et al, 1996, Reis and Combs,
2000; Graf et al, 2005). The lack of consistency with the amount of concentrate supplementation
on ruminal pH of grazing dairy cows suggests that there is not a simple relationship between
amount of concentrate supplemented and ruminal pH.

The objectives of this experiment were to assess the impact of varying levels of
concentrate supplementation on production performance, ruminal pH and fermentation products,
nutrient utilization and fiber digestibility in grazing dairy cows.

**MATERIALS AND METHODS**

*Experimental Design, Animals and Diet*

Six ruminally cannulated Holstein cows (3 primiparous and 3 multiparous) were
randomly assigned to three supplement treatments within a Latin rectangle design. Average BW
was 543 ± 45.4 kg at the beginning of the experiment and 570.6 ± 46.1 kg at the end of the
experiment. At the start of the experiment the cows were 147 ± 14.3 DIM. The experiment
consisted of three 21-day periods with 11 days of adaptation and 10 days of sampling. The
experiment was approved by the West Virginia University Animal Care and Use Committee and
was conducted at the West Virginia University Research Farm from June 25 to August 26, 2008. The treatments consisted of 3 levels of concentrate supplementation: **C4**: 4 kg concentrate/d, **C8**: 8 kg concentrate/d and **C12**: 12 kg concentrate/d, on a DM basis. The concentrate supplement consisted of (DM basis): 75.18% ground corn grain, 18.02% soybean meal, 3.0% sugarcane molasses, 2.0% hydrolyzed feather meal, 0.6% limestone, 0.5% salt, 0.4% vitamin premix, and 0.3% calcium diphosphate. Assuming a pasture intake of 14 kg DM/d combined with the lowest level of supplementation the diet met NRC (2001) requirements for cows 130 DIM and producing 23 kg milk/d. The cows were milked twice daily at 0830 and 1930 hrs and fed half the daily allotment of their respective concentrate supplement using a Calan gate feeding system (American Calan Inc., Northwood, NH) directly after each milking. A trace mineral salt was offered ad libitum each day after the evening milking and cows had ad libitum access to water on the pasture. Cows had access to pasture approximately 21 h per day.

The pasture was a 5 acre natural, unfertilized field that was previously cut for hay 3 weeks prior to the start of the experiment. Total herbage mass of the pasture averaged 420 kg DM/acre and was measured by sampling the field 6 times with a 0.5 m² quadrant. Pasture growth on the field was not uniform, but approximately one third of the field was mechanically clipped again 14 d into the experiment (21 d prior to cows gaining access to this area) in order to maintain pasture quality. Pasture composition was estimated by sampling the field 10 times with a 0.5 m² quadrant and consisted of (DM basis): 57.9% grasses, 16.9% legumes and 25.5% weeds. The cows were allowed access to 0.2 acres of fresh pasture per day, providing 14 kg of fresh pasture DM/cow/d available to them. Front and back polywire temporary fences were moved once daily after the morning milking to allow for even pasture use. The fences were moved so the cows had access to a new 0.2 acre paddock along with access to the previous day’s paddock.
Sample Collection

Concentrate and pasture samples were collected twice per period. Pasture samples were collected using a 0.5 m² quadrant and for each pasture sample, 5 random sub-samples were cut to a stubble height of 6 cm, collected and pooled from the next day’s paddock.

Lanthanum (La) in solution was used as a marker to estimate fecal output (Hartnell and Satter, 1979) and was ruminally dosed after each milking throughout the entire study to provide 0.8 g of La per cow per day. Ten fecal samples per cow per period were collected at differing times during the last 4 days of sampling to represent a 24 h period to account for diurnal variation. The samples were immediately frozen, then later dried at 60° C, pooled by period for each cow and ground through a 1-mm screen with a Wiley Mill grinder (Arthur H. Thomas, Philadelphia, PA).

Ruminal degradation of pasture and concentrate supplement were measured using in situ bags made of Dacron polyester cloth with a pore size of 52 ± 5 µm. Pasture samples used in the in situ procedure were collected from the rumens of two cows that grazed the pasture immediately before the first period. Rumens of the two cows were evacuated and the animals were allowed to graze for 45 min. Pasture consumed by the cows was then removed from the rumen and immediately ground with dry ice through a 4-mm screen with a chilled Wiley mill grinder (Arthur H. Thomas, Philadelphia, PA) and then frozen. Large bags (10 x 20 cm) containing 15 g of pasture samples obtained using the rumen evacuation method and small bags (5 x10 cm) containing 5 g of the concentrate supplement were weighed in duplicate, placed in larger mesh bags, soaked in warm water for 10 min and ruminally incubated for times 0, 3, 6, 12, 18, 24, 48, 72, and 96 h occurring during the first 5 days of sampling. After removal from the
rumen, bags were washed under cold, running tap water and then frozen for later analysis. Upon
thawing Dacron bags were rinsed in cold water. The time 0 h bags were not placed in the
rumen, but were subject to the same rinsing procedure. The bags were then dried at 60° for 48 h.

Continuous pH data was collected using a submersible Lethbridge Research Centre pH
measuring system (LRCpH, Dascor, Escondido, CA). Indwelling systems were proven an
accurate and precise method to measure changes in pH over time (Penner et al, 2006). The
LRCpH was inserted, with two 1 kg weights attached to prevent shifting, into the ventral sac of
the rumen for 96 h. Before insertion into the rumen pH readings were recorded in standard
buffers of pH 4 and pH 7. Readings recorded every 30 seconds included pH, rumen temperature
and battery voltage. Data transfer from the logger to a computer were conducted every 24 h and
occurred after the morning milking. During this time the logger was removed, logging was
disabled, the data was downloaded and then the logger was returned to the respective cow.
Standardizations of the pH electrodes were conducted after 48 and 96 h; readings in standard
buffer solutions of 4 and 7 were recorded and then the data was downloaded. The shift in
millivolt readings from the electrodes between the start and end standardizations were assumed
to be linear and were used to convert millivolt readings to pH units.

Rumen fluid was sampled (in relation to morning feeding) at times 0, 2, 3, 6, 9, 12, 14,
15, 18, and 21 -h during the last day of sampling for determination of VFA and ammonia
concentrations. Rumen fluid was obtained by grab samples of the digesta from the anterior,
medial and posterior ventral locations of the rumen and then strained through 4 layers of
cheesecloth. Samples of 10 ml, done in duplicate, were acidified with 0.5 ml of H₂SO₄ and
frozen for later VFA analysis. Another 10 ml sample was acidified with 0.2 ml of H₂SO₄ and
frozen for later determination of ammonia concentrations.
Microbial protein synthesis was not measured directly, instead the urinary excretion of the purine derivatives allantoin and uric acid were used as an estimate of microbial N flow to the duodenum. Creatinine analysis was utilized to estimate the total urine output. Spot samples of urine were collected at different times throughout sampling days. The number of collected urine samples ranged from 1-5 samples with an average of 3 samples per cow per period. The samples were immediately frozen for later analysis.

Milk samples were collected and milk yields were recorded at each milking for the last 4 days of each experimental period.

**Laboratory Analysis and Calculations**

Concentrate and pasture DM was determined by oven drying at 60°C for 48 h. Analytical DM of the concentrate, pasture and fecal matter were determined by oven drying at 100°C for 24 h (AOAC, 1995). Ether extraction of the concentrate and pasture was performed according to AOAC (1995) using a Soxtec Foss Tecator (Foss Analytical, Hillerød, Denmark). Ash content of concentrate, pasture and fecal matter were determined using the procedure described by AOAC (1995). Samples were ashed at 500° C for 16 h. NDF and ADF content in the concentrate, pasture and fecal matter were determined using an Ankom 200 Fiber Analyzer (Ankom Technology Corp, Macedon, NY). Crude protein content in the concentrate, pasture and fecal matter were analyzed according to AOAC (1995) using an automated Tecator digestion system (Tecator Inc., Herndon, VA). The sugars of the concentrate, pasture and fecal samples were determined by the extraction procedure adapted from Derias (1961). Reducing sugars were determined spectrophotometrically with potassium ferricyanide. The starch content of concentrate, pasture and fecal matter were determined by the procedure of Smith (1969).
Lanthanum concentrations were determined by dry ashing ground fecal samples at 500°C for 16 h. The ashed samples were then dissolved in 70% nitric acid, diluted to 1:50 in dH₂O and filtered twice through 42 µm filter paper. Lanthanum concentrations were then determined by inductively coupled plasma spectroscopy (ICP). Fecal output was calculated based on La concentration in feces.

The estimated pasture intake for each cow was based on the presence of indigestible ADF (IADF) in pasture, concentrate and fecal samples collected each period. IADF was determined as the ADF remaining after 144 h of in vivo incubation. Using fecal output and the percentage of IADF in the feces the total IADF output was calculated for each cow in each period. Intake of pasture IADF was calculated as the difference between total IADF output and intake of concentrate IADF. Pasture intake was then calculated based on the IADF content of the pasture samples collected each period.

The fractional rate of DM disappearance (DMD) of pasture and concentrate in situ was calculated as the slope of the natural log transformed DMD versus time. Also, the effect of diet on DMD and NDF disappearance (NDFD) of pasture and concentrate at 24 h was analyzed separately.

Analysis of VFA concentrations in the rumen fluid were performed using the gas chromatographic separation procedure (Anonymous, 1975). The gas chromatograph was a Varian model 3300 with an FID detector (Varian, Inc., Palo Alto, CA). The column was a 2 m × 2 mm glass column packed with 10% SP-1200/1% H₃HPO₄ on 80/100 chromosorb WAW (Supelco, Inc., Bellefonte, PA). Ammonia in the rumen fluid was analyzed according to AOAC (1995) using an automated Tecator digestion system (Tecator Inc., Herndon, VA).
Frozen urine samples were thawed out and 20 ml of urine were diluted with 80 ml of dH$_2$O. Samples were then diluted to a final dilution of 1:50 for analysis of allantoin and uric acid. A dilution of 1:40 was used for analysis of creatinine and uric acid samples that were too dilute for analysis using the 1:50 dilution. Allantoin concentration was determined colorimetrically using the method described by Chen and Gomes (1992); however, 1M HCl was used instead of 0.5 M HCl in order to keep the pH below 3. Uric acid in urine was determined colorimetrically using a diagnostic uric acid reagent kit (Biovision Inc, Mountain View, CA). Creatinine concentrations in urine were determined colorimetrically using a diagnostic creatinine reagent kit (Biovision Inc, Mountain View, CA).

Urine output (L/d) was calculated as body weight (kg) x creatinine excretion rate (mg/kg of body weight/d) divided by creatinine concentration (mg/L). One mean daily creatinine excretion rate of 29.0 mg/kg of BW per d was used based on data from Valadares et.al (1999). Purine absorption and intestinal flow of microbial N was calculated using the assumptions and equations given by Chen and Gomes (1992). The quantitative relationship between absorption of microbial purines (mmol/d) and excretion of purine derivatives in urine can be described by the following equation:

\[ Y = 0.85X + (0.0385 W^{0.75}), \]

where \( W^{0.75} \) represents the metabolic body weight (kg) of the animal. The slope of 0.85 represents the recovery of absorbed purines as purine derivatives in urine. The component within parenthesis represents the net endogenous contribution of purine derivatives to total excretion after correction for the utilization of microbial purines by the animal. The following factors were used for the calculation of intestinal flow of microbial N (g N/d) from the microbial purines absorbed (mmol/d): digestibility of microbial purines was assumed to be 0.83; the N
content of purines was 70 mg N/mmol; and the ratio of purine-N:total N in mixed rumen microbes was taken as 11.6:100. Thus the microbial N was calculated as:

\[
\text{Microbial N supply (g/d)} = \frac{X \times 70}{0.83 \times 0.116 \times 1000} = 0.727 \times X
\]

This assumes that the purine:protein ratio in mixed rumen microbes was unchanged by dietary treatment.

The milk samples were sent to Dairy One (Ithaca, New York) for determination of lactose, protein, % milk fat and SNF.

**Statistical Analyses**

Data was analyzed using the mixed model procedure of SAS 9.1 (2001). Data that did not have repeated measures over time were analyzed using a model including treatment as a fixed effect and period and cow as random effects.

Ruminal variables that had repeated measures over time (pH, ammonia, VFA concentrations and percentages, and acetate:propionate ratio) were analyzed using a model that included treatment, feeding (morning or evening) and hours post feeding as fixed variables along with their two- and three-way interactions. Period, cow and cow by period were included as random effects in the model. Based on model fitting statistics a Compound Symmetry covariance structure was used for these analyses.

Statistical analysis was conducted using a Kenward-Roger adjustment and reported as least squared means. Separation of means was performed on data with significant treatment differences and tendency for treatment differences, and a Tukey’s adjustment was used to make treatment comparisons. Linear and quadratic effects of increasing levels of concentrate
supplementation were tested using orthogonal contrasts. Differences were considered significant at $P \leq 0.05$ and considered a trend at $0.05 < P \leq 0.10$.

**RESULTS AND DISCUSSION**

*Dry Matter Intake and Nutrient Digestibilities*

As expected, concentrate intake increased linearly and was 3.5, 6.4 and 8.4 kg/d for the increasing levels of supplement offered (C4, C8, and C12, respectively). One specific cow did not eat any concentrate throughout the entire experiment resulting in lower than expected average concentrate intakes for each of the treatments. At the highest level of supplementation, C12 group concentrate intake was statistically similar to the C8 group, with only a few cows consuming all of the concentrate offered in the C12 treatment. Pasture intake decreased linearly with increasing levels of supplement ($P < 0.05$; Table 3); with cows fed the C12 diet consuming 18.8% less pasture than cows fed the C4 diet. The estimates for pasture DMI, which were 14.9 kg/d for the lowest supplementation level and 12.1 kg/d for the highest level of supplementation, are comparable to grazing studies with increasing levels of supplementation (Reis and Combs, 2000) and supplementation of greater than 5 kg corn supplement/d (Carruthers and Neil, 1997; Bargo et al, 2002a). On average pasture intake decreased 0.63 kg for every kilogram of concentrate fed, which is higher than the SR of 0.55 and 0.40 reported by Bargo et al (2002a) and Reis and Combs (2000), respectively. This difference might be caused by the higher level of concentrate fed in the current study compared to the 9 and 10 kg DM/cow/d fed in the studies by Bargo et al (2002a) and Reis and Combs (2000), respectively. It has been reported that SR increased by 0.03 kg DM/kg DM for each additional concentrate kg DM supplemented (Stockdale, 2000). Substitution rates vary, but in general, pasture DMI decreases about 0.5 to
0.9 kg for each kilogram of grain fed (Reis and Combs, 2000). Pasture quality also affects SR; the higher the pasture quality the higher the SR due to increased pasture DMI (Penno et al, 2006). In the current study a high SR of 0.63 kg pasture/kg concentrate was seen with medium quality pasture. The total intake of DM and OM increased linearly with increasing concentrate supplement ($P < 0.04$ and $0.03$, respectively; Table 3). Intake of NDF did not change due to treatment; however a trend toward a linear decrease in ADF intake with increasing levels of concentrate supplementation was observed ($P < 0.10$). Because of the lower NDF and ADF content of the concentrate compared to pasture the NDF and ADF intakes decreased numerically with increased concentrate intakes. The intakes of CP and starch plus WSC increased linearly with increasing levels of concentrate supplement ($P < 0.01$ and $0.004$, respectively). The increasing intakes of CP and starch plus WSC can be explained by their high content in the concentrate (18 and 62.9%, respectively). There was no effect of level of supplementation on digestibility of any of the nutrients (Table 3), however digestibility of starch plus WSC did decrease numerically when more than 4 kg of concentrate was supplemented ($P = 0.12$). This numerical decrease in starch digestibility was accompanied by a linear increase in fecal starch plus WSC content ($P=0.002$) with increasing levels of supplementation. The effect of supplementation on digestibility in this study is not in accordance with other research. Bargo et al (2002a) and Reis and Combs (2000) found increasing digestibilities of DM and OM with supplementation, which did not occur in the present study. Reis and Combs (2000) reported an increase in starch digestibility with increasing levels of supplementation which should result in increased DM digestibility. Contrary, we observed a numerical decrease in starch digestibility in the current study. Although we did not measure rate of passage the increase in DMI observed with increasing levels of supplementation might be expected to increase rate of passage. The
majority of starch is degraded in rumen, so an increased rate of passage would have a negative impact on starch digestion.

**Milk Production and Composition**

Milk production increased linearly with increasing levels of concentrate supplement \((P < 0.02\) Table 4). Cows fed the C12 diet produced 12.3% more milk than the cows fed the C4 diet. Milk fat percentage was not different due to treatment, but milk fat yield increased linearly \((P < 0.02)\) with increasing concentrate supplement due to the increase in milk yield. This is not in agreement with other studies which show a decrease in milk fat percentage where grazing cows were supplemented with greater than 5 kg/cow/day (Berzaghi et al, 1996; Carruthers and Neil, 1997; Reis and Combs, 2000; Bargo et al, 2002a). Reis and Combs (2000) reported linear reductions in milk fat percentage from 3.89, 3.50, to 3.08% with increasing levels of concentrate supplementation from 0, 5, to 10 kg/d. For all but the Carruthers and Neil (1997) study, the decrease in milk fat percentage may be associated with lower acetate to propionate ratios in supplemented cows, which was not seen in the current study (Table 5). In the current study acetate to propionate ratio ranged from 3.8 to 4.1; well above the ratios reported in other studies where supplementation resulted in lower milk fat percentage. Carruthers and Neil (1997) reported a low acetate to propionate ratio average for all treatments of 2.1 compared with a ratio average of 3.3 from supplemented cows in other studies (Reis and Combs, 2000; Bargo et al, 2002a).

Both milk protein percentage and yield were not different due to treatment. A numerical increase was shown in milk protein yield from 0.53 to 0.61 kg/d in cows on the C4 treatment compared to cow on the C12 treatment. However, in other research (Carruthers and Neil, 1997;
Reis and Combs, 2000; Bargo et al, 2002a), milk protein yield and percentage increased with corn supplementation. Reis and Combs (2000) and Bargo et al (2002a) reported an increase in the production of propionic acid with supplementation, indicating an increase in availability of fermentable energy obtained with corn supplementation. Absorption of amino acids represents the main substrate for milk protein. An increase in propionate would be expected to decrease the use of amino acids used for gluconeogenesis, thereby making more amino acids available for milk protein synthesis. Because of the unchanged propionate concentrations (Table 5), as well as unaffected microbial protein synthesis (Table 7), increased milk protein percentage was not seen in this study. Lactose percentage was similar across treatments, whereas lactose yield increased linearly with increasing levels of supplement ($P < 0.01$). Corn supplementation did not affect milk lactose content; therefore, the greater lactose yield directly reflects the difference in milk production.

**Ruminal Fermentation Characteristics**

Ruminal ammonia concentrations were not affected by varying levels of concentrate supplement (Table 5) and averaged 9.97 mg/dl which is well above the 5 mg/dl necessary to maximize microbial growth (Satter and Sylter, 1974). Other studies report decreasing ammonia concentrations with increasing levels of supplementation (Reis and Comb, 2000) and concentrate supplementation greater than 5 kg/day (Carruthers and Neil, 1997; Bargo et al, 2002a). The unaffected ammonia concentrations in this study may be due to the high CP content of the concentrate (18 %) compared to concentrates fed in other studies. However, feeding and hours post feeding had an effect on ruminal ammonia concentrations (Figure 1). Ammonia concentration increased from 0 to 2 h after the morning feeding, but then decreased, whereas no
increase was observed following the evening feeding. The increase in ammonia concentration after the morning feeding may be associated with cows given access to a new paddock directly after the morning concentrate feeding. Grazing behavior was not recorded, but high grazing bouts of new pasture were observed at this time which could explain the observed increase in ruminal ammonia concentration.

Total VFA concentrations were not affected by treatment, but the effect of feeding on VFA concentration was not similar across treatments (Table 5). Total VFA concentrations were higher after the evening feeding compared to the morning feeding for the C12 treatment compared to the C4 and C8 treatments. The increase in VFA concentration after the evening feeding for the C12 group may be due to a possible decrease in pasture intake for this treatment along with a decrease in rumination, resulting in less dilution of rumen contents compared to other treatments. Also, differences were seen in total VFA concentration due to hours post feeding (P < 0.006). In general, VFA concentrations increased after each feeding of concentrate and then decreased again prior to the next feeding (Figure 2). Acetate and propionate concentrations were not different due to treatment, but differences were seen due to hours post feeding (P < 0.01) similar to the ones observed for total VFA concentration (Figure 3 and 4, respectively). Since propionate is the major end product of starch fermentation an increase in propionate concentrations were expected, but not seen in this study which is not in accordance with other studies reporting increasing propionate concentrations with supplementation (Reis and Combs, 2000; Bargo et al, 2002a). The unaffected propionate concentration may be associated with a lower percentage of corn grain in the concentrate fed in the current study compared to other studies (75.2% in the current study vs. 90.5% in Reis and Combs, 2000) as well as the fact that the corn grain was very coarsely ground (high amount of whole kernels),
resulting in lower starch digestibility compared to more finely ground corn. Whereas Reis and Combs (2000) reported starch and sugar digestibilities of 86.0 and 86.7% when supplementing with 5 and 10 kg/cow/d, respectively, the average digestibility of starch and WSC was only 79.7% in the current study. Acetate and propionate concentrations were higher after the evening feeding compared to the morning feeding for the C12 treatment compared to the C4 and C8 treatments. An interaction between feeding and hours post feeding was shown to affect acetate concentrations with a greater increase in acetate concentration following the evening feeding compared to the morning feeding. Butyrate concentrations increased linearly with increasing levels of concentrate supplement ($P < 0.05$) which agrees with results from other studies (Carruthers and Neil, 1997; Reis and Combs, 2000 and Bargo et al, 2002a). Propionate had higher concentrations after the evening feeding compared to the morning feeding; this was especially pronounced for the C12 treatment. Butyrate concentrations were higher for the C12 treatment after the evening feeding compared to the morning feeding, whereas this did not occur in the C4 or C8 treatments. Acetate to propionate ratios did not change due to treatment, but an interaction between feeding and hours post feeding showed a higher acetate to propionate ratio occurring from 0 to 2 h post feeding for the morning feeding compared to the evening feeding at the same times ($P < 0.05$). In the current study acetate to propionate ratio ranged from 3.8 to 4.1; well above the ratios reported in other studies (Berzaghi et al, 1996; Carruthers and Neil, 1997; Reis and Combs, 2000; Bargo et al, 2002a; Sayers et al, 2005). The higher acetate to propionate ratio seen in this study may be attributed to the unaffected propionate concentrations as well as the numerical decrease in starch digestibility. The increase in fecal starch plus WSC concentrations indicates the lower availability of the starch provided in the concentrate. Another reason for the higher acetate to propionate ratio may be that the acetate concentrations were
higher in the current study than what was previously reported (Berzaghi et al, 1996; Carruthers and Neil, 1997; Reis and Combs, 2000; Sayers et al, 2005). The high acetate concentration found in the current could be related to the higher NDF intake compared to the intakes reported by Berzaghi et al (1996) and Reis and Combs (2000).

Mean ruminal pH was unaffected by diet, but did decrease numerically from 6.37 to 6.27 when level of concentrate supplementation increased from 4 to 8 and 12 kg DM/d (Table 6). These pH values are lower than the findings of Reis and Combs (2000) who reported a mean ruminal pH of 6.68, but are consistent with Bargo et al (2002a) findings of a mean ruminal pH of 6.27 in supplemented cows. The high mean ruminal pH in the current study is likely related to the medium quality of the pasture grazed (Table 2). Fiber, in the grazing animals diet, increases saliva flow by stimulating chewing and rumination (Allen, 1997), therefore the rumen contents are diluted and only slightly acidic even with increased VFA production. The effect of supplementation on rumen pH vary among studies; Carruthers and Neil (1997) and Bargo et al (2002a) found decreased ruminal pH in supplemented cows where as Berzaghi et al (1996) and Reis and Combs (2000) found no differences in ruminal pH of supplemented cows compared to pasture only cows. Daily minimum pH was also unaffected by diet, but similarly to mean ruminal pH decreased numerically when level of supplementation was increased above 4 kg DM/d. Number of daily bouts with a pH less than 5.8 tended to increase with increasing level of supplementation with 1.1 events per day in the C4 group to 7.5 events per day in the C12 group (P = 0.09). The mean length of a bout ≤ 5.8 was unaffected by diet, however the amount of time spent below 5.8 increased linearly from 27.1 min/d in the C4 group to 162.4 min/d in the C12 group with increasing levels of concentrate supplement (P < 0.02). Mean area of a bout spent
below pH 5.8 was not affected by treatment, but increased numerically from 73.8 to 128.8 min x pH units/d with increasing levels of supplementation.

**Ruminal Degradation of Pasture and Concentrate**

Increasing the level of supplementation did not affect 24 h in situ ruminal DM degradation of concentrate (Table 7), whereas 24 h in situ ruminal degradation of pasture DM decreased linearly from 48.6 to 42.3%, as did degradation of pasture NDF (from 15.5 to 9.0%) with increasing levels of concentrate supplement ($P < 0.007$ and 0.03, respectively). Reis and Combs (2000) also reported a reduction in the potentially degradable fraction with increasing levels of supplementation, but had no effect on apparent total tract fiber digestibility. An increased proportion of starch in the diet resulted in more pronounced diurnal patterns of VFA concentrations (Figure 2) and increased time spent below pH 5.8, which is not favorable for fiber digestion. The fact that total tract NDF digestibility did not differ due to treatment indicates that post-ruminal digestion compensated for the decrease in ruminal DM and NDF degradation.

**Microbial Yield**

Daily excretion of allantoin and uric acid were not affected by treatment (Table 7), resulting in similar estimated microbial N supplies, which averaged 589.4 g/d of microbial N, as well as similar allantoin/creatinine ratios, which averaged 7.14, for the three levels of supplementation. This is not in accordance with other studies (Carruthers and Neil, 1997; Bargo et al, 2002a) which reported an increase in microbial protein synthesis with increasing levels of supplementation. Carruthers and Neil (1997) reported a microbial N supply of 248 g/d in the unsupplemented cows vs. 286.5 g/d in cows fed a concentrate supplement. Bargo et al (2002a)
reported an allantoin/creatinine ratio of 3.03 in pasture only cows vs. 3.31 in cows fed a concentrate supplement, indicating an increased microbial protein supply in the supplemented cows. The microbial N supply and allantoin/creatinine values reported in the current study are high when compared to other studies. This could be due to a large variation in urine samples collected per cow (1-5 samples/cow) or due to a large variation in values from sample to sample within and between cows.

CONCLUSION

Increasing the amount of grain supplement in the diet decreased pasture intake, but increased overall DMI. This was associated with an increased milk production, with no changes in fat, protein or lactose content with increasing levels of concentrate supplementation.

Mean ruminal pH and daily minimum pH was not affected by increasing levels of concentrate supplementation. However, an increased proportion of starch in the diet resulted in increased time spent below pH 5.8 with a trend for increasing number of bouts ≤ 5.8, which is not favorable for fiber digestion. This resulted in a reduction in the in situ degradability of DM and NDF at 24 hrs, however, total tract digestibility was not affected by supplementation. Total VFA and ammonia concentrations were not affected by treatment although diurnal variations were shown. Microbial N supply was not affected by treatment, but was higher than what other studies have previously reported. In the current study high levels of supplementation of 12 kg DM/cow/d had negative effects on rumen fermentation and digestibility and may not be beneficial in a pasture based system.
REFERENCES


Table 1. Composition of concentrate supplement

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground corn grain</td>
<td>75.2</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>18.0</td>
</tr>
<tr>
<td>Sugarcane molasses</td>
<td>3.0</td>
</tr>
<tr>
<td>Hydrolyzed feather meal</td>
<td>2.0</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.6</td>
</tr>
<tr>
<td>Salt</td>
<td>0.5</td>
</tr>
<tr>
<td>Vitamin premix(^1)</td>
<td>0.4</td>
</tr>
<tr>
<td>Calcium diphosphate</td>
<td>0.3</td>
</tr>
</tbody>
</table>

\(^1\) Vit A: 9,920,624.9 IU/kg, Vit. D\(_3\): 2,204,585.5 IU/kg, Vit. E: 4,409.2 IU/kg
Table 2. Chemical composition of pasture and concentrate

<table>
<thead>
<tr>
<th></th>
<th>DM</th>
<th>CP</th>
<th>NDF</th>
<th>ADF</th>
<th>Ether Extract</th>
<th>WSC²</th>
<th>Starch</th>
<th>NE₅/₃</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percent of DM</td>
<td>Mcal/ kg DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pasture</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>21.6 ± 3.27¹</td>
<td>14.6 ± 2.29</td>
<td>44.3 ± 2.47</td>
<td>30.6 ± 2.73</td>
<td>2.9 ± 0.64</td>
<td>13.1 ± 0.41</td>
<td>1.38 ± 0.02</td>
<td>1.26 ± 0.05</td>
</tr>
<tr>
<td>Period 2</td>
<td>21.0 ± 2.57</td>
<td>17.2 ± 3.17</td>
<td>42.0 ± 3.89</td>
<td>31.4 ± 1.26</td>
<td>3.0 ± 0.67</td>
<td>11.0 ± 1.35</td>
<td>0.6 ± 0.48</td>
<td>1.31 ± 0.08</td>
</tr>
<tr>
<td>Period 3</td>
<td>25.1 ± 1.33</td>
<td>15.2 ± 1.21</td>
<td>47.2 ± 0.35</td>
<td>31.6 ± 1.25</td>
<td>2.9 ± 0.14</td>
<td>11.0 ± 0.49</td>
<td>0.7 ± 0.56</td>
<td>1.19 ± 0.01</td>
</tr>
<tr>
<td>Average</td>
<td>22.7 ± 2.3</td>
<td>15.7 ± 1.4</td>
<td>44.5 ± 2.6</td>
<td>31.2 ± 0.5</td>
<td>2.9 ± 0.1</td>
<td>11.7 ± 1.2</td>
<td>0.9 ± 0.4</td>
<td>1.25 ± 0.1</td>
</tr>
<tr>
<td><strong>Concentrate</strong></td>
<td>89.8 ± 0.2</td>
<td>18.0 ± 0.8</td>
<td>12.5 ± 1.5</td>
<td>5.2 ± 0.5</td>
<td>3.6 ± 0.3</td>
<td>6.7 ± 0.6</td>
<td>56.2 ± 0.7</td>
<td></td>
</tr>
</tbody>
</table>

¹Standard deviation
²Water soluble carbohydrates
³Based on Mertens (1983): NE₅/Mcal/ kg DM = (1.0055 – 0.0098 * % NDF) ÷ 0.454.
Table 3. Feed intake and digestibility of grazing dairy cows fed increasing levels of a concentrate supplement

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intake, kg/d</th>
<th>Effects, P ≤</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>C4</td>
<td>C8</td>
</tr>
<tr>
<td>Pasture</td>
<td>14.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.0&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total DM</td>
<td>18.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>OM</td>
<td>16.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NDF</td>
<td>7.0</td>
<td>7.1</td>
</tr>
<tr>
<td>ADF</td>
<td>4.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>CP</td>
<td>2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Starch and WSC&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.8&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

% Digestibility

<table>
<thead>
<tr>
<th>DM</th>
<th>OM</th>
<th>NDF</th>
<th>ADF</th>
<th>CP</th>
<th>Starch and WSC</th>
<th>Fecal Starch plus WSC, % of DM</th>
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</thead>
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<tr>
<td>54.9</td>
<td>57.9</td>
<td>39.9</td>
<td>38.1</td>
<td>55.0</td>
<td>83.5</td>
<td>8.28&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>54.9</td>
<td>57.4</td>
<td>37.8</td>
<td>36.6</td>
<td>56.0</td>
<td>77.6</td>
<td>14.49&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>55.6</td>
<td>57.7</td>
<td>40.3</td>
<td>39.7</td>
<td>56.2</td>
<td>78.0</td>
<td>16.54&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>3.39</td>
<td>3.28</td>
<td>4.84</td>
<td>4.03</td>
<td>1.85</td>
<td>3.58</td>
<td>2.41</td>
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<tr>
<td>0.94</td>
<td>0.97</td>
<td>0.66</td>
<td>0.59</td>
<td>0.73</td>
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<tr>
<td>0.76</td>
<td>0.94</td>
<td>0.88</td>
<td>0.60</td>
<td>0.47</td>
<td>0.08</td>
<td>0.0009</td>
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<td>0.87</td>
<td>0.82</td>
<td>0.38</td>
<td>0.39</td>
<td>0.79</td>
<td>0.23</td>
<td>0.20</td>
</tr>
</tbody>
</table>

1<sup>C4</sup> = 4 kg concentrate, <sup>C8</sup> = 8 kg concentrate, <sup>C12</sup> = 12 kg concentrate.
2<sup>Water soluble carbohydrates</sup>
<sup>a,b</sup> Least square means in the same row with differing subscripts differ (P < 0.05).
Table 4. Milk production and milk composition of grazing dairy cows fed increasing levels of a concentrate supplement

<table>
<thead>
<tr>
<th>Treatments¹</th>
<th>C4</th>
<th>C8</th>
<th>C12</th>
<th>SEM</th>
<th>Effects, ( P \leq )</th>
<th>Diet</th>
<th>Linear</th>
<th>Quadratic</th>
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<tbody>
<tr>
<td>Milk, kg/d</td>
<td>19.9(^b)</td>
<td>21.6(^{ab})</td>
<td>22.7(^a)</td>
<td>2.37</td>
<td></td>
<td>0.02</td>
<td>0.01</td>
<td>0.63</td>
</tr>
<tr>
<td>Fat</td>
<td>3.4</td>
<td>3.7</td>
<td>3.7</td>
<td>0.15</td>
<td></td>
<td>0.24</td>
<td>0.15</td>
<td>0.39</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kg/d</td>
<td>0.66(^b)</td>
<td>0.80(^a)</td>
<td>0.82(^a)</td>
<td>0.09</td>
<td></td>
<td>0.02</td>
<td>0.01</td>
<td>0.13</td>
</tr>
<tr>
<td>Protein</td>
<td>2.8</td>
<td>2.8</td>
<td>2.8</td>
<td>0.17</td>
<td></td>
<td>0.51</td>
<td>0.83</td>
<td>0.27</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kg/d</td>
<td>0.53</td>
<td>0.6</td>
<td>0.61</td>
<td>0.06</td>
<td></td>
<td>0.11</td>
<td>0.06</td>
<td>0.28</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.5</td>
<td>4.6</td>
<td>4.6</td>
<td>0.07</td>
<td></td>
<td>0.19</td>
<td>0.11</td>
<td>0.40</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kg/d</td>
<td>0.85(^b)</td>
<td>0.98(^a)</td>
<td>1.03(^a)</td>
<td>0.11</td>
<td></td>
<td>0.01</td>
<td>0.01</td>
<td>0.26</td>
</tr>
</tbody>
</table>

\(^a,b\) Least square means in the same row with differing subscripts differ \((P < 0.05)\).

¹C4 = 4 kg concentrate, C8 = 8 kg concentrate, C12 = 12 kg concentrate
Table 5. Ruminal fermentation variables of grazing dairy cows fed increasing levels of a concentrate supplement

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SEM</th>
<th>Effects, $P &lt;$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C4</td>
<td>C8</td>
</tr>
<tr>
<td>Ammonia, mg/dl</td>
<td>9.6</td>
<td>9.6</td>
</tr>
<tr>
<td>VFA, mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>108.9</td>
<td>113.6</td>
</tr>
<tr>
<td>Acetate</td>
<td>72.8</td>
<td>73.5</td>
</tr>
<tr>
<td>Propionate</td>
<td>20.3</td>
<td>21.9</td>
</tr>
<tr>
<td>Butyrate</td>
<td>13.0$^b$</td>
<td>15.2$^a$</td>
</tr>
<tr>
<td>A/P$^3$</td>
<td>3.9</td>
<td>3.8</td>
</tr>
</tbody>
</table>

1$^{C4} = 4$ kg concentrate, $C8 = 8$ kg concentrate, $C12 = 12$ kg concentrate.

2Effects: $D = $ Diet, $F = $ Feeding, $HPF = $ hours post-feeding, $D \times F = $ diet by feeding interaction, $D \times HPF = $ diet by hours post-feeding interaction, $D \times F \times HPF = $ diet by feeding by hours post feeding interaction, $L = $ linear contrast, $Q = $ quadratic contrast

3Acetate to propionate ratio

$^{a,b}$ Least square means in the same row with differing subscripts differ ($P < 0.05$).
Table 6. Ruminal pH of grazing dairy cows fed increasing levels of a concentrate supplement

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C4</th>
<th>C8</th>
<th>C12</th>
<th>SEM</th>
<th>Effects, P ≤</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Diet</td>
</tr>
<tr>
<td>Mean ruminal pH</td>
<td>6.37</td>
<td>6.27</td>
<td>6.27</td>
<td>0.08</td>
<td>0.20</td>
</tr>
<tr>
<td>Daily minimum pH</td>
<td>5.86</td>
<td>5.75</td>
<td>5.74</td>
<td>0.08</td>
<td>0.30</td>
</tr>
<tr>
<td>Number of bouts ≤ 5.8 /d</td>
<td>1.1</td>
<td>4.5</td>
<td>7.5</td>
<td>2.58</td>
<td>0.09</td>
</tr>
<tr>
<td>Time spent ≤ 5.8, min/d</td>
<td>27.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>162.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.72</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean length of bout ≤ 5.8, min</td>
<td>10.5</td>
<td>15.9</td>
<td>12.8</td>
<td>4.86</td>
<td>0.71</td>
</tr>
<tr>
<td>Mean area of bout ≤ 5.8</td>
<td>73.8</td>
<td>116.3</td>
<td>128.8</td>
<td>54.89</td>
<td>0.67</td>
</tr>
</tbody>
</table>

<sup>1</sup>C4 = 4 kg concentrate, C8 = 8 kg concentrate, C12 = 12 kg concentrate.

<sup>a,b</sup>Least square means in the same row with differing subscripts differ (P < 0.05).
Table 7. Purine derivative excretion of grazing dairy cows fed increasing levels of a concentrate supplement

<table>
<thead>
<tr>
<th></th>
<th>Treatment $^1$</th>
<th>SEM</th>
<th>Effects, P ≤</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C4</td>
<td>C8</td>
<td>C12</td>
</tr>
<tr>
<td>Uric Acid, mmol/d</td>
<td>46.2</td>
<td>45.4</td>
<td>56.2</td>
</tr>
<tr>
<td>Allantoin, mmol/d</td>
<td>947.1</td>
<td>750.0</td>
<td>1002.6</td>
</tr>
<tr>
<td>Total, mmol/d</td>
<td>993.2</td>
<td>795.3</td>
<td>1057.6</td>
</tr>
<tr>
<td>Absorption, mmol/d</td>
<td>848.6</td>
<td>680.4</td>
<td>903.2</td>
</tr>
<tr>
<td>Microbial N supply, g/d</td>
<td>616.9</td>
<td>494.7</td>
<td>656.6</td>
</tr>
<tr>
<td>Urine output, L/d</td>
<td>76.7</td>
<td>53.9</td>
<td>71.8</td>
</tr>
<tr>
<td>Allantoin (A), mg/L</td>
<td>2346.9</td>
<td>2350.7</td>
<td>2478.3</td>
</tr>
<tr>
<td>Creatinine (C), mg/L</td>
<td>317.6</td>
<td>355.4</td>
<td>387.6</td>
</tr>
<tr>
<td>A/C ratio</td>
<td>7.70</td>
<td>6.78</td>
<td>6.94</td>
</tr>
</tbody>
</table>

$^1$C4 = 4 kg concentrate, C8 = 8 kg concentrate, C12 = 12 kg concentrate.
Table 8. Ruminal degradation at 24 h of pasture and concentrate of grazing dairy cows fed increasing levels of a concentrate supplement

<table>
<thead>
<tr>
<th></th>
<th>Treatment¹</th>
<th>SEM</th>
<th>Effects, P ≤</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C4</td>
<td>C8</td>
<td>C12</td>
</tr>
<tr>
<td>% DMD²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentrate</td>
<td>62.8</td>
<td>61.6</td>
<td>59.6</td>
</tr>
<tr>
<td>Pasture</td>
<td>48.6ᵃ</td>
<td>46.5ᵃ</td>
<td>42.3ᵇ</td>
</tr>
<tr>
<td>% NDF disappearance</td>
<td>15.5ᵇ</td>
<td>18.3ᵇ</td>
<td>9.0ᵇ</td>
</tr>
</tbody>
</table>

¹C4 = 4 kg concentrate, C8 = 8 kg concentrate, C12 = 12 kg concentrate.
²Dry matter disappearance
ᵃᵇ Least square means in the same row with differing subscripts differ (P < 0.05).
Figure 1. Hours post feeding pattern of ammonia concentration of grazing dairy cows fed increasing levels of a concentrate supplement. (SEM = 2.29)
Figure 2. Daily pattern of total VFA concentration of grazing dairy cows fed increasing levels of a concentrate supplement. (SEM = 5.76)

↓ Indicates concentrate feeding
Figure 3. Daily pattern of acetate concentration of grazing dairy cows fed increasing levels of a concentrate supplement. (SEM = 3.39)

\( \downarrow \) Indicates concentrate feeding
Figure 4. Daily pattern of propionate concentration of grazing dairy cows fed increasing levels of a concentrate supplement. (SEM = 1.3)

ดาวน์arrow Indicates concentrate feeding
Figure 5. Daily pattern of butyrate concentrations of grazing dairy cows fed increasing levels of a concentrate supplement. (SEM = 1.39)

↓ Indicates concentrate feeding